Making things easier

How to improve antiviral drug treatment for children

Diane Bastiaans

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Proefschrift

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General introduction

Medicines for children

Paediatric patients cannot simply use the same drug formulations and dosages as adult patients. The appropriateness of a formulation is determined by the characteristics of the patient to be treated with the drug, the specific drug and the dose to be used, and the environment in which it has to be administered. Children are characterized by gaining in height and weight until they grow up beyond adolescence, and by the size and function of their organs changing over time [1]. The right dose has to be determined for children taking into account the disease they suffer from, their age and stage of development. This often results in the need for different amounts of drugs relative to their weight, body surface area or maturation of organ functions, and different formulations depending on their age and development. Oral administration of drugs is generally considered the most convenient method to administer drugs, also for children. For paediatric oral drug therapy several aspects have to be considered [2]. Since the dose might have to be changed while children grow and develop, drug formulations with the possibility of flexible dosing are preferred. Different aspects determine acceptability, such as food restrictions, size of tablets, amount of drug to be taken and the palatability [2,3]. Palatability is determined by the taste, texture and smell of the formulation [4]. It can be expected that the lower the acceptability of the drug formulation, the more important also the dosing frequency is. Which type of formulation is appropriate for a child changes during its growth and development [5]. Liquid formulations are generally considered most appropriate for the youngest children, slowly changing to the use of solid formulations, such as tablets and capsules, for older children.

When no appropriate paediatric formulations are available, (hospital) pharmacists can attempt to make medicines more child friendly, such as preparing capsules or a liquid out of tablets with the 'inappropriate' strength, or by compounding using the pure active pharmaceutical ingredient [6,7]. Excipients have to be used to be able to develop such a new formulation. For use in children, certain excipients used in adult drug formulations should be avoided, if possible [4,8]. For example excipients as alcohol and propylene glycol can have serious adverse effects in children [4,9,10]. Not all drug formulations can be changed into a child friendly formulation. For example, some active pharmaceutical ingredients have to be protected from degradation in the gastrointestinal tract or can

damage the mucosa of the oesophagus [11, 12]. Also, the rate and extent of absorption of the active pharmaceutical ingredient can be changed when manipulating the formulation. This may lead to higher or lower drug levels in the blood, resulting in toxicity or loss of efficacy.

Efficacy of drug treatment is studied with a given formulation. It is generally accepted that efficacy will be comparable when exposure to the drug from different formulations is comparable [13, 14]. Exposure is determined by investigating the pharmacokinetics of the drug. Pharmacokinetics describes the absorption, distribution, metabolism and elimination of the drug in relation to time. When new formulations with the same drug are developed, bioequivalence studies are performed to investigate whether the use of these formulations results in comparable pharmacokinetics of the drug.

Comparison of pharmacokinetic parameters of a drug is also used to determine whether a different dosing regimen can be used, for example whether the total daily dose can be administered once daily, instead of divided in multiple doses over the day. This can be done by administering the drug using the dosing regimens of interest to the target population, or by extrapolation of data from different populations or different dosing regimens to predict exposure in the target population.

Viral infections in children

Viruses are small organisms that can infect living cells and can cause disease in the (human) host [15]. The infection can be acute (e.g., influenza) or chronic [e.g., infection by hepatitis B virus or human immunodeficiency virus (HIV)]. Viral infections can also become latent, which means the virus remains quiescent until specific signals trigger the virus to become active. Cold sores are an example of a reactivation of herpes simplex virus latently present in the host [15]. Most viral infections are self-limiting and do not need any antiviral treatment in immunocompetent children. Infections with herpes viruses, such as herpes simplex virus and varicella zoster virus, require treatment only when they cause a severe disease as seen in neonates and immunocompromised patients [16–18]. Immunocompromised patients are also at risk of reactivation of herpes viruses latently present. In those scenarios, antiviral drugs may be used either therapeutically or prophylactically to prevent reactivation [19]. Antiviral drugs used in the Netherlands for herpes simplex virus and varicella zoster virus infections in children are aciclovir and valaciclovir. Bioavailability of aciclovir after oral administration is low (approximately 20%), highly variable and dose

dependent [20]. Therefore, oral administration of aciclovir involves frequent and high dosing, and treatment of serious infections is given by intravenous administration [21]. Intravenous therapy carries several risks, such as line related infections and it requires hospitalization or extensive use of home health service. Valaciclovir is an oral prodrug of aciclovir with equal efficacy, a similar safety profile and a higher, more reliable bioavailability, with a resulting lower dosing frequency than aciclovir [22, 23]. However, an appropriate paediatric formulation for oral administration is lacking for valaciclovir.

Children infected with human immunodeficiency virus (HIV) require antiviral treatment to prevent the development of acquired immune deficiency syndrome (AIDS). It is estimated that worldwide about 2.6 million children under the age of 15 years were living with HIV in 2014, of which 3,300 were living in Western & Central Europe and North America [24]. With current knowledge and treatment options, HIV-infected children can expect to have full adult life, but with the necessity to continue treatment lifelong. To effectively treat an HIV-infection, a combination of at least three drugs needs to be used: combination antiretroviral treatment, or cART [25]. It is recommended to treat all HIV-infected children independent of their immune states and the presence of overt disease [25–27]. Antiretroviral drugs can be divided into several classes based on their mechanism of action. To prevent the virus becoming resistant to antiretroviral drugs, it is paramount to maintain long-term adherence with at least two classes of antiretroviral drugs. The need of combination therapy combined with the need of long-term adherence puts a challenge on the patient and its caregivers (parents, carers and clinicians).

Aim and outline of this thesis

The aim of this thesis is to investigate how pharmacotherapy with antiviral drugs can be optimized taken into account the above-mentioned issues, to ensure safe and effective treatment for children suffering from acute and chronic viral infections.

In the first part, the focus is on simplifying the dosing schedule of two antiretroviral drugs as part of cART, lopinavir and darunavir. It is investigated whether a once-daily dosing schedule can be used instead of a twice-daily regimen. In **chapter 1**, a large international randomized clinical trial investigating the efficacy, safety and pharmacokinetics of once- versus twice-daily lopinavir/ritonavir is described. The effectiveness of the off-label use of once-daily lopinavir/ritonavir in a real-life setting is analysed and

described in **chapter 2**. In **chapter 3** the exposure to once-daily darunavir in children is investigated by collecting pharmacokinetic data in HIV-infected children in the Netherlands. The calculated and approved once-daily dosing regimen will thus be validated. Combining pharmacokinetic data of children from different ages offers the possibility to characterise age-related changes in pharmacokinetics. A population pharmacokinetic model was built for lamivudine in which results from several pharmacokinetic studies and therapeutic drug monitoring data were combined (**chapter 4**).

The second part of this thesis focuses on the drug formulation. A review summarizing the published data about the influence of the formulation on the pharmacokinetics of antiretroviral drugs is presented in **chapter 5**. The pharmacokinetics of a new paediatric tablet of lopinavir combined with ritonavir, developed by a pharmaceutical company, is studied in **chapter 6**. The last two chapters focus on the development of a new paediatric formulation of valaciclovir. First, the pharmaceutical development and the bioequivalence assessment of the new paediatric valaciclovir formulation are described (**chapter 7**). Secondly, the palatability is investigated, using in vitro (electronic tongue) and in vivo (paediatric and adult taste panel) methods (**chapter 8**).

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Part 1   Dosing regimen – pharmacokinetics

Making things easier

1 Once- vs. twice-daily lopinavir/ritonavir in HIV-1-infected children

Paediatric European Network for Treatment of AIDS (PENTA)

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Abstract

Objective
To evaluate whether once-daily (q.d.) lopinavir/ritonavir is noninferior to twice-daily (b.i.d.) dosing in children.

Design
International, multicentre, phase II/III, randomized, open-label, noninferiority trial (KONCERT/PENTA18/ANRS150).

Setting
Clinical centres participating in the PENTA, HIV-NAT and PHPT networks.

Participants
Children/adolescents with HIV-1 RNA viral load less than 50 copies/ml for at least 24 weeks on lopinavir/ritonavir-containing antiretroviral therapy.

Intervention
Children were randomized to continue lopinavir/ritonavir b.i.d. or change to q.d.

Main outcome measure
Confirmed viral load $\geq$ 50 copies/ml by 48 weeks (12% noninferiority margin).

Results
One hundred seventy-three children were randomized in the KONCERT trial (86 q.d., 87 b.i.d.); 46% men, median (IQR) age 11 (9, 14) years, CD4% 33 (27, 38)%. By week 48, 97 and 98% of time was spent on q.d. and b.i.d., respectively (one q.d. child lost at week 4). Twelve q.d. vs. seven b.i.d. children had confirmed viral load $\geq$ 50 copies/ml within 48 weeks; estimated difference in percentage with viral load rebound 6% [90%CI $(-2, 14)$]. Numbers of children with grade 3/4 adverse events (11 vs. 7) or major resistance mutations (3 vs. 2) were similar, q.d. vs. b.i.d. (both $P > 0.3$). Among 26 children in an intrasubject lopinavir/ritonavir pharmacokinetic substudy, lower daily exposure (AUC$_{0-24}$ 161 h×mg/l vs. 224 h×mg/l) and lower C_{last} (1.03 mg/l vs. 5.69 mg/l) were observed with q.d. vs. b.i.d. dosing.

Conclusion
Noninferiority for viral load suppression on q.d. vs. b.i.d. lopinavir/ritonavir was not demonstrated. Although results, therefore, do not support routine use of q.d. lopinavir/ritonavir, lack of safety concerns or resistance suggest that q.d. dosing remains an option in selected, adherent children, with close viral load monitoring.

1.1 Introduction

Antiretroviral drugs have changed HIV-1 infection from a life-threatening disease to a chronic infection. However, adherence to therapy remains a key determinant of disease outcome. For perinatally HIV-infected children, who face a lifetime on treatment, maintaining long-term adherence is often a challenge. Simplification of treatment, including decreasing the frequency of dosing, is likely to increase convenience and enhance adherence to antiretroviral therapy (ART) [1]. Although several q.d. regimens have been shown to have noninferior efficacy and safety in adults [2], resulting in Food and Drug Administration (FDA) and European Medicines Agency (EMA) approval and widespread use in clinical practice, fewer antiretroviral drugs are licensed to be taken q.d. by children.

Protease inhibitors are potential candidates for q.d. dosing. They have a high genetic barrier to development of resistance [3] and when coadministered with ritonavir, resulting in increased absorption and/or prolonged terminal elimination half-life, have increasing potential for decreased dosing frequency. The coformulation of ritonavir-boosted lopinavir (lopinavir/r) in one tablet (also available as a smaller paediatric formulation) also enhances convenience of dosing. Various studies have supported the licensing of q.d. dosing of lopinavir/r for HIV-infected adults [2, 4–7]. However, based on the currently available evidence in children, paediatric treatment guidelines recommend lopinavir/r to be taken twice daily (b.i.d.) [8, 9]. Small studies using q.d. lopinavir/r oral solution or soft gel capsules in children showed high interpatient variability in lopinavir pharmacokinetic parameters and low trough levels [10, 11]. Reduced variability in lopinavir pharmacokinetics in adults and children has been observed after administration of the tablet formulation, suggesting that this formulation could be more appropriate for q.d. dosing [12, 13]. Here we report the results of KONCERT (PENTA18/ANRS150), the first randomized controlled trial evaluating the safety, efficacy and pharmacokinetics of lopinavir/r tablets dosed q.d. vs. b.i.d. following FDA body-weight band dosing guidelines in virologically suppressed ART-experienced children and adolescents.

1.2 Methods

1.2.1 Study design and participants

KONCERT was an open-label, multicentre, randomized trial (ISRCTN 02452400, EudraCT 2009-013648-35) in HIV-infected children aged below 18 years who had a stable $CD4^+$ cell count on combination ART containing

b.i.d. lopinavir/r and had been virologically suppressed (viral load < 50 copies/ml) for at least 24 weeks (single viral load < 400 copies/ml allowed). In addition, eligibility required that children had viral load less than 50 copies/ml at screening, weighed $\geq$ 15 kg and were able to swallow tablets. Kaletra tablets were used throughout. If required, lopinavir/r dose was adjusted at screening in line with the US FDA dosing guidelines based on bodyweight band [total daily dose: 400/100 mg lopinavir/r (15 to $\leq$ 25 kg), 600/150 mg (25 to $\leq$ 35 kg) or 800/200 mg (> 35 kg)] [14]. Children were randomized 1 : 1 to continue taking lopinavir/r b.i.d. or to take their total daily lopinavir/r in a single dose. Parents/guardians and adolescents provided written consent, younger children gave assent according to their age and knowledge of HIV status. The study received approval from ethics committees and regulatory bodies in each participating country and clinical site.

Randomization was stratified by weight band (as above) and participation in the pharmacokinetic substudy. The computer-generated sequentially numbered randomization list (with variable block sizes) was preprepared by the trial statistician and securely incorporated within the database at the Trials Unit. Randomization was undertaken via a web service accessed by the clinician or Trials Unit, who could access the next allocation but not the whole list.

1.2.2 Outcome measures

The primary endpoint was a viral load at least 50 copies/ ml (confirmed within 4 weeks) within the first 48 weeks of follow-up. Primary endpoints for the pharmacokinetic substudies were pharmacokinetic parameters of lopinavir/r [area under the curve (AUC), $C_{\max}$, C_{last}] [1], comparing b.i.d. (week 0) to historical pharmacokinetic data, and [2] comparing q.d. (week 4) to b.i.d. (week 0) in the same children. Analysis of endpoint [1] has previously been described [15]. Secondary outcomes included the following: viral load at least 400 copies/ml (confirmed) within 48 weeks; number of major HIV-1 RNA mutations in those with viral rebound; change in CD4 cell count/percentage from baseline to 48 weeks; adherence to, acceptability of, and changes made to the ART regimen; ART-related grades 3 and 4 clinical or laboratory adverse events [16, 17].

1.2.3 Data collection and follow-up procedures

Follow-up visits were scheduled at weeks 4, 8 and 12, then 12 weekly until the last child reached week 48 (Figure 1.1). Viral load was measured at each study visit; children with viral load at least 50 copies/ml returned within 4 weeks for retest of viral load. Assessment of adherence to treatment and a resistance test were requested when children had a confirmed viral load at least 50 copies/ml. T-cell lymphocyte subsets were performed at all visits; biochemistry and haematology were performed 12-weekly; blood lipids were measured at weeks 0, 24 and 48; adherence questionnaires were given to carers and children at weeks 0, 4, 12, 24 and 48; acceptability questionnaires were completed at baseline and if children switched from q.d. to b.i.d. dosing. At each study visit, a plasma sample was stored for subsequent assessment of population lopinavir/r pharmacokinetics.
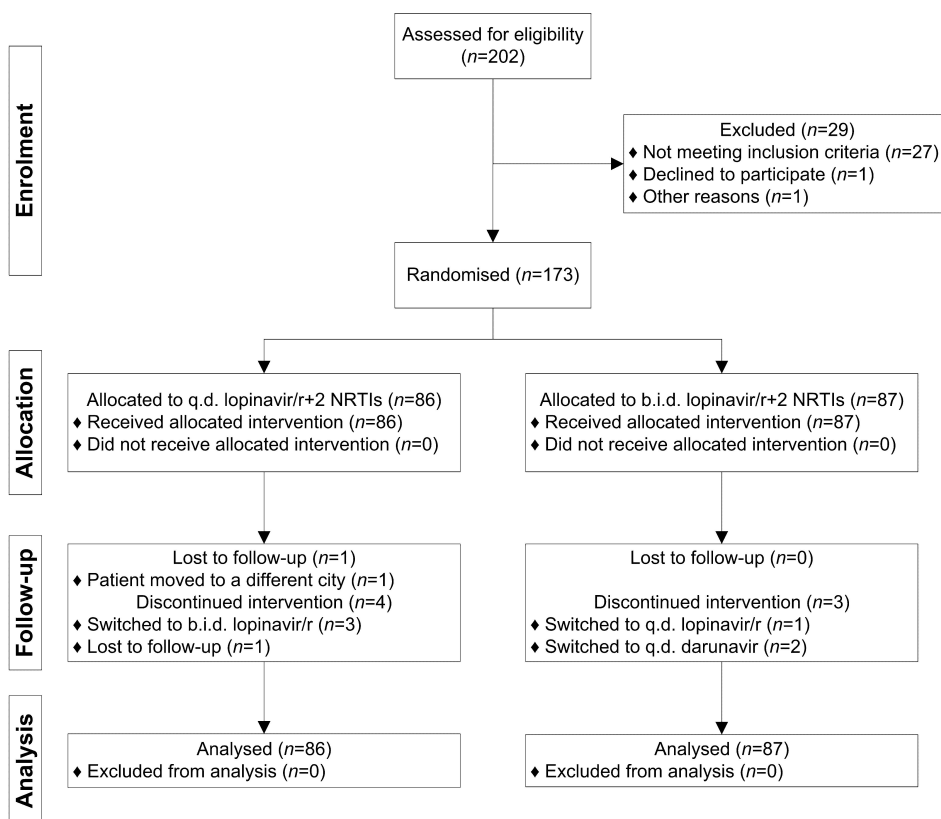
Figure 1.1: Trial profile.

1.2.4 Pharmacokinetic substudy

Children who consented were enrolled in a pharmacokinetic substudy, until a minimum of 16 children in each stratification weight band had evaluable pharmacokinetic data. Children with nonevaluable pharmacokinetic results were followed within the main study, but excluded from the pharmacokinetic analysis. Lopinavir pharmacokinetics were determined at week 0 in both arms, and at week 4 if randomized to q.d. dosing. Prior to the day of pharmacokinetic assessment, children took the paediatric lopinavir/r tablet (100/25 mg) formulation for at least seven days, following the FDA-recommended weight band-based dosing. On the pharmacokinetic assessment day, 2 ml of blood was taken before observed intake of lopinavir/r in the morning (T_0) and at 2, 4, 6, 8 and 12 (week 0, b.i.d.) or 24 h (week 4, q.d.) after the dose. Plasma concentrations were determined using a validated ultrahigh performance liquid chromatography assay with UV detection derived from a previously published assay [18]. Lopinavir pharmacokinetic parameters were determined using noncompartmental analysis (WinNonlin/ Phoenix version 6.3; Pharsight Corporation, Mountain View, California, USA): AUC_{0-24} [area under the plasma concentration-time curve calculated (linear up-log down method) over a dosing interval from time 0 to 24 h after dosing], $C_{\max}$ (maximum observed plasma concentration), $T_{\max}$ (time of maximum observed plasma concentration), C_{last} (last observed drug concentration) and clearance (CL/F). The intensive pharmacokinetic analyses were performed at the Department of Pharmacy, Radboud University Medical Center, Nijmegen, the Netherlands.

Lopinavir concentrations were also determined on available stored plasma samples at the screening visit and at weeks 0, 4, 8, 12, 24, 36 and 48 on all children. This was done to investigate the effect of having lopinavir plasma concentration below the lower limit of quantification (LLOQ = 0.10 mg/l) at any visit on virological rebound. Pharmacokinetic analyses for these stored samples were performed at Radboud University Medical Center, except for samples in Thailand which were performed at the PHPT-AMS laboratory, Chiang Mai University, Thailand. Both laboratories participate in an international interlaboratory quality control programme for therapeutic drug monitoring of antiretroviral drugs [19].

1.2.5 Statistical analyses

A target enrolment of 160 children (80 in each arm) provided at least
80% power to exclude a noninferiority margin of 12% for the difference
between the two arms in the proportion of children reaching the primary
endpoint, assuming a 10% virological rebound rate and onesided $\alpha = 0.05$.
An Independent Data Monitoring Committee reviewed interim data for
safety and efficacy three times during the study.

All comparisons between randomized arms (q.d. vs. b.i.d.) were
intention-to-treat, with follow-up censored at week 52 or last follow-up
date (if before the week 48 visit). The proportion of children experiencing
virological rebound by week 48 in each arm was estimated using the Kaplan-
Meier method, with 90% confidence intervals (CIs) for the difference in
proportions calculated using bootstrap standard errors [20]. Two prespeci-
fied sensitivity analyses of the primary outcome were completed: adjusting
for baseline stratification factors, and censoring follow-up at the time of
lopinavir/r treatment modification (change in dose, > 7-day interruption or
permanent discontinuation; a 'per-protocol' analysis). A post-hoc analysis
adjusting for chance imbalance between arms in viral load and CD4% at
baseline was also performed.

Change in CD4% and other continuous laboratory outcomes from
baseline to 48 weeks were analyzed using normal regression, adjusting
for the baseline measurement and stratification factors. Major resistance
mutations known to confer resistance to antiretroviral drugs not seen in
any pretrial resistance tests were summarized by drug class. Categorical
variables were compared using Fisher's exact tests; rates were estimated
using Poisson regression. All P values were two sided and all statis-
tical calculations were performed using STATA (Stata Statistical Software,
Release 13; StataCorp LP, College Station, Texas, USA).

All paired evaluable pharmacokinetic assessments [on b.i.d. (week 0)
and q.d. (week 4)] in children randomized to q.d. were included. Within
subject ratios of AUC_{0-24}, clearance (CL/F/kg), C_{max} and C_{last} for q.d.
vs. b.i.d. dosing were calculated. AUC_{0-24} for b.i.d. dosing was calcu-
lated as $2 \times AUC_{0-12}$. An overall geometric mean ratio (GMR) for each
pharmacokinetic parameter was calculated after log-transformation of the
within-subject ratios; 90%CIs were calculated (using the t-distribution)
using the bioequivalence crossover design tool approach within the Phoenix
WinNonlin software package (with fixed effects in the model specification).
A GMR with a 90%CI including 1.0 and falling entirely within 0.80–1.25
was considered as bioequivalence for AUC_{0-24} and C_{max}. Relative risk

ratios were calculated comparing the likelihood of virological rebound for children with at least one sample with lopinavir concentration levels below LLOQ to those children with all samples $\geq$ LLOQ.

1.3 Results

1.3.1 Baseline characteristics

Between August 2010 and August 2012, 173 children were randomized (86 allocated to q.d., 87 to b.i.d.) (Figure 1.1); 80 children from Europe, 59 from Thailand and 34 from South America; participants were from 49 clinical centres in 12 countries. Fifty-three took part in the pharmaco-kinetic substudy, 27 randomized to the q.d. arm; 46, 50 and 77 children were in the 15 to 25 kg, > 25 to 35 kg, > 35 kg weight bands, respectively.

Baseline demographics were similar in the two arms (Table 1.1); median (IQR) age was 11.0 (8.7, 14.3) years and 94 (54%) were female. More children in the q.d. arm had advanced HIV disease, lower CD4% and a viral load at least 50 copies/ml at baseline (Table 1.1). Pretrial ART exposure was comparable between arms; 35 (20%) children were on their first-line regimen at baseline, and half had been exposed to three different antiretro-viral drug classes. The children were on a variety of NRTI backbones at baseline (44% zidovudine + lamivudine or emtricitabine, 20% abacavir + lamivudine or emtricitabine, 16% tenofovir + any other NRTI, 20% other); 29% of backbone NRTIs were taken as q.d. dosing (28% q.d. arm, 30% b.i.d. arm); this proportion increased over the time of the trial.

1.3.2 Follow-up and antiretroviral therapy received

One q.d. child withdrew consent at week 4; all other children completed 48 weeks follow-up and are included in all analyses. In total, 98 and 97% of follow-up time was spent on q.d. and b.i.d. dosing of lopinavir/r in the q.d. and b.i.d. arms, respectively. Twenty-nine (17%) children made changes to their ART regimen in the first 48 weeks of follow-up [20 (23%) q.d., 9 (10%) b.i.d.]. In the q.d. arm, two children switched back to b.i.d. lopinavir/r dosing (at week 1 and 39), 17 children changed their NRTI backbone (66% to q.d. regimens), and one child did both at week 8. In the b.i.d. arm, one child switched to q.d. lopinavir/r dosing at week 38 and eight children changed their NRTI backbone.

Table 1.1: Baseline characteristics

	Once-daily	Twice-daily	Total
Children randomized: n	86	87	173
Men: n (%)	41 (48)	38 (44)	79 (46)
Age (years): median (IQR)	10.8 (8.7, 14.2)	11.2 (9.0, 14.5)	11.0 (8.7, 14.3)
[range]	[4.3, 17.6]	[3.8, 17.7]	[3.8, 17.7]
Ethnic origin: n (%)			
White	27 (31)	17 (20)	44 (25)
Black: African or other	17 (20)	29 (33)	46 (27)
Mixed black/white	5 (6)	6 (7)	11 (6)
Asian/Thai	31 (36)	30 (34)	61 (35)
Other	6 (7)	5 (6)	11 (6)
Vertically infected: n (%)	86 (100)	84 (97)	170 (98)
CDC stage: n (%)			
N or A	28 (33)	39 (45)	67 (38)
B or C	58 (68)	48 (55)	106 (61)
Viral load (HIV-1 RNA) $\geq$ 50 copies/ml at randomization*:			
n (%)	12 (14)	4 (5)	16 (9)
Median [range]	120 [51, 91 201]	135 [57, 270]	120 [51, 91 201]
CD4%: mean (sd)	32.0 (6.5)	33.9 (8.6)	32.9 (7.7)
Weight (kg): median	33.3	32.2	33.1
(IQR)	(24.6, 42.0)	(23.9, 43.8)	(24.6, 42.6)
[range]	[15.0, 72.5]	[15.6, 68.9]	[15.0, 72.5]
Baseline ART			
first regime: n (%)	18 (21)	17 (20)	35 (20)
Exposed to three classes			
of ART: n (%)	41 (48)	46 (53)	87 (50)

* All $<$ 50 copies/ml at screening.

1.3.3 Primary outcome

Nineteen children (12 q.d., seven b.i.d.) experienced confirmed viral
rebound at least 50 copies/ml during 48 weeks of follow-up; all but one
rebound (q.d.) was considered by the treating clinician to be adherence
related. The estimated percentage of children with viral rebound by 48
weeks was 14% [95%CI $(8, 24\%)$] in the q.d. arm vs. 8% [(95%CI $(4, 16\%)$]
in the b.i.d. arm, an estimated difference between arms of 6% [(90%CI
$(-2, 14\%)$, bootstrap $P = 0.19$] (Figure 1.2). The upper 90% confidence
limit of 14% was greater than the predefined noninferiority margin of 12%.
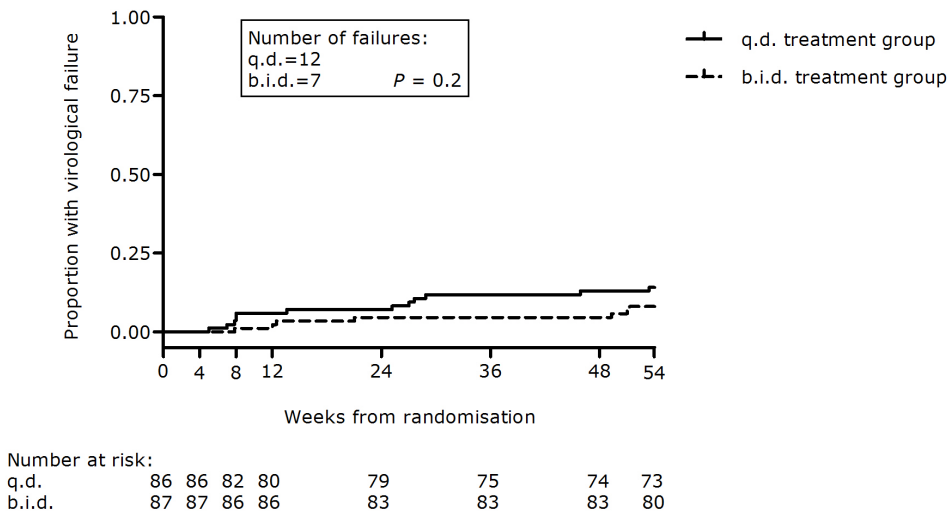
Figure 1.2: Time to virological failure.

Results were similar after adjustment for stratification factors – estimated
difference between arms of 6% [90%CI $(-2, 14\%)$, bootstrap $P = 0.20$]
– and for per-protocol analyses wherein follow-up for eight children was
censored as a result of treatment modification – estimated difference
between arms of 5% [90%CI $(-3, 13\%)$, bootstrap $P = 0.27$]. A post-hoc
analysis adjusting for the chance imbalance between arms in viral rebound
at baseline, reduced the estimated difference in proportion rebounding to
4% [90%CI $(-4, 11\%)$, bootstrap $P = 0.39$], bringing the upper 90% confi-
dence limit just within the noninferiority margin.

Fifteen children [nine (75%) q.d., six (86%) b.i.d.] remained on the same
dosing regimen of lopinavir/r after rebound, the majority of whom went

on to resuppress [seven (78%) q.d., four (67%) b.i.d.]. Two children (q.d.) returned to b.i.d. dosing (one of whom resuppressed during follow-up) and two (one q.d., one b.i.d.) discontinued lopinavir/r after rebound (both resuppressed).

1.3.4 Secondary outcomes

Viral rebound defined as at least 400 copies/ml was observed in 11 children (eight q.d., three b.i.d.); the estimated difference between arms in the probability of rebounding by 48 weeks was 6% [90%CI $(0, 12\%)$, $P = 0.10$].

Genotypic resistance tests were available in 18 (12 q.d., 6 b.i.d.) children with rebound within 48 weeks; major new resistance-associated mutations were detected in five (three q.d., two b.i.d.; one b.i.d. did not resupress < 50 copies/ml during follow-up). Major protease inhibitor mutations were detected in none of the children on q.d. vs. two children on b.i.d. lopinavir/r (L90M, M46I + V82A). The M184V mutation was detected in one child from each arm; three children (two q.d., one b.i.d.) had at least one thymidine-associated mutation.

Mean changes in CD4% from baseline to week 48 were similar in both arms: 0.4% for the q.d. arm and 0.1% for the b.i.d. arm [difference 0.3%, 95%CI $(-1.0, 1.7\%)$, $P = 0.61$]. Changes in biochemistry, haematology and lipid measurements were also minimal and comparable (data not shown).

There were no new CDC stage C events or deaths reported during the trial. Three new stage B events were reported (two q.d.: pneumonia and herpes zoster; one b.i.d.: cholecystitis).

There were no significant differences between the trial arms for any of the clinical safety endpoints (Table 1.2).

Fifteen serious adverse events in 14 children occurred during the first 48 weeks of the trial [episodes (children): nine (8) q.d., six (6) b.i.d., Fisher's exact test: $P = 0.6$], none of which were fatal or life threatening. All reported serious adverse events were as a result of hospitalization; only one event, diarrhoea reported during the first week of the trial in a child taking q.d. lopinavir/r, was considered possibly related to lopinavir/r by the treating clinician. The incident risk ratio for q.d. relative to b.i.d. was 1.72 [95%CI $(0.63, 4.66)$, GEE Poisson regression $P = 0.29$].

Twenty-two grade 3 or 4 clinical or laboratory adverse events in 18 children were reported: [episodes (children): 13 (11) q.d., 9 (7) b.i.d., Fisher's exact test: $P = 0.3$]. Three children experienced adverse events that led to treatment modification: two children on q.d. with nausea and vomiting changed back to b.i.d. dosing at weeks 1 and 8; one child on b.i.d.

Table 1.2: Summary of adverse events to week 48 assessment

	Once-daily episodes (children)	Twice-daily episodes (children)	Total episodes (children)	P value*
Total adverse events	271 (73)	232 (76)	503 (149)	0.7
Grades 1 and 2 adverse events	256 (70)	222 (76)	478 (146)	0.3
Grades 3 and 4 adverse events	13 (11)	9 (7)	22 (18)	0.3
Gastrointestinal disorders	2 (2)	2 (1)	4 (3)	1.0
Infections and infestations	4 (4)	3 (3)	7 (7)	0.7
Laboratory investigations†	5 (4)	3 (2)	8 (6)	0.4
Blood and lymphatic system disorders	0 (0)	1 (1)	1 (1)	1.0
Hepatobiliary disorders	1 (1)	0 (0)	1 (1)	0.5
Nervous system disorders	1 (1)	0 (0)	1 (1)	0.5
Adverse events leading to treatment modification	4 (2)	1 (1)	5 (3)	0.6
Serious adverse events	9 (8)	6 (6)	15 (14)	0.6
Gastrointestinal disorders	2 (2)	1 (1)	3 (3)	0.6
Infections and infestations	5 (5)	4 (4)	9 (9)	0.75
Respiratory, thoracic and mediastinal disorders	0 (0)	1 (1)	1 (1)	1.0
Hepatobiliary disorders	1 (1)	0 (0)	1 (1)	0.5
Surgical and medical procedures	1 (1)	0 (0)	1 (1)	0.5
SAE rate per 100 person-years (95%CI)	9.5 (4.7, 19.0)	6.7 (3.0, 14.9)	8.0 (4.8, 13.6)	0.6**

SAE, serious adverse event.
* Fisher's exact test.
** Poisson regression.
† Abnormal laboratory values without reported associated clinical symptoms.

had neutropenia at week 4 and substituted abacavir for zidovudine.

Both children and carers reported a preference for q.d. dosage of lopinavir/r; 120 of 140 (86%) children and 128 of 144 (89%) carers completing the acceptability questionnaire at trial enrolment thought q.d. dosing would be easier than b.i.d. dosing. This preference persisted at the end of trial, when 50 of 68 (74%) of children and 45 of 64 (70%) of carers reported a preference. Combining responses to adherence questionnaires completed by children or carers at each trial visit during the first 48 weeks (89% completion rate), missing a dose within 3 days of the clinic visit was only reported on 20 occasions [14 (3.5%) q.d. vs. 6 (1.5%) b.i.d., GEE logistic regression: $P = 0.2$].

1.3.5 Pharmacokinetic analysis

Intraindividual, paired comparison of lopinavir twice-daily and once-daily dosing

Twenty-six out of 27 children randomized to the q.d. arm in the pharmacokinetic substudy had evaluable full pharmacokinetics at weeks 0 and 4. Tables 1.3 (a) and (b) show child demographic data and pharmacokinetic parameters for lopinavir, respectively. Fifteen (58%) children on q.d. dosing at week 4 compared with all children on b.i.d. dosing at baseline had a C_{last} above 1.0 mg/l, a measurement associated with optimal virological response in b.i.d. regimens [9]. The GMR (90%CI), q.d. vs. b.i.d., of lopinavir AUC_{0-24} and lopinavir C_{max} were calculated as 0.72 (0.62, 0.83) and 1.13 (1.00, 1.26), respectively. Neither falling within the 80–125% limits required for bioequivalence.

Routine measurement of lopinavir plasma concentrations

Most children (76 q.d., 74 b.i.d.) had eight samples available during the initial 48 weeks of follow-up for determination of lopinavir plasma concentration (19 children had seven samples, 1 had six, 2 had five, 1 had three). Overall, 28 (16.2%) children had at least one lopinavir concentration that was below the LLOQ of 0.10 mg/l: 21 (24.4%) q.d. vs. 7 (8.0%) b.i.d., Fisher's exact test $P = 0.004$. A higher proportion of children reaching the primary endpoint of viral rebound had at least one lopinavir plasma concentration < LLOQ [11 (57.9%) at least 1 sample < LLOQ vs. 8 (42.1%) no samples < LLOQ: 9 q.d. 2 b.i.d., Fisher's exact test $P = 0.03$].

The overall relative risk (95%CI) of viral rebound, stratified by randomized arm, given at least one lopinavir concentration < LLOQ was 7.61

(2.95, 19.69). A trend was observed of an increasing proportion experiencing virological rebound when the number of samples with concentrations < LLOQ increased: 5.5% with no samples < LLOQ, 21.4% with one sample < LLOQ and 57.1% with two or more samples < LLOQ.

Table 1.3 (a): Within-children pharmacokinetic substudy in 26 children randomized to once-daily arm – Baseline characteristics

| | Weight band | | | |
	15–25 kg	25–35 kg	> 35 kg	Total
Children: n	7	8	11	26
Men: n (%)	4 (57)	5 (63)	3 (27)	12 (46)
Age (years):				
median	7.1	10.6	14.3	12.8
(IQR)	(6.7, 8.7)	(9.5, 15.0)	(13.5, 15.4)	(8.7, 14.7)
[range]	[4.4, 8.9]	[6.3, 16.0]	[12.7, 16.8]	[4.4, 16.8]
Weight (kg):				
median	19.4	30.7	42.0	32.1
(IQR)	(19.0, 23.1)	(29.8, 32.1)	(38.5, 49.5)	(24.1, 41.0)
[range]	[15.0, 24.1]	[26.4, 33.8]	[36.0, 72.5]	[15.0, 72.5]
BMI (kg/m^2):				
median	15.1	15.7	17.7	16.5
(IQR)	(14.4, 15.7)	(14.8, 18.0)	(17.4, 20.6)	(15.1, 18.6)
[range]	[11.5, 15.8]	[14.5, 19.4]	[16.0, 27.6]	[11.5, 27.6]
Vertically infected:				
n (%)	7 (100)	8 (100)	11 (100)	26 (100)
Ethnic origin: n (%)				
White	0 (0)	1 (13)	1 (9)	2 (8)
Black: African or other	2 (29)	3 (38)	2 (18)	7 (27)
Mixed black/white	1 (14)	1 (13)	0 (0)	2 (8)
Asian/Thai	4 (57)	3 (38)	7 (64)	14 (54)
Other	0 (0)	0 (0)	1 (9)	1 (4)

1.4 Discussion

KONCERT is the first randomized controlled trial in children and adolescents to investigate the safety and efficacy of q.d. vs. b.i.d. dosing of lopinavir/r. Children from a wide age-range were included, and all main ethnic groups were represented. The rate of virological rebound, defined as confirmed viral load at least 50 copies/ml at any time within 48 weeks, was low in both arms. However, noninferiority of lopinavir/r q.d. vs. lopinavir/r b.i.d. dosing, when taken as part of combination ART, was not

Table 1.3 (b): Within-children pharmacokinetic substudy in 26 children randomized to once-daily arm – Pharmacokinetic parameters for lopinavir on once-daily (week 4) and twice-daily (week 0) dosing

	n	Once-daily geometric mean (95%CI)[c]	Twice-daily geometric mean (95%CI)[c]	Once-/twice-daily geometric mean ratio (90%CI)
AUC_{0-24} (h×mg/l)[a]				
Total:	26	160.9 (138.4, 187.0)	223.9 (194.8, 257.4)	0.72 (0.62, 0.83)
Weight:				
15–25 kg	7	172.6 (121.3, 245.7)	232.1 (153.3, 351.4)	
25–35 kg	8	159.3 (120.6, 210.5)	256.8 (209.3, 315.2)	
> 35 kg	11	155.0 (116.8, 205.6)	198.1 (159.8, 245.5)	
C_{max} (mg/l)				
Total:	26	14.0 (12.7, 15.6)	12.5 (11.1, 14.0)	1.13 (1.00, 1.26)
Weight:				
15–25 kg	7	15.5 (12.4, 19.4)	13.5 (9.8, 18.7)	
25–35 kg	8	15.0 (12.2, 18.5)	14.1 (11.4, 17.3)	
> 35 kg	11	12.5 (10.7, 14.7)	10.9 (9.3, 12.6)	
C_{last} (mg/l)				
Total:	26	1.03 (0.61, 1.75)	5.69 (4.58, 7.07)	0.18 (0.12, 0.27)
Weight:				
15–25 kg	7	0.91 (0.27, 3.07)	4.92 (2.65, 9.16)	
25–35 kg	8	0.93 (0.38, 2.26)	6.65 (5.22, 8.47)	
> 35 kg	11	1.20 (0.42, 3.44)	5.57 (3.73, 8.32)	
Clearance (l/(h×kg))[b]				
Total:	26	0.115 (0.099, 0.134)	0.084 (0.074, 0.095)	1.37 (1.19, 1.57)
Weight:				
15–25 kg	7	0.112 (0.076, 0.165)	0.085 (0.062, 0.117)	
25–35 kg	8	0.120 (0.091, 0.158)	0.076 (0.062, 0.094)	
> 35 kg	11	0.114 (0.086, 0.150)	0.089 (0.071, 0.113)	
T_{max}(h)[c]				
Total:	26	4.0 (2.0, 8.0)	3.5 (0.0, 12.0)	
Weight:				
15–25 kg	7	4.0 (2.0, 8.0)	3.8 (0.0, 4.1)	
25–35 kg	8	4.0 (2.0, 6.0)	2.8 (1.7, 4.0)	
> 35 kg	11	4.0 (2.0, 8.0)	3.4 (1.7, 12.0)	

[a] AUC_{0-24} for b.i.d. dosing = AUC_{0-12}×2.
[b] Clearance calculated as
CL/F/kg = dose (mg)/[AUC_{0-24}(h×mg/l)×body weight (kg)].
[c] For T_{max} median values (minimum, maximum) are reported.

demonstrated; 6% more children in the q.d. arm experienced viral load rebound within the first 48 weeks, and the upper bound of the CI of 14% was outside the predetermined noninferiority bound of 12%. This difference was partially explained by the chance imbalance between arms in viral rebound which occurred between screening and baseline. However, even after adjustment the upper bound of the CI was 11%, only just within the predefined 12% margin of noninferiority. No significant safety issues were demonstrated and there were no differences between arms in development of resistance mutations.

The within patient pharmacokinetic substudy showed that administration of lopinavir/r paediatric tablets q.d. resulted in lower daily exposure to lopinavir and a lower C_{last} compared with b.i.d. dosing in the same child. In adults, higher exposure (AUC$_{0-24}$ 206.5 h×μg/ml), but comparable C_{max} (14.8 μg/ml) has been observed after q.d. dosing of 800/200 mg lopinavir [21]. Elimination half-life ($t_{1/2}$) was comparable with values found in adults: mean $t_{1/2}$ (sd) in our study was 6.0 h (3.0 h) for q.d. and 7.7 h (3.0 h) for b.i.d., compared with 6.1 h (2.5 h) and 8.6 h (4.2 h) in adults, respectively [5]. Previous smaller paediatric studies have reported that the AUC$_{0-24}$ of lopinavir after q.d. dosing of lopinavir/r using various formulations (solution, soft-gel capsules and tablets) lies between 150 and 215 h×mg/l, and C_{last} between 1.6 and 5.8 mg/l [10–12, 22–25]. In our larger study, the AUC was at the lower end of this range and C_{last} below it. This cannot be explained by lower dose, as the median lopinavir dose received by children in the pharmacokinetic study was 19.0 mg/kg or 537 mg/m^2 q.d., which is comparable or higher than the doses received by children in the other studies. In addition, exposure to lopinavir from tablets in adults was shown to be significantly higher than from softgel capsules, although the 90%CI of the GMR was reported to be within the bioequivalence range [13].

Additional findings from this trial reflect 'real life' dosing, as not only were formal 'within-child' pharmacokinetic studies undertaken, but also sparse random sampling in all children attending clinic throughout the 48 weeks. We demonstrated that more children in the q.d. treatment group had at least one undetectable ($<$ LLOQ) lopinavir plasma concentration: 24.4% q.d. vs. 8.0% b.i.d. Further we observed a pharmacokinetic/pharmacodynamic relationship, with the overall risk of viral load rebound being over seven-fold greater among children with at least one lopinavir concentration $<$ LLOQ, and twice as high in q.d. vs. b.i.d. children (9.3 vs. 4.6). These findings together with the results of the within-child pharmacokinetics show that lopinavir is less forgiving when

children are dosed q.d., and thus if children are nonadherent, there is a higher chance of virological rebound. Despite this during the trial, nine out of 12 children on q.d. who rebounded later resuppressed, and seven of the nine remained on q.d. lopinavir/r. Although drug concentration measurements demonstrated that missed q.d. doses had a greater risk of viral rebound, reassuringly due to the relatively high resistance barrier of ritonavir-boosted lopinavir, development of new mutations remained low, and similar to b.i.d. dosing.

Both children and carers reported a preference for taking lopinavir/r q.d., but data from the adherence questionnaires suggests that a small number of children may miss more doses on q.d.

In resource-rich countries, other q.d. boosted protease inhibitor treatments are now widely available for children, but in resource poor situations, which carry the burden of the epidemic, lopinavir/r remains the mainstay of paediatric protease inhibitor based therapy (Habiyambere V, WHO ARV use survey, 2014, personal communication) [26], and the findings of the trial are particularly relevant to these settings.

In conclusion, based on the combination of viral load rebound and pharmacokinetic results in the KONCERT trial, q.d. lopinavir/r cannot be routinely recommended as a simplification option for children with suppressed viral load on b.i.d. lopinavir/r. However, among selected adherent children for whom regular viral load monitoring is available, q.d. dosing remains an option, as we have demonstrated that it is both safe and not associated with any increased risk of developing resistance mutations.

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Conflicts of interest

There are no conflicts of interest.

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Making things easier

2 Sustained viral suppression in HIV-infected children on once-daily lopinavir/ritonavir in clinical practice

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Abstract

Background

The use of lopinavir/ritonavir once daily (LPV/r q.d.) has not been approved for children. Good short-term clinical, virological and immunological outcomes have been observed in children on LPV/r q.d.

Methods

We evaluated the long-term effectiveness of a LPV/r q.d. containing regimen in HIV-1 infected children in clinical practice. Selected children (aged 0–18 years) with an undetectable HIV-1 RNA viral load ($<$ 50 copies/ml) for at least 6 months on a twice-daily (b.i.d.) LPV/r-containing regimen switched to LPV/r q.d. The main outcome measures were the percentage of patients with an undetectable HIV-1 viral load each subsequent year after switch to LPV/r q.d. (on treatment and last observation carried forward (LOCF)), and virological failure during follow-up ($>$ 400 copies/ml twice within 6 months). Also the exposure to LPV on the initial once-daily dosing regimen was determined.

Results

Forty children (median age 6.5 years; range: 1.0, 17) were included. Median follow-up was 6.3 years (range 1.0, 10.3). During yearly follow-up, the percentage of children with an undetectable viral load varied between 82–100% (on treatment) and 83–93% (LOCF). Five children (12.5%) met the criteria for failure. CD4+ and CD8+ counts remained stable at normal values. Geometric mean LPV AUC_{0-24} was 169.3 h×mg/l and C_{last} 1.35 mg/l. Adverse events were encountered in eight patients, were mainly gastro-intestinal and in these cases no reason to stop treatment.

Conclusion

A once-daily LPV/r containing regimen in HIV-1 infected children with intensive clinical and therapeutic drug monitoring is well tolerated and has good long-term clinical, virological, and immunological outcome.

2.1 Introduction

Lopinavir boosted with ritonavir (LPV/r) is the only protease inhibitor (PI) available as tablet and liquid combination formulation for children under the age of 3 years [1,2]. LPV/r is an effective protease inhibitor and has a high barrier of resistance. Furthermore it is generally well tolerated [2,3]. Therefore, LPV/r is currently recommended as first-line protease inhibitor in combination with other antiretroviral drugs in the treatment of HIV-1-infected children aged < 6 years [1]. LPV/r is approved by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) to be used twice daily (LPV/r b.i.d.) in children. For adult patients a once-daily regimen (LPV/r q.d.) has also been approved with virological and immunological responses comparable to a twice-daily regimen [2,4–10]. For children < 18 years LPV/r q.d. has not been approved.

Seven studies on the pharmacokinetics and clinical efficacy of LPV/r q.d. in HIV-infected children have been published in the past decade [11–17]. These studies show that LPV/r q.d. can result in an adequate exposure to LPV, but that there is a high interindividual variability in pharmacokinetics, especially in young children. Despite frequently reported subtherapeutic trough levels ($C_{last} < 1.0$ mg/l), most of the children in these studies showed good short-term clinical, virological and immunological outcomes (follow up between 6 and 12 months). To answer the question whether once-daily LPV/r is non inferior to twice-daily LPV/r, a prospective randomized controlled trial on LPV/r q.d. versus b.i.d. was initiated within the PENTA network, and results have recently been published [16]. This study was inconclusive concerning the noninferiority of q.d. LPV/r compared with b.i.d. LPV/r. Although recommended to be dosed twice daily, the PENTA group suggests that LPV/r may be used once daily in specific cases where adherence is monitored and routine clinical follow-up is available [1,16].

Two of the pharmacokinetic studies on LPV/r q.d. were initiated in our center in 2002 [15,17]. After completion of these studies, patients who were on LPV/r q.d. were allowed to continue LPV/r q.d. as long as their viral loads were undetectable. Subsequently, we gave selected children that had not participated in the study the chance to switch to LPV/r q.d. on off-label use basis. To ensure that children had adequate exposure to LPV, therapeutic drug monitoring (TDM) was used. This study reports the results of a long-term follow-up of clinical, virological and immunological response to a TDM controlled regimen of q.d. LPV/r in 40 HIV-1-infected children.

2.2 Methods

2.2.1 Study design and population

All children included in the previously published pharmacokinetic studies on LPV/r q.d. were included in the current long-term follow up study [15, 17]. After completion and publication of the pharmacokinetic study on LPV/r q.d., also children not included in the pharmacokinetic study in our center were offered to switch to (off-label) LPV/r q.d. Inclusion criteria were an expected good compliance and an undetectable viral load during the previous 6 months. Children with a follow-up shorter than 12 months were excluded from this study. Data were retrospectively collected and derived from data obtained in long-term follow-up studies in which all patients participated. The long-term follow-up study protocols were approved by the medical ethics committee of the ErasmusMC University Medical Center Rotterdam, the Netherlands. Written informed consent was obtained from patients and their parents.

2.2.2 Treatment and clinical follow-up

Treatment-experienced children were initially switched to a regimen containing LPV/r 460 mg/m^2 q.d. based on the results of the previous pharmacokinetic study or to a higher or lower dose if the LPV dose in their b.i.d. regimen had been adjusted to a higher or lower dose because of pharmacokinetic evaluation [15]. The daily dose of LPV q.d. was equivalent to the daily dose the child received b.i.d. Children visited the clinic for follow-up and counseling with one of the nurse practitioners and/or pediatricians every 3 months. During the clinical visit HIV-1 viral load, CD4+ and CD8+ T-cell count, liver and kidney function, whole blood cell count and LPV plasma levels were determined, and urinalysis was performed. Lipid profile was analyzed once a year.

Follow-up for this study stopped when children discontinued LPV/r q.d. or when they were lost to follow-up, eg because of transfer to adult care.

2.2.3 Therapeutic drug monitoring

Within 2–4 weeks after switch to the once-daily regimen, intensive therapeutic drug monitoring (TDM) was performed as standard of care. Children came to the clinic without having taken their LPV/r that day. Samples were taken before observed intake and at 2, 4, 6, 8, 12, 18 and 24 hours after intake. Pharmacokinetic parameters of LPV for the

total group were determined using noncompartmental analysis (Phoenix, WinNonlin version 6.3. Pharsight Corporation, Mountain View, CA, USA): AUC_{0-24} (area under the plasma concentration-time curve (linear up-log down method) over a dosing interval from time 0 to 24 hours after dosing), C_{max} (maximum observed plasma concentration), C_{last} (last observed drug concentration) and clearance (CL/F). LPV concentration was considered subtherapeutic if C_{last} was measured or predicted to be < 1.0 mg/l [18]. If needed, the dose was adjusted using the individuals curve and population data. TDM was then repeated on a single sample, preferably a trough sample until plasma concentration of LPV was deemed therapeutic [15]. Furthermore, during follow-up, single plasma levels were monitored every three months.

LPV plasma concentrations were determined using a validated ultrahigh performance liquid chromatography assay with ultraviolet detection derived from a previously published assay [19]. The analysis was performed at the Department of Pharmacy, Radboud University Medical Center Nijmegen, the Netherlands.

2.2.4 Statistical analysis

Primary outcome of the study was the percentage of children with an undetectable HIV-1 viral load (< 50 copies/ml) each subsequent year after starting LPV/r q.d. Secondary outcomes were 1) sustained viral suppression (< 50 copies/ml) rates during three-monthly follow-up visits, 2) time to virological failure during follow-up, 3) immunological and clinical response including adverse events, and 4) exposure (AUC_{0-24}) to LPV on the initial once-daily dosing regimen.

The percentage of children with an undetectable HIV-1 viral load was calculated with an on-treatment and last observation carried forward (LOCF) analysis. A Kaplan-Meier curve was constructed to evaluate time to failure. For this analysis, failure was defined as confirmed viral rebound with an HIV-1 viral load > 400 copies/ml at least twice within 6 months. Additionally, a single confirmed HIV-1 viral load > 400 copies/ml resulting in termination of the LPV/r q.d. regimen was also considered as failure [20]. Next to absolute counts, CD4+ and CD8+ T cell counts were calculated as percentages of age-specific reference values, because absolute CD4+ and CD8+ T cell reference values are age related [21].

For statistical analysis SPSS version 21.0 (IBM® SPSS® Statistics) and Excel 2010 (Microsoft) was used.

2.3 Results

2.3.1 Population

Between March 2002 and January 2014, 43 treatment-experienced HIV-1-infected children switched to LPV/r q.d. of whom 40 could be included in our study. Reasons for exclusion were: follow-up period shorter than 12 months ($n = 1$), detectable viral load during the half year before switch ($n = 1$) and age > 18 years at start of LPV/r q.d. ($n = 1$). Baseline characteristics are summarized in Table 2.1. Figure 2.1 shows the number of children on treatment with LPV/r q.d. per year from 2002 through 2013.
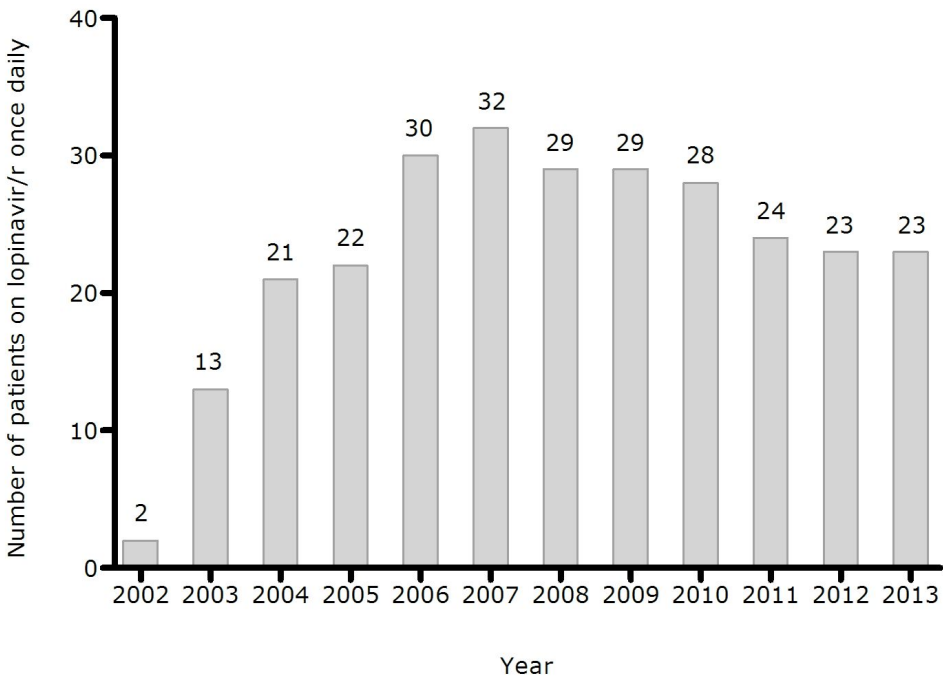
Figure 2.1: Number of children on LPV/r q.d. per year.

The median duration of follow-up was 6.3 years (range 1.0, 10.3). First yearly follow-up was at exact 12 months after starting LPV/r q.d. The interval of each subsequent yearly follow-up was aimed at 12 months but varied from 9–15 months depending on scheduled appointments.

Table 2.1: Baseline characteristics of HIV-1-infected children in this study of clinical response to lopinavir/ritonavir once daily ($n = 40$)

Characteristic	Value	
Sex (male/female)	23/17	
Age in years (range)[†]	6.3 (1.0, 17)	
Route of HIV transmission		
Vertical	33	
Blood contact	2	
Sexual abuse	1	
Unknown	4	
CDC HIV disease classification[‡]		
N1, N2, N3	7, 3, 3	
A1, A2, A3	2, 4, 1	
B1, B2, B3	2, 5, 1	
C1, C2, C3	1, 1, 10	
Plasma HIV-1 RNA level[†] (copies/ml)	50 (< 50, 145)	
HIV RNA < 50 copies/ml	37	
HIV RNA > 50 copies/ml	3	
CD4+ T cell value[†]		
Absolute cell count ($\times 10^6$ cells/ml)	920 (340, 2760)	
Percentage of normal for age	93 (34, 267)	
CD8+ T-cell value[†]		
Absolute cell count ($\times 10^6$ cells/ml)	920 (240, 2280)	
Percentage of normal for age	137 (30, 413)	
Total T-cell value[†]		
T-cell count ($\times 10^6$ cells/ml)	2055 (670, 5450)	
Percentage CD4+ of total T-cell count	48 (26, 70)	
cART regimen prior to LPV/r q.d.		
3TC/AZT/LPV/r b.i.d.	19	
3TC/AZT/IDV/r b.i.d.	6	
LPV/r/EFV b.i.d.	5	
3TC/AZT/ABC	2	
3TC/ABC/LPV/r b.i.d.	2	
Other combinations	6	
Combinations with LPV/r q.d.	Initial	After switch
3TC/ABC	18	33
3TC/AZT	15	2
EFV	5	3
TDF/FTC	2	2

[†] at start LPV/r q.d. regimen
[‡] at start antiretroviral therapy
Data are presented as number of patients or median (range) values. CDC, centers of disease control and prevention; b.i.d., twice-daily; q.d., once-daily; 3TC, lamivudine; AZT, zidovudine; LPV/r, lopinavir/ritonavir; IDV/r, indinavir/ritonavir; EFV, efavirenz; ABC, abacavir; TDF, tenofovir disoproxil fumarate; FTC, emtricitabine

2.3.2 Treatment

All children received combination antiretroviral therapy (cART) prior to starting LPV/r q.d. (Table 2.1 with a mean duration of 3.4 years (range 0.7, 9.6 years). Nineteen (48%) of them used a combination of lamivudine, zidovudine and LPV/r twice daily. If children switched from cART not containing LPV/r, they started their regimen with LPV/r b.i.d. before switching to LPV/r q.d.

At start of the once-daily regimen, 15 children (38%) started with the liquid formulation, 15 (38%) with soft-cell capsules and 10 (25%) with tablets. Median dose at start of the regimen was 465 mg/m^2 (range 275, 637 mg/m^2). During follow-up, in 31 children dose was adjusted 120 times. Median number of dose changes per child was 2 (range 0, 10). Reasons for changing dose were growth (increase in body surface area) (50.8%), low plasma concentrations of LPV (25%) or change of LPV/r formulation (20.8%). In 2 children LPV/r dose was lowered once because of too high concentrations of LPV with risk of toxicity. Most doses were adjusted in children who started the once-daily regimen on LPV/r liquid and were based on growth. Backbone therapy was changed 13 times in 13 children (33% of all children) during follow-up. Simplification was the reason for changing the nucleoside reverse transcriptase inhibitor backbone medication in all cases.

Eight children (20%) discontinued the once-daily regimen. Five of them switched to a one pill-once a day regimen with efavirenz/emtricitabine/ tenofovir DF for simplification reasons. One child switched cART because of toxicity of the regimen (see below). Two children stopped cART (1 because of religious beliefs and 1 because of adherence problems due to severe depression).

2.3.3 Virological and immunologic response to therapy

Figure 2.2 shows the percentage of children with an undetectable viral load (< 50 copies/ml) for each subsequent year after starting LPV/r q.d. At start of the LPV/r q.d. regimen 37 (93%) children had an undetectable viral load. Three children had a single detectable viral load < 400 copies/ml at start, after being undetectable for 6 months or more. These 3 children had an undetectable viral load again during follow-up after this initial detectable viral load. During follow-up the percentage of children with an undetectable viral load varied between 82–100% (on treatment) and 83–93% (LOCF).

In 13 children (33%) HIV-RNA was detectable (> 50 copies/ml) at least
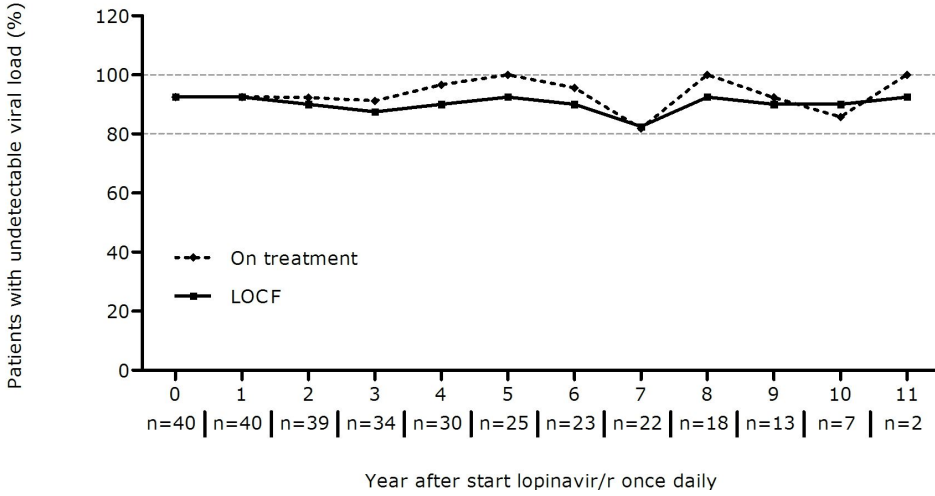
Figure 2.2: Percentage of children with a viral load < 50 copies/ml during follow-up; $t_0 =$ start LPV/r q.d.; $n =$ number of children on LPV/r q.d. at yearly time-point; LOCF, Last Observation Carried Forward.

once during three-monthly follow-up. In 6 of these children viral load did not exceed 400 copies/ml. In 7 children viral load was higher than 400 copies/ml at least once during follow-up. Figure 2.3 shows the Kaplan-Meier curve for time to virological failure. During the entire follow-up period a total of 5 (12.5%) children met the criteria for failure (viral load > 400 copies at least twice within 6 months during follow-up). All children returned to an undetectable viral load within one year after being detectable. All children who had a detectable viral load during follow-up, admitted to have adherence problems to therapy at that time.

At baseline median absolute CD4+ and CD8+ counts were 920×10^6 cells/ml (range 340, 2760) and 920×10^6 cells/ml (range 240, 2280), respectively. These absolute counts corresponded with a median percentage of age-specific reference values of 93% (CD4+) and 136% (CD8+). During follow-up median CD4+ counts remained stable at normal levels. Additionally, median CD8+ counts also remained at normal levels during follow-up. Median percentage CD4+ of total T-cell count was 48% (range 26, 70) and was 48% (range 23, 70) during follow-up.
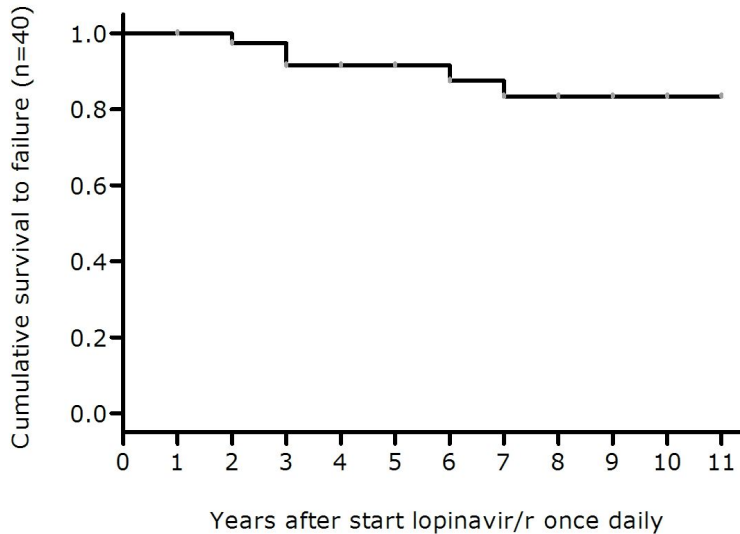
Figure 2.3: Kaplan-Meier curve for time to virological failure; virological failure defined as a viral load > 400 copies/ml two or more times within 6 months, or viral load > 400 copies/ml resulting in discontinuation of LPV/r q.d.

2.3.4 Therapeutic drug monitoring

Full pharmacokinetic curves after switch to LPV/r q.d. were available for 39 out of 40 children. Geometric mean (95%CI) area under the curve (AUC_{0-24}) for LPV was 169.3 (144.9, 197.8) h×mg/l, C_{max} 12.5 (11.0, 14.3) mg/l and C_{last} 1.35 (0.86, 2.12) mg/l, see also Table 2.2. A total of 15 children (38%) had a $C_{last} < 1.0$ mg/l. Ten of these 15 children (67%) had their dose changed after baseline pharmacokinetic analysis. Two children had their curves repeated after which no dose change was needed and 3 children remained on the same dose because virological and immunological response to the initial dose was good.

Out of the 15 children who had $C_{last} < 1.0$ mg/l at baseline, 2 children had a detectable viral load after switching to LPV/r q.d. One child ($C_{last} = 0.41$ mg/l) had a viral load of 52 copies/ml that returned to an undetectable load at the next measurement without the need of changing the dose. One child ($C_{last} = 0.58$ mg/l) had a viral load of 453 copies/ml, which returned to an undetectable viral load 4 months after dose was increased based on the results of pharmacokinetic analysis. Out of the 24 children who had $C_{last} > 1.0$ mg/l at baseline, 1 child had a detectable viral load after switch

Table 2.2: Geometric mean (95%CI)* pharmacokinetic parameters of lopinavir ($n = 39$)

AUC_{0-24} (h×mg/l)	169.3	(144.9, 197.8)
C_{max} (mg/l)	12.5	(11.0, 14.3)
C_{last} (mg/l)	1.35	(0.86, 2.12)
Clearance (l/(h×kg))§	0.104	(0.088, 0.123)
T_{max} (h)*	6.2	(0.0, 18.6)

§ Clearance calculated as
CL/F/kg = dose (mg)/[AUC_{0-24}(h×mg/l)×body weight (kg)]
* For T_{max} the median value (minimum, maximum) is reported.

to LPV/r q.d. This child ($C_{last} = 6.27$ mg/l) experienced a viral load of 110 copies/ml but returned to an undetectable load within 3 months without the need of changing the dose.

2.3.5 Adverse events and laboratory findings

Adverse events were encountered in 8 children (20%). Seven experienced gastrointestinal side effects such as nausea and/or diarrhea. For these children, symptoms were mild and there was no need to stop treatment. One child stopped treatment because of persistent elevated lipid-levels, with highest measured cholesterol of 9.4 mmol/l and triglycerides of 7.8 mmol/l. In other children lipid profiles varied, but were mainly within range of the reference values. A total of 31 lipid profiles (32 triglycerides profiles) were available at baseline. 191 lipid profiles were analyzed during follow-up (24 profiles missing in 19 patients during follow-up). Mean cholesterol at start ($n = 31$) of the once-daily regimen was 4.7 mmol/l (range 2.9, 7.2) and during follow-up ($n = 40$) 4.9 mmol/l (range 2.8, 9.4). Values for triglycerides were a mean of 1.38 mmol/l (range 0.58, 5.34) at start ($n = 32$) of the regimen and 1.54 mmol/l (range 0.42, 7.83) during follow-up ($n = 40$).

2.4 Discussion

In this long-term follow-up observational cohort study in 40 patients we show that a once-daily LPV/r containing regimen can result in a high virological suppression rate (83–93% undetectable viral load with median follow-up of 6.3 years, range 1.0, 10.3). During the years of follow-up 27 (68%) HIV-1-infected children had continuously suppressed viral loads < 50 copies/ml, when treated with LPV/r q.d. Out of the children with

virological rebound, 6 children experienced a viral load < 400 copies/ml. Out of the children with virological rebound > 400 copies/ml, five children met the criteria for failure (viral load > 400 copies/ml within 6 months at least twice). All children with a detectable viral load, regardless of absolute count, returned to an undetectable viral load within a year after the viral rebound. CD4+ and CD8+ counts in children remained stable at normal values during follow-up.

The suppression rate in our study, is either comparable to or higher than the results of real-life cohort studies on the long term follow-up of HIV-1 infected children treated with a twice-daily LPV/r containing regimen [22,23]. Our results are also comparable to the virological response rates on different, and variable cART regimens found in two nationwide cohort studies from European countries comparable to our setting [20, 21]. A long-term follow-up study performed in 997 HIV-1 infected children in the United Kingdom and Ireland reported an optimal virological response rate in 92% of the children after 12 months of follow-up, but with estimated higher failure rates as follow-up progressed. The other study was performed in the Dutch cohort where 89% of 210 children treated with cART had undetectable viral loads during a median follow-up period of 8.7 years. The 40 children included in the current study are part of these 210 children.

To our knowledge there are no other studies that report on the long-term (> 1 year) real-life virological and immunological response to LPV/r q.d. in children. Some studies report on the short-term results of LPV/r q.d., with response rates between 57 and 100% [11, 12, 14]. Based on results from a recent large randomized trial comparing LPV/r q.d. with twice-daily dosing of LPV/r in treatment-experienced children performed by the PENTA network (KONCERT), the 2015 PENTA guidelines on the treatment of HIV-1 infected children advise that LPV/r should be dosed twice daily, but that once-daily dosing may be used in selected children by whom adherence is monitored and routine clinical follow-up is available [1, 16]. The KONCERT study reports that 86% of the children maintained viral suppression after 48 weeks on a once-daily regimen vs 92% on twice-daily dosing, (an estimated difference between arms of 6% [90%CI −2%, 14%], bootstrap $P = 0.19$). A baseline imbalance between groups in viral rebound and CD4-percentage complicated the interpretation of these results. Our results support PENTAs statement that in a monitored clinical setting and in children with expected good adherence to therapy, LPV/r q.d. can be a good alternative in treating children.

All children in our study who failed on therapy admitted to have adher-

ence problems at that time. We recognize the risk of virological failure in a once-daily regimen when children are non-adherent to therapy. We therefore stress the importance of adherence when a once-daily dosing regimen is considered. The possible higher chance of virological failure when being non-adherent has been suggested previously [12, 16]. Counseling by trained nurses, addressing adherence issues, and a strict failure protocol will also have supported our children to achieve virological suppression.

Performing intensive TDM might have been a key to detect under or over dosing and possible non-adherence and thus increase virological response rates in our cohort. We performed intensive pharmacokinetic evaluation in all children about 2 weeks after start of LPV/r q.d. and TDM every 3 months. Exposure to LPV was comparable to what was found in other pediatric studies [11, 13–15, 17]. LPV trough concentrations > 1.0 mg/l are associated with better virological outcome in subjects treated with LPV/r b.i.d. [18]. We showed that both children with C_{last} below this limit as well as children that were above this limit experienced viral rebounds. These findings are comparable to results from other studies [11, 12].

Once-daily LPV/r was well tolerated, also in the younger age group that had to take the liquid formulation with poor palatability. This might be explained by the LPV/r experienced population. Intolerability is mostly reported in treatment-naïve children [6]. In our study lipid profiles remained stable and within the normal range in most children. This is similar to findings in other studies [11, 16].

A limitation of this study is the population that was selected based on expected good adherence and pre-existing viral suppression, which makes the results not generalizable to unselected populations. Additionally, because of the retrospective nature of the study, no control group could be included.

LPV/r is approved to be used twice daily only in children, and once-daily use is therefore considered off-label [2, 7]. When drugs are used off-label, safety and efficacy have not been reviewed by regulatory authorities, and need to be evaluated for each patient [24, 25]. When a drug is used off-label in real-life setting in such a relatively large population as ours we feel it is important to evaluate outcome in a structured manner and to report the results found.

In conclusion, we suggest that, based on our results, especially for younger children in whom LPV/r is the only boosted PI option, LPV/r q.d. can be a good alternative for b.i.d. dosing in selected cases with pre-existing undetectable viral load, expected good adherence, and intensive

clinical and therapeutic drug monitoring.

Acknowledgements

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Making things easier

3 Administration of the approved darunavir once-daily dosage in children results in lower than predicted exposure

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Abstract

Background

The approved dosing recommendations for children 6–12 years old are based on a modelling and simulation procedure by the company. The aim of this study was to validate the proposed dosing recommendation for once-daily darunavir/ritonavir in HIV-infected children 6–12 years of age.

Methods

This pharmacokinetic study is a multicenter phase-1 trial in HIV-infected children and has been performed by paediatric HIV-centres in the Netherlands. Children took darunavir tablets following the approved dose. A 24h pharmacokinetic curve was collected after observed intake. Pharmacokinetic parameters (area under the plasma concentration-time curve (AUC), maximum plasma concentration (C_{max}), last observed plasma concentration in dosing interval (C_{last})) were determined using noncompartmental analysis and were compared to historical data.

Results

Twelve children were included. The geometric mean (%CV) AUC_{0-24} was 63.1 (33%) h×mg/l, C_{max} 5.6 (34%) mg/l and C_{last} was 1.5 (44%) mg/l. The lower limit of the one sided 90%CI of the AUC_{0-24} was 55.7 h×mg/l, which is 62% of the adult value (89.7 h×mg/l). Ten out of the 12 children had an AUC_{0-24} below the adult target value, of which eight had an AUC below 0.8 of the adult target value. C_{last} of all of the children was found to be adequate.

Conclusion

The AUC of darunavir in children 6–12 years was substantially lower than predicted by the population pharmacokinetic model, which was used for approval of the once-daily dosing regimen of darunavir/ritonavir in children. Since trough levels were above the target value, the treatment was considered adequate.

3.1 Introduction

Antiretroviral treatment that can be administered once daily instead of twice daily is generally preferred for the treatment of HIV-infected children [1, 2]. Protease inhibitors that are approved to be used once daily in children are atazanavir, with or without ritonavir, and recently also darunavir boosted with ritonavir (darunavir/r). Dosing recommendations for darunavir/r once daily in children have been derived from results from several pharmacokinetic studies in children [3–5]. These included once-daily pharmacokinetic data from the DIONE trial in adolescents (12–18 years) demonstrating a slightly lower darunavir plasma exposure, but comparable virologic response and safety profile compared to treatment-naive adults, and results from children in the ARIEL trial (3–6 years) who received the darunavir suspension [3, 4]. A population pharmacokinetic model was built from data of these paediatric studies combined with adult data [6]. This model was used to predict exposure to darunavir using different potential dosing regimens. The dosing regimen that was predicted to approach the adult exposure best, was chosen to be included as dose recommendation in the label [6].

The predicted exposure after administration of the recommended once-daily dose has not yet been studied in the target population. Because of the lack of clinical and supporting pharmacokinetic data, once-daily darunavir is not yet recommended for children under the age of twelve years in current paediatric guidelines [1]. To fill this gap in information the aim of this study was to describe the pharmacokinetics for the recommended once-daily darunavir/r dose in children 6–12 years old and to determine whether exposure is comparable to the target in adults.

3.2 Methods

This pharmacokinetic study was an open label, multi-center phase I trial in HIV-infected children (6–12 years of age) who were treated with darunavir/r once daily as part of their current combination antiretroviral treatment. The trial has been performed by paediatric HIV-centres in the Netherlands and was approved by the medical ethical committee of the Radboud university medical center (CMO Arnhem-Nijmegen, NCT02285478).

3.2.1 Population and treatment

Children could be included when they were using darunavir/r once-daily in the approved dose: 600/100 mg if 15–30 kg; 675/100 mg if 30–40 kg; 800/100 mg if > 40 kg). Furthermore, they must have had an undetectable viral load (< 50 copies/ml) for at least 6 months, had a body weight of 15 kg or more and were able to swallow intact tablets. Children who previously failed on a protease inhibitor-containing regimen could not be included. The use of concomitant drugs was not allowed unless permission was granted by the trial team. Other exclusion criteria were: inability to understand the nature and extent of the trial and the procedures required; a documented history of sensitivity to darunavir or ritonavir medicinal products or its excipients and a relevant history that might interfere with drug absorption, distribution, metabolism or excretion.

3.2.2 Sample size

Darunavir plasma exposure should be similar compared to adults when using the approved dosing regimen. The target geometric mean area under the plasma concentration time curve over a dosing interval (AUC_{0-24}) in adults is 89.7 mg×h/l. A variation coefficient of 29% in AUC_{0-24} has been observed in adolescents [3]. Based on these values it was aimed to include 12 children to be able to determine with a power of 80.3% that the lower limit of the 90% one sided confidence interval of the geometric mean of the AUC is higher than 0.8 of the value found in adults. This was based on a simulation study in SAS$^{®}$ performed to establish the power of the study for varying sample sizes. For each choice of sample size 10 000 simulations were performed.

3.2.3 Pharmacokinetic assessment

Prior to the day of pharmacokinetic assessment children took darunavir tablets in the morning for at least 3 days. Children came to the clinic fasting and without having taken the morning dose of darunavir/r. Darunavir was administered with a breakfast. A 24h pharmacokinetic curve (7 or 8 samples) was collected after observed intake. Samples (2 ml of blood) were taken just before dosing and at 2, 3, 4, 6, 8, 12 and 24h post ingestion. For logistic reasons (eg travelling distance to hospital), the 12h sample was allowed to be left out.

Plasma concentrations of darunavir and ritonavir were determined using a validated ultra-high performance liquid chromatography assay with UV

detection derived from the previously published assay [7]. The analysis was performed at the Department of Pharmacy, Radboud university medical center, Nijmegen, the Netherlands. The analytical range of the assay for darunavir was 0.10–30 mg/l and for ritonavir 0.045–30 mg/l. The intraday and interday precision for both assays ranged from 0.6 to 4.3% (coefficient of variation) and 0.3 to 2.4%, respectively. The percentage accuracy of the assay ranged from 98.2 to 105.6%.

3.2.4 Acceptability questionnaire

Acceptability of a drug regimen is important for adherence. Aspects such as dosing frequency, ease of administration and palatability are important for acceptability. Therefore, during the day of the pharmacokinetic assessment children and their parents were asked about the preference for the current or previous antiretroviral regimen. They were also asked what the main reason for the preference was.

3.2.5 Pharmacokinetic and statistical analysis

Darunavir and ritonavir pharmacokinetic parameters were determined using noncompartmental analysis (Phoenix$^{®}$ WinNonlin$^{®}$ version 6.4, Pharsight Corporation, Mountain View, CA, USA). The actual sampling times were used for pharmacokinetic analysis. Pharmacokinetic parameters of interest were AUC$_{0-24}$ (area under the plasma concentration-time curve calculated over a dosing interval from time 0 to 24 hours after dosing (linear up-log down trapezoidal method)), maximum observed plasma concentration (C_{max}), time of C_{max} (T_{max}), drug concentration 24 hours post-dose (C_{24}), clearance (CL/F/kg) and elimination half life ($t_{1/2}$).

3.3 Results

3.3.1 Population

Twelve children were enrolled between July 2015 and August 2016, of which seven were girls. Median (range) age of the included children was 8.9 (6.3, 11.7) years and weight was 26.6 (22.4, 45.0) kg. Further demographic data of the children are presented in Table 3.1. Seven children used darunavir/r 600/100 mg, two 675/100 mg and three 800/100 mg. The median (range) darunavir dose administered to the children was 22.6 (17.8, 26.8) mg/kg. All children used abacavir and lamivudine once daily as

antiretroviral background regimen. The use of other concomitant medication was reported for two children (one child: levetiracetam, valproic acid, clobazam; one child: triptorelin). None of these drugs are known to influence the metabolism of darunavir or ritonavir. Three children used vitamin D. One child suffered from anxiety starting 4 weeks after switch to darunavir/r and 2 weeks after the pharmacokinetic assessment, resulting in hallucinations at 6 weeks after start. Darunavir/r was stopped and the child fully recovered within 4 days after discontinuation. This child used 600 mg (24 mg/kg) darunavir.

Table 3.1: Demographic data of the children at the day of the pharmacokinetic assessment ($n = 12$)

	median (range)
Sex (female/male)	7/5
Age (years)	8.9 (6.3, 11.7)
Weight (kg)	26.6 (22.4, 45.0)
Darunavir dose (mg/kg)	22.6 (17.8, 26.8)
Ritonavir dose (mg/kg)	3.8 (2.2, 4.5)
Dosing regimen darunavir/r (n)	
600/100 mg (15–30 kg)	7
675/100 mg (30–40 kg)	2
800/100 mg ($\geq$ 40 kg)	3

3.3.2 Darunavir pharmacokinetics

The calculated pharmacokinetic parameters of all of the children ($n = 12$) were used for the final pharmacokinetic model. The geometric mean (%CV) AUC_{0-24} was 63.1 (33%) h×mg/l, C_{max} 5.6 (34%) mg/l and C_{last} was 1.5 (44%) mg/l, see also Table 3.2. The mean plasma concentration time profile is shown in Figure 3.1. It was predefined that exposure would be adequate, when the lower limit of the 90% one sided confidence interval of the geometric mean of the AUC_{0-24} was higher than 0.8 of the value of adults (0.8×89.7 = 71.8 h×mg/l). The lower limit of the one sided 90%CI was 55.7 h×mg/l, which is 62% of the adult value. Therefore, this target was not reached. The geometric mean AUC_{0-24} was 70% of the AUC_{0-24} found in adults. Ten out of the 12 children (83%) had an AUC_{0-24} below the adult target value, of which eight (67%) had an AUC below 80% of the adult target value, see also Figure 3.2.

C_{last} of all of the children was measured between 23.1 and 25.1 hours after the observed intake and all were above 0.55 mg/l (range: 0.69, 2.38 mg/l), which is the target for protease inhibitor experienced patients.

Table 3.2: Darunavir pharmacokinetic parameters after once-daily dosing in HIV-infected children ($n = 12$)

	geometric mean (CV%)	
AUC_{0-24} (h×mg/l)	63.1	(33%)
C_{max} (mg/l)	5.6	(34%)
C_{last} (mg/l)	1.5	(44%)
Clearance (l/h×kg)	0.36	(40%)

AUC_{0-24}: Area under the plasma concentration time curve over 24 hours;
C_{max}: maximum plasma concentration;
C_{last}: last observed plasma concentration within the dosing interval;
Clearance: (CL/F/kg) = dose/(AUC_{0-24}×body weight).
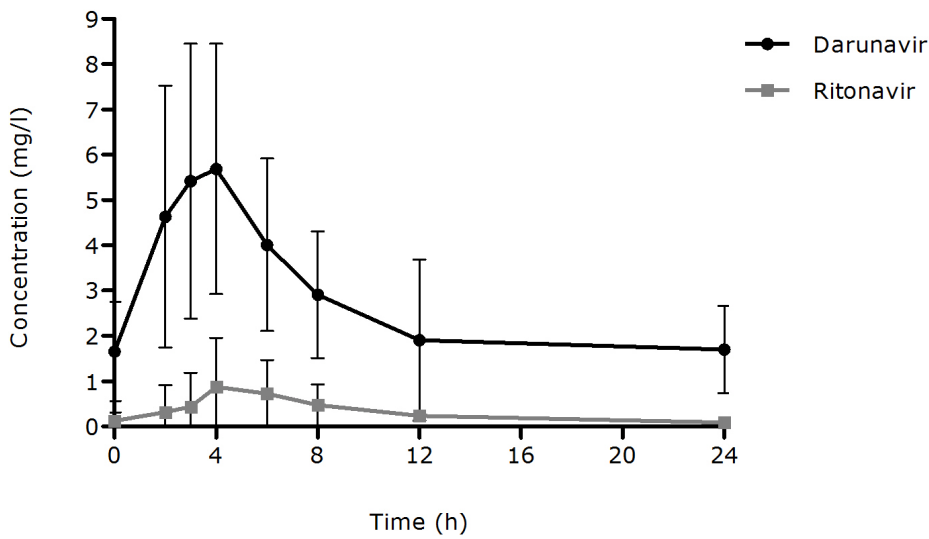
Figure 3.1: Mean (± SD) plasma concentration time curve.

3.3.3 Acceptability

All children completed the questionnaire together with their parents. For all children the antiretroviral regimen used before darunavir once daily
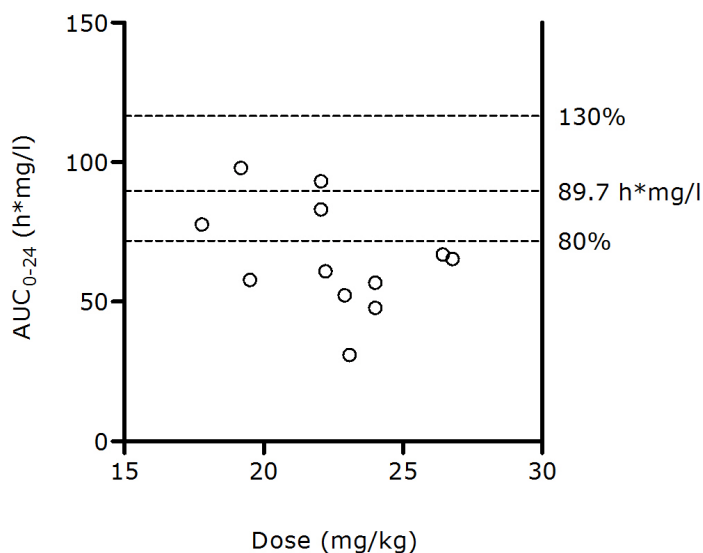
Figure 3.2: Area under plasma concentration time curve versus dose in comparison to the adult target value of 89.7 h×mg/l.

was lopinavir/r administered as tablets once daily ($n = 3$) or twice daily ($n = 9$). Ten of the children and ten of the parents thought it was easier or much easier to take darunavir/r once daily, compared to the previous dosing regimen. One child and two of the parents did not think it was a difference. One child had more difficulties with it, because the child had to get used to the taste. A once-daily regimen compared to a twice-daily regimen was preferred by most of the subjects ($n = 11$), one did not think it made a difference. The main reason for preference for once-daily use was the lower impact on daily activities ('Child doesn't have to be woken up for medication', 'Child doesn't have to take medicines to social activities'). The preferred moment to take the medication was the evening for eight of the children and in the morning for four of the children.

3.4 Discussion

A lower than predicted exposure (AUC) to darunavir was observed in HIV-infected children, aged 6–12 years, using the recommended once-daily dosing regimen. The by the EMA and FDA approved dosing regimen was based on a population pharmacokinetic model, that predicted a mean AUC in children between 80 and 130% of the adult value of 89.7 h×mg/l [6]. The

geometric mean exposure in our paediatric population was 63.1 h×mg/l, which is 70% of the adult value. For only four of the twelve children, an exposure between the predicted limits was found. However, since C_{last} of all of the children was above 0.55 mg/l, it is our opinion that exposure is adequate and that the once-daily dose recommendations can be maintained.

This is the first study to report on the pharmacokinetics of the approved once-daily dosing regimen of darunavir/r in children aged 6–12 years. Two studies investigated the exposure to darunavir in adolescents, one in children under the age of 6 years [3, 6, 8]. The observed exposure in adolescents treated with once-daily darunavir/r 800/100 mg in the DIONE trial was slightly lower than observed in adults [3]. The geometric mean AUC_{0-24} was 80.7 mg×h/l, which is 90% of the adult target. A paediatric study in Thailand ($n = 8$) found also a lower exposure to darunavir, in children aged 11–19 years [8]. The dose used in this study was lower than the approved dosing regimen. A higher exposure was observed in 10 treatment-experienced children in the ARIEL study who switched from twice- to once-daily dosing of darunavir after 24 weeks of therapy. Children used the liquid formulation for which bioequivalence compared to the commercial tablets has been shown under fed conditions in adults [9]. Pharmacokinetic parameters were determined after two weeks and darunavir mean AUC_{0-24} was 128% of the adult AUC_{0-24} [9]. Children in this study were administered a higher bodyweight based dose (40/7 mg/kg darunavir/r, or 600/100 mg from 15 kg), which could partly explain the higher exposure.

It can be debated whether AUC is the best parameter to determine the efficacy of darunavir, since for most protease inhibitors efficacy is found to be correlated with the trough level [2]. However, for darunavir no correlation has been found between the observed trough levels (nor AUC) and efficacy, but in clinical studies trough levels remained widely above the median effective concentration for wild-type HIV-1 [10].

The bioavailability of darunavir increases from about 37% to 82% when a single dose darunavir 600 mg is combined with 100 mg ritonavir in HIV-negative volunteers [10]. This indicates an important effect from ritonavir on darunavir absorption and/or first pass metabolism. In the population pharmacokinetic model the ritonavir concentration was not included as covariate. However, most of the subjects included in the population pharmacokinetic model used darunavir/r twice daily instead of once daily. Next to this, children have a lower gastro-intestinal volume than adults, which might influence the amount of darunavir that is dissolved and avail-

able for absorption. Especially for a low-solubility class drug such as darunavir, this might also partly explain the observed lower exposure [11].

In conclusion we found that the AUC of darunavir in children 6–12 years was substantially lower than predicted by the population pharmacokinetic model, which was used for approval of the once-daily dosing regimen of darunavir/r in children. Since trough levels were above the target value, the treatment was considered adequat.e

Acknowledgements

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Making things easier

4 Dose evaluation of lamivudine in HIV-infected children aged 5 months – 18 years based on a population pharmacokinetics analysis

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Abstract

Aim

The objectives of this study were to characterise age-related changes in lamivudine pharmacokinetics in children and evaluate lamivudine exposure, followed by dose recommendations for subgroups in which target AUC_{0-24} is not reached.

Methods

Population pharmacokinetic modelling was performed in NONMEM using data from two model building datasets and two external datasets ($n = 180$ (aged 0.4–18 years, bodyweight 3.4–60.5 kg); 2 061 samples (median 12 per child)) in which a daily oral dose ranging from 60–300 mg (3.9–17.6 mg/kg) was given. The model was validated both internally and externally. AUC_{0-24} was calculated per individual.

Results

A two-compartment model with sequential zero-order and first-order absorption best described the data. Apparent clearance and central volume of distribution was 13.2 l/h and 38.9 l for a median individual of 16.6 kg, respectively. Bodyweight was identified as covariate on apparent clearance and volume of distribution using nonlinear functions. The external evaluation supported the predictive ability of the final model. In 94.5% and 35.8% of the children with a bodyweight $>$ and $<$ 14 kg, respectively, the target AUC_{0-24} was reached.

Conclusion

Bodyweight best predicted the developmental changes in apparent lamivudine clearance and volume of distribution. For children with a bodyweight $<$ 14 kg, the dose should be increased to 10 mg/kg/day if the adult target for AUC_{0-24} is aimed for. In order to identify whether bodyweight influences bioavailability, clearance and/or volume of distribution, future analysis including data on intravenously administered lamivudine is needed.

4.1 Introduction

Lamivudine is a nucleoside reverse transcriptase inhibitor (NRTI) which is widely used as part of the treatment of human immunodeficiency virus (HIV)-infected children. It is currently recommended as part of first-line NRTI backbone together with either a protease inhibitor or non-nucleoside reverse transcriptase inhibitor [1]. Lamivudine is available in solid and liquid dosage forms, as well as in single entity and fixed dose combination products. Several issues have been raised concerning the treatment with lamivudine, such as bioavailability issues [2–6] and possible underdosing in the youngest age group [7–9].

Several studies on the pharmacokinetics of lamivudine in children have been performed. Most studies applied noncompartmental analysis [4, 5, 8–15] and some developed a population pharmacokinetic model [7, 16–20]. Most of these studies were based on a small number of children, narrow age ranges or the relationship between parameters and covariate was fixed a priori. None of the models has been validated externally, in other words the models have not been evaluated in how well they generalize to new data, which have not been included in the model-building dataset.

As highlighted before, dosing in children should be based on the understanding of the developmental changes in the pharmacokinetic and/or pharmacodynamic relation of drugs instead of applying the adult mg/kg dose to children [21–23]. For lamivudine, the area under the daily plasma concentration-time curve (AUC_{0-24}) is mainly used as a surrogate for the intracellular exposure to lamivudine triphosphate. In adults, an average AUC_{0-24} of 8.9 mg×h/l is reached after administration of a daily dose of 300 mg [2]. Also in children, this value is used as a target for lamivudine exposure [7, 15, 16, 24].

The objectives of this study were to characterise age-related changes in lamivudine pharmacokinetics in infants, children and adolescents, and to test how well this model can be generalized to new patients not included in the model-building dataset. Based on the developed population pharmacokinetic model, lamivudine exposure upon currently used dosing recommendations was evaluated and, when necessary, a new dose will be calculated for subgroups in which target AUC_{0-24} was not reached.

4.2 Methods

4.2.1 Patients and treatment

Model building was based on data from two datasets of in total 85
children using lamivudine twice daily. The first dataset consisted of 64
children, aged 0.5–14.9 years, who participated in the CHAPAS1 trial [25].
CHAPAS1 was an open, randomized controlled phase I/II trial designed
to assess the appropriate dosing of and adherence to Triomune®. Lamivu-
dine was administered in a fixed-dose combination tablet of lamivudine,
nevirapine and stavudine. Daily dosing was based on acquiring an appro-
priate nevirapine dose. Daily lamivudine dose varied between 60 and 240
mg (6.3–17.6 mg/kg). In the second dataset, 21 children, aged 1.7–18.0
years, were included in whom therapeutic drug monitoring on lopinavir
was performed as part of routine clinical care. In the available samples,
lamivudine concentrations were also determined. Lamivudine was dosed
orally according to the PENTA guideline valid at that time [26]. Daily
lamivudine dose varied between 80 and 300 mg (5.1–10.5 mg/kg) for the
children included in this cohort. An overview of the patient characteristics
is given in Table 4.1.

External evaluation of the model was performed with two external
datasets [8, 14, 15, 27]. The first external dataset consisted of 24 children,
aged 1.6–17.3 years, in whom therapeutic drug monitoring on lopinavir was
performed. Lamivudine concentrations were determined on samples from
different occasions (range: 1, 10 occasions). Sixteen of these 24 children
participated in the RONDO trial [27]. Lamivudine was dosed both once and
twice daily according to the PENTA guideline valid at that time and daily
dose varied between 90 and 300 mg (3.9–10.2 mg/kg) [26]. The second
external dataset consisted of 77 children, aged 0.4–12.8 years, who were
included in three studies: PENTA13 [8], PENTA15 [15] and ARROW [14].
All three studies were cross-over studies to compare the pharmacokinetics of
once-daily lamivudine dosing versus twice-daily dosing. Daily dose varied
between 60 and 300 mg (4.9–15.0 mg/kg). An overview of the patient
characteristics is given in Table 4.1. Data on different dosing occasions of
six children were included in both the model building dataset as well as in
the dataset for external validation.

4.3 Blood sampling and assay

For all children included in the model building datasets and the second
external dataset, at least one complete concentration-time profile after

dosing was available (≥ 6 samples). This also applied for 15 children (63%) included in the first external dataset. Sampling was performed until the end of the dosing interval.

For the data from the CHAPAS1 trial, PENTA trials and therapeutic drug monitoring study, lamivudine concentrations were measured using a high-performance liquid chromatography assay with ultraviolet detection [28]. The lower limit of quantification was 0.05 mg/l. Data below the limit of quantification were excluded (M1 method [29]). For the data from the ARROW trial, lamivudine was measured using a high-performance liquid chromatography assay with tandem mass spectrometry detection. The lower limit of quantification was 0.0025 mg/l.

4.3.1 Pharmacokinetic analysis and model evaluation

Model building was performed in four different steps: 1) testing of both a one- and two-compartment model and different absorption models in order to select a structural model; 2) selection of a statistical model; 3) covariate analysis; and 4) model evaluation. For oral absorption, a zero-order and first-order model, a lag time model, transit compartment model, and combined absorption models were evaluated [30].

Discrimination between structural models was achieved by comparison of the objective function value (OFV) and the total number of parameters. A decrease in the OFV of more than 3.8 points was considered statistically significant for the structural model ($P < 0.05$ based on χ^2 distribution). The goodness-of-fit plots (observed versus both individual- and population-predicted concentrations and both time and population predictions versus conditional weighted residuals) were evaluated. Improvement of individual plots, confidence intervals of the parameter estimates and the correlation matrix were also assessed.

4.3.2 Covariate analysis

Covariates were plotted against individual post hoc parameter estimates and the weighted residuals to visualise potential relationships. The covariates bodyweight, age, height and formulation were evaluated. Potential covariates were separately implemented in the model, using a linear or power equation

$$P_i = P_p \times \left(\frac{\text{cov}_i}{\text{cov}_{\text{median}}} \right)^k,$$

where P_i represents the individual parameter estimate of the ith subject,

Table 4.1: Patient characteristics of the children in the two model building
datasets and the two datasets used for external validation.
Values are expressed as median [range]

| | Model building | | |
	Dataset 1	Dataset 2	Total
Number of children	64	21	85
Age (years)	7.0 [0.5, 14.9]	8.0 [1.7, 18.0]	7.2 [0.5, 18.0]
Bodyweight (kg)	16.3 [3.4, 29.0]	27.8 [10.5, 59.2]	16.6 [3.4, 59.2]
Number of dose (mean per child)	64 (1)	21 (1)	85 (1)
Number of observations (mean per child)	433 (6.8)	205 (9.8)	638 (7.5)
Daily dose (mg)	120 [60, 240]	200 [80, 300]	120 [60, 300]
Daily dose (mg/kg)	8.9 [6.3, 17.6]	7.8 [5.1, 10.5]	8.5 [5.1, 17.6]
Formulation	FDC: 64	NA: 21	FDC: 64 NA: 21

* 16 of the 24 children in this dataset were part of the RONDO trial; 6 of the 24
children were also included in model building dataset 2 on a different occasion;
FDC: fixed dose combination tablet; NA: not available

P_p is the population parameter estimate, cov is the covariate and k is the exponent. k was fixed at 1 for a linear function or estimated for a power function. The framework proposed by Krekels et al. to systematically evaluate the descriptive and predictive performance of a paediatric model was used as a guide to discriminate between different covariate models [31]. A decrease in OFV of at least 7.8 was applied to evaluate covariates in forward inclusion. In backward deletion, a more stringent P-value of < 0.001 was used (a decrease in OFV of at least 10.83 points). When two or more covariates were found to significantly improve the model, the covariate causing the largest decrease in OFV was kept in the model. In order to be retained in the model, additional covariates had to reduce this OFV further. The clinical relevance of a covariate relationship was also considered [31]. In order to confirm the final covariate model, individual and population parameter estimates were plotted against the most predictive covariate to evaluate whether the individual predicted parameters were equally distributed around the population predicted parameters.

Table 4.1: Patient characteristics of the children in the two model building datasets and the two datasets used for external validation. Values are expressed as median [range] (continued)

	External validation			Total
Dataset 1*	Dataset 2	Total		
24	77	101		180
8.4 [1.6, 17.3]	5.8 [0.4, 12.8]	6.0 [0.4, 17.3]		6.6 [0.4, 18.0]
30.2 [11.0, 54.1]	17.4 [7.4, 60.5]	18.2 [7.4, 60.5]		17.0 [3.4, 60.5]
88 (3.7)	153 (2.0)	241 (2.4)		326 (1.8)
232 (9.7)	1 191 (15.5)	1 423 (14.1)		2 061 (11.5)
200 [90, 300]	150 [60, 300]	160 [60, 300]		150 [60, 300]
7.3 [3.9, 10.2]	8.6 [4.9, 15.0]	8.2 [3.9, 15.0]		8.2 [3.9, 17.6]
Solution: 17	Solution: 63	Solution: 80		FDC: 64
Tablet: 9	Tablet: 90	Tablet: 99		Solution: 80
NA: 62		NA: 62		Tablet: 99
				NA: 83

4.3.3 Internal evaluation procedure

The final model was evaluated using two methods: the bootstrap method and the normalised prediction distribution error (NPDE) method [31–34]. The bootstrap analysis was used to evaluate the stability and precision of the model. The model building dataset was resampled to produce 2000 new datasets of the same size, containing a different combination of individuals. The final model was sequentially fitted to all of these newly generated datasets. The parameter estimates were summarised in terms of median values and 90% confidence intervals, and were compared with the estimates obtained from the model building datasets.

The accuracy of the model was evaluated with the NPDE method [33, 34]. Each observation was simulated 1000 times, after which the observed and simulated concentrations were compared. The software assembled the quantiles of each observation in its predicted distribution, on the basis of the simulated values. The observations and predictions were decorrelated.

The NPDEs were then obtained after the inverse function of the normal cumulative density function was applied.

4.3.4 External evaluation procedure

External evaluation of the model was performed with two external datasets, as described above [8, 14, 15, 27]. These datasets were not included when the model was fitted to the data.

The final pharmacokinetic model was used to simulate concentrations for each data point in the two external datasets. Additionally, the final pharmacokinetic model was used to compute the NPDE for each of the external datasets [33, 34]. Finally, the parameters of the final model were re-estimated on the basis of the two model building datasets combined with both external datasets.

4.3.5 Evaluation of currently used dosing guidelines

For the first available dosing occasion per individual, AUC_{0-24} was noncompartmentally derived from the estimated individual parameter estimates. Target AUC_{0-24} was 8.9 mg×h/l, the AUC_{0-24} that is obtained in adults after once-daily administration of 300 mg [2]. Based on the results, a dose adaptation was proposed for subgroups of children not reaching the target AUC_{0-24}.

4.3.6 Software

The pharmacokinetic analysis and evaluation procedures were performed using the nonlinear mixed-effects modelling software NONMEM version 7.3 (Icon Development Solutions, Hanover, MD). Tools like PsN version 4.2.0 [35] (University of Uppsala, Sweden), Pirana version 2.9.0 (Pirana Software & Consulting BV, Amsterdam, the Netherlands) and R version 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria) were used to visualise and evaluate the models. For the NPDE analysis, the NPDE software package in R was used [34].

4.4 Results

4.4.1 Population pharmacokinetic model building

Model building was based on 638 observations from 85 children while external evaluation was based on 1 423 observations from 101 children

(Table 4.1). A two-compartment model with sequential zero-order and first-order absorption best described the data. A two-compartment model was preferred over a one-compartment model, since the model building data, and especially the highest concentrations, were more accurately described with a two-compartment model. The final model was parameterised in terms of a zero-order absorption phase (D1), which was followed by a first-order absorption process (K_a) (i.e. sequential zero-order and first-order absorption model), apparent clearance (CL/F), inter-compartmental clearance (Q/F) and the apparent volumes of distribution of the central compartment (V_2/F) and peripheral compartment (V_3/F). Because there were difficulties estimating reliable values for V_3/F, the model was simplified by equalising V_3/F to V_2/F. The residual variability was best described using a combined additive and proportional error model.

4.4.2 Systematic covariate analysis

The covariate analysis identified bodyweight as the most important covariate for both CL/F and V_2/F. The exponent for the effect of bodyweight on CL/F was 0.506 (20.2%) and for the effect on V_2/F 0.489 (32.3%). The parameter estimates for the simple and final model are shown in Table 4.2. In Figure 4.1, the individual estimates of variability (ETA) of CL/F and V_2/F are plotted against bodyweight for the simple and final models. A significant part of the inter-individual variability is explained after inclusion of bodyweight as a covariate, with a decrease of 19% in the inter-individual variability of CL/F and 15% in the inter-individual variability of V_2/F (Table 4.2). After inclusion of bodyweight, no other covariates (i.e. age, height or formulation) could be identified ($P > 0.05$).

4.4.3 Internal evaluation of the final pharmacokinetic model

Table 4.2 gives an overview of the parameter estimates of the simple and final model, together with the values obtained from the bootstrap analysis. The median estimated values based on the bootstrap were within 10% of the values obtained in the final model. In Figures 4.2a and 4.2b, observed versus individual and population-predicted concentrations are given for the final model, while in Figure 4.2c a histogram of the NPDE is shown. No trend was seen in the NPDE versus time or versus predicted concentrations (results not shown). Figure 4.5 shows that the data in different weight groups are well described.

Table 4.2: Population parameter estimates of the i) simple and ii) final pharmacokinetic model based on two model building datasets, iii) the values obtained after bootstrap of the final pharmacokinetic model and iv) the parameter estimates after combining the model building data with the two external datasets

Parameter	Simple pharmaco-kinetic	Final pharmaco-kinetic	Bootstrap final pharmacokinetic model	Model building data and external data
Fixed effects				
$CL/F_{16.6\,kg}$ (l/h)	13.2 [4.8]	13.2 [4.2]	13.2 [12.1–14.3; 4.3]	13.1 [2.8]
θ in $CL/F_{16.6\,kg}$ $\times(BW/16.6)^{\theta}$		0.506 [20.2]	0.524 [0.298–0.716; 21.9]	0.372 [18.6]
$V_2/F_{16.6\,kg} = V_3/F_{16.6\,kg}$ (l)	37.0 [7.6]	38.9 [7.0]	37.8 [30.3–43.9; 8.4]	36.6 [6.8]
θ in $V_2/F_{16.6\,kg}$ $\times(BW/16.6)^{\theta}$		0.489 [32.3]	0.531 [0.127–0.836; 34.9]	0.581 [19.8]
Q/F (l/h)	2.09 [17.7]	2.02 [13.4]	2.14 [1.53–3.76; 24.1]	2.65 [11.3]
D1 (h)	0.697 [15.1]	0.847 [10.3]	0.823 [0.493–1.05; 16.1]	0.655 [9.7]
K_a (h^{-1})	2.47 [11.5]	3.41 [17.3]	3.16 [1.56–6.78; 37.3]	1.73 [12.1]
Inter-individual variability				
ω^2 (CL/F)	0.468 [7.5]	0.379 [7.9]	0.374 [0.319–0.436; 16.3]	0.336 [6.4]
ω^2 (V_2/F)	0.645 [6.9]	0.550 [9.0]	0.559 [0.467–0.686; 19.7]	0.435 [7.2]
Omega block (CL/F – V_2/F)	0.848	0.820	0.812 [0.797–0.813]	0.718
ω^2 (D1)	0.815 [10.7]	0.704 [10.5]	0.721 [0.560–0.986; 29.6]	0.796 [9.0]
Residual variability				
σ^2 (proportional)	0.133 [9.2]	0.147 [9.3]	0.144 [0.118–0.174; 19.5]	0.301 [5.2]
σ^2 (additive)	0.0477 [10.9]	0.0450 [11.4]	0.0443 [0.0340–0.0542; 22.2]	0.088 [27.9]

Data presented as value [%RSE]; bootstrap results presented as median [95%CI; %RSE (sd/mean)]; θ: parameter of interest; ω^2, σ^2: variance, BW: bodyweight; CI: confidence interval; CL/F: apparent clearance for a typical individual with BW of 16.6 kg; D1: duration of zero-order absorption; K_a: rate constant of first-order absorption; Q/F: inter-compartmental clearance; $V_2/F_{16.6}$: volume of distribution of the central compartment for a typical individual with BW of 16.6 kg; $V_3/F_{16.6}$: volume of distribution of the peripheral compartment for a typical individual with BW of 16.6 kg
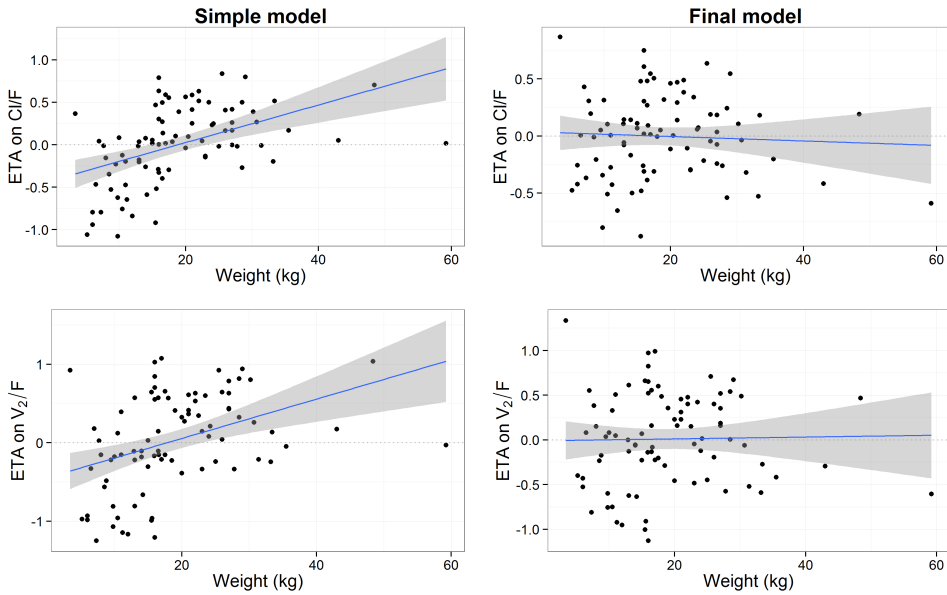
Figure 4.1: Inter-individual variability (*dots*) for the simple (left) and final model (right) for apparent clearance (ETA on CL/F) and apparent volume of distribution (ETA on V_2/F) versus weight (two model building datasets) with trendline (*blue line*) and 95% confidence interval (*grey area*).

4.4.4 External evaluation of the final pharmacokinetic model

The predictive performance of the final model was evaluated using two external datasets (Table 4.1) [8, 14, 15, 27]. In Figure 4.2, observed versus individual predicted concentrations (Figures 4.2d and 4.2g) and observed versus population-predicted concentrations (Figures 4.2e and 4.2h) are given for both external datasets. Additionally, the histograms of the NPDE are shown in Figures 4.2f and 4.2i. While the final model is able to predict the data in external dataset 2 without bias, a slight bias is seen for external dataset 1, in which the sampling was more sparse, compared to the model building datasets. This bias is observed in Figure 4.2e, which shows observed versus population predicted concentrations, as well as in Figure 4.2f.

Combined analysis of the two model building datasets and both external datasets revealed that fairly similar parameter values were obtained (Table 4.2). The concentrations in all four datasets could be well described by this model without bias and with adequate precision (Figure 4.6).

4.4.5 Evaluation of currently used dosing guidelines

94.5% of the children with a bodyweight above 14 kg reached the adult target AUC_{0-24} of 8.9 mg×h/l with the currently administered daily dose. However, this did not hold for all children with a bodyweight below 14 kg (Figure 4.3). If the daily dosage for these children is increased to at least 10 mg/kg/day, it is expected that most children will have adequate exposure to lamivudine (64.2% before dose adaptation, 92.5% thereafter; Figure 4.4).
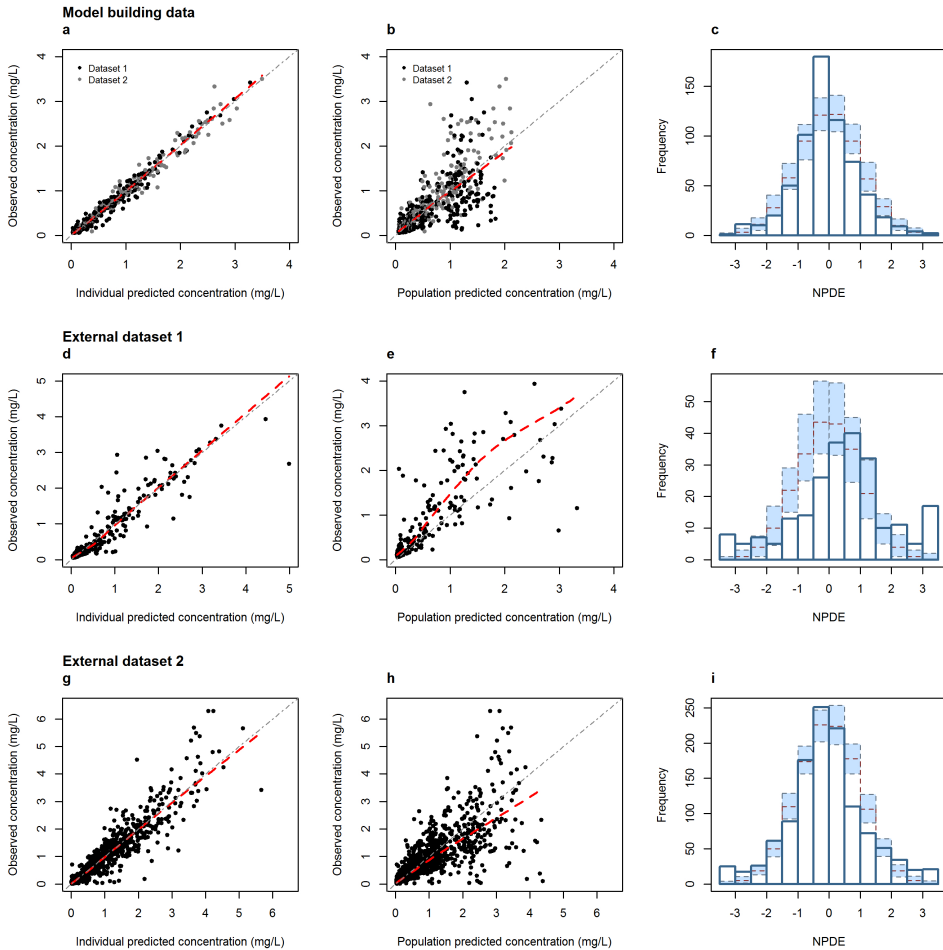
Figure 4.2: Observed versus individual predicted concentrations and observed versus population predicted concentrations of (a–b) the two model building datasets, (d–e) external dataset 1 and (g–h) external dataset 2. The histograms show the distribution of the normalized prediction distribution error (NPDE) of the (c) model building datasets, (f) external dataset 1 and (i) external dataset 2.
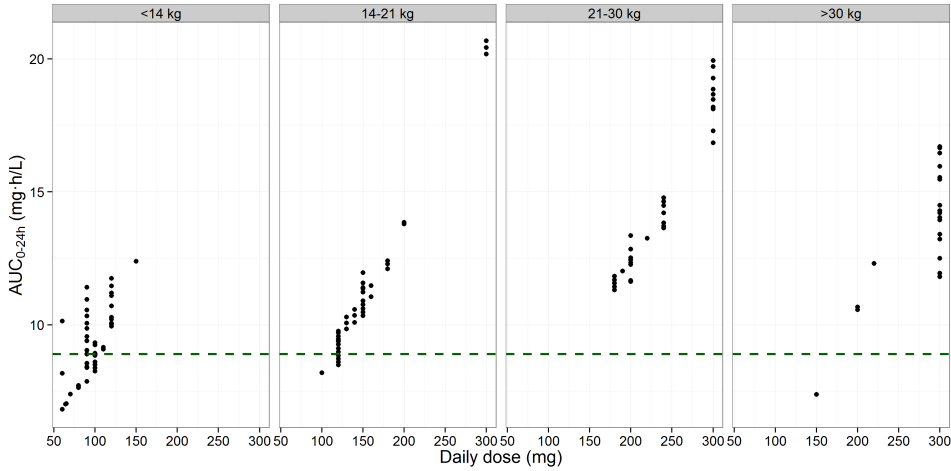
Figure 4.3: Simulated AUC_{0-24} versus daily dose administered (mg) split by bodyweight: $\leq$ 14, 14–21 kg, 21–30 kg and > 30 kg. The dotted line indicates an AUC_{0-24} of 8.9 mg×h/l (adult target for once-daily dosing). Vertically occurring sequences of dots occur because of fixed dose tablets.
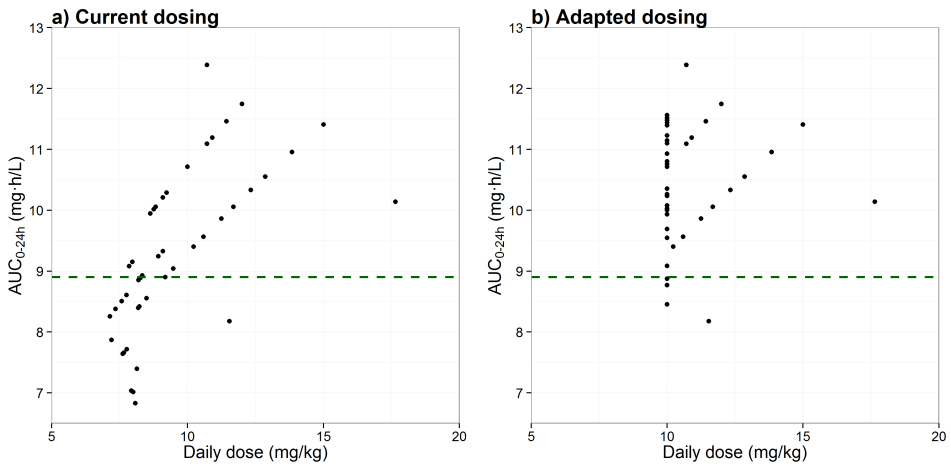
Figure 4.4: Simulated AUC_{0-24} versus daily dose administered (mg/kg bodyweight) for children with a bodyweight $\leq$ 14 kg after administered dose (a) and adapted dose where a minimum of 10 mg/kg is administered (b). The dotted line indicates an AUC_{0-24} of 8.9 mg×h/l (adult target for once-daily dosing).

4.5 Discussion

A model-based approach has been applied in order to describe the pharmacokinetics of lamivudine in children. The model was based on a large and relatively rich dataset, since for most of the children at least six samples within one dosing interval were available. Also, the full paediatric age range is covered, with a large proportion of children below the age of 3 years ($n = 16$ (18.8%) in the dataset used for model building; $n = 41$ (22.8%) in total). The two-compartment model used to describe the data is in agreement with previously developed models [7, 16, 20, 36]. In order to obtain reliable estimates of the peripheral volume, the model had to be simplified by stating that the central and peripheral volume of distribution were equal to each other (Table 4.2), however this did not lead to reduced descriptive or predictive value (Figures 4.2 and 4.5).

Bodyweight best predicted the developmental changes in both apparent lamivudine clearance and apparent central volume of distribution. This is consistent with previous studies [7, 16–20]. Although the typical parameter estimates for an individual of 16.6 kg are comparable, the estimations of both scaling exponents were lower in our analysis [7, 16, 18–20, 37]. Remarkable is the difference in the relationship between apparent volume of distribution and bodyweight. The majority of the performed studies fixed this relationship a priori to 1 [7, 16, 18–20]. Piana et al. found an exponent of 0.635, which seems close to the exponent of 0.489 we identified (Table 4.2) [17].

The stability of the final model was indicated by the NPDE and the bootstrap as well as the ability to predict external dataset 2 accurately. For external dataset 1, the predictive performance was slightly biased. This may be explained by the sparse nature of the data available in that dataset. When the data from all datasets were combined and analysed together, the data in external dataset 1 were described without any bias (Figure 4.6).

The target AUC_{0-24} of 8.9 mg×h/l that has been identified in adults was reached in 85.6% of the children. However, 35.8% of the children with a bodyweight below 14 kg did not reach this target. It was shown previously that lamivudine exposure was lower in the youngest group of children compared to older and heavier children [7–9, 37]. For children with a bodyweight below 14 kg we calculated that the target AUC_{0-24} can be reached with a dose of at least 10 mg/kg/day, based on the expected apparent clearance. The same dose was also proposed by Bouazza et al. for children with a bodyweight < 17 kg [7]. We chose our cut-off bodyweight in line with the approved dosing regimens of the antiretroviral drugs frequently

used in children and with the data found in our model [1].

In the model, the absorption phase was relatively difficult to describe, which can partly be explained by the limited data available for that part of the concentration-time profile. In most paediatric population pharmacokinetic models for lamivudine, a first-order absorption model is used [7, 16, 18, 19, 36]. However, also a delay in absorption with either a lag-time or transit compartments has been described [20, 37]. During model building, all these absorption models, as well as a zero-order absorption, were tested. A sequential zero- and first-order absorption model was finally found to best describe the data.

A limitation of the current study is that we could not fully study the influence of the formulation on the pharmacokinetics. As shown before, the type of drug formulation can affect the lamivudine exposure significantly in children [4, 5, 19, 24]. However, similar to previous population pharmacokinetic studies, the formulation used by the children could not be identified as a possible covariate in our study as information on the formulation used was not complete for all of the children [16, 37, 38]. Next to this, we could not study the influence of renal function in this analysis. Lamivudine is a renally excreted drug and it has been shown in adults that renal function can affect the pharmacokinetics of lamivudine [2, 3, 39–41]. Even though in several paediatric studies, serum creatinine could not be identified as possible covariate for clearance, we could not study this covariate as information on renal function was incomplete [16, 18, 19].

In conclusion, lamivudine pharmacokinetics was best described by a two-compartment model with sequential zero-order and first-order absorption. Bodyweight was found as covariate on apparent clearance and apparent central volume of distribution, both in a nonlinear function. The model generalizes well to patients not included in the model-building dataset. In order to identify whether these (nonlinear) changes result from changes in bioavailability, clearance and/or volume of distribution, future analysis, which includes intravenously administered lamivudine, is warranted. The results of this study suggest that the currently recommended dose for children with a bodyweight below 14 kg should be increased to at least 10 mg/kg/day in order to reach an AUC_{0-24} of 8.9 mg×h/l.
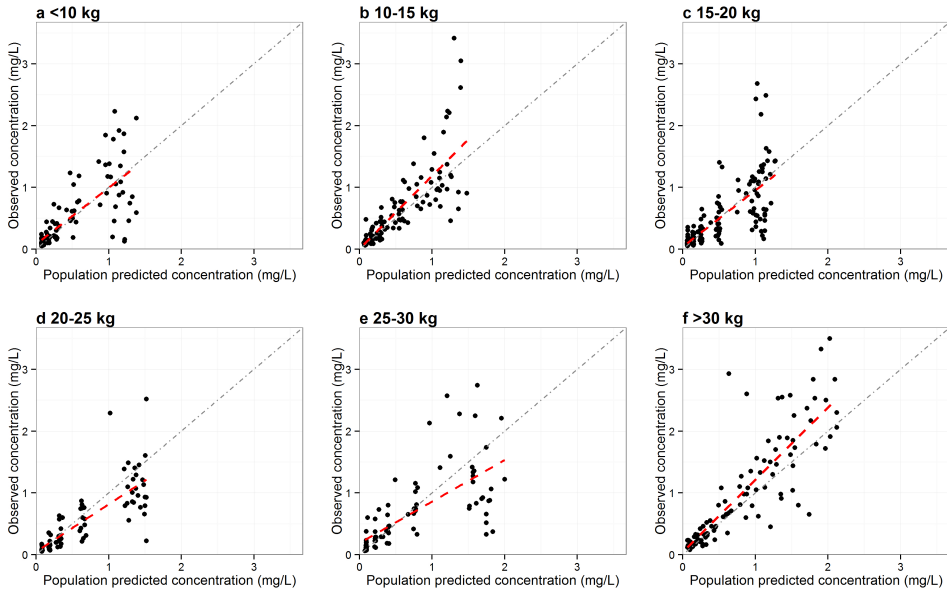
Figure 4.5: Diagnostic plots (observed versus predicted concentrations in the model building data) of the final model split by bodyweight: a: < 10 kg, b: 10–15 kg, c: 15–20 kg, d: 20–25 kg, e: 25–30 kg, f: > 30 kg. Dots indicate the observed concentration versus population predicted concentration, grey dotted lines show $x = y$, red striped lines show trend line.
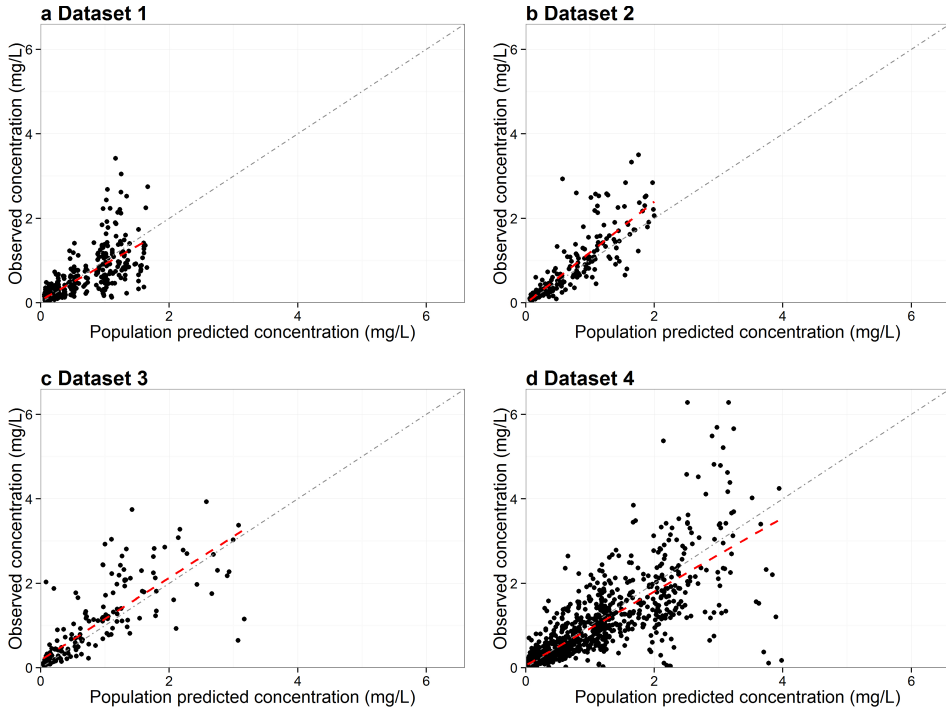
Figure 4.6: Diagnostic plots of the model based on both the two model building and the two external datasets: observed versus population predicted concentrations split by dataset: a: dataset 1, b: dataset 2, c: dataset 3, d: datset 4. Dataset 1 and 2 have been used for model building, datset 3 and 4 for external validation. Dots indicate the observed versus population predicted concentration, grey lines show $x = y$, red lines show trend line.

Competing interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: CK had support from NWO and TI Pharma for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous 2 years; no other relationships or activities that could appear to have influenced the submitted work.

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Part 2   Paediatric formulations

5 The role of formulation on the pharmacokinetics of antiretroviral drugs

Diane E.T. Bastiaans, Tim R. Cressey, Herman Vromans, and David M. Burger

Abstract

Introduction

A multitude of antiretroviral drug formulations are now available for HIV-infected adults and children. These formulations include individual and co-formulated drugs, many of which are also supplied in generic versions. Many antiretroviral drugs have a low aqueous solubility and poor bioavailability. Drug formulation can significantly affect bioavailability, and given the increasing number of new formulations and drug combinations, it is important to be aware that formulation can influence the pharmacokinetics of antiretroviral drugs.

Areas covered

This review provides an overview of studies assessing the pharmacokinetics of different antiretroviral drug formulations in adults and children, including fixed-dose combinations. For some antiretroviral drugs, differences in pharmacokinetics have been described, with largest differences in exposure when a liquid formulation is compared to a tablet or capsule formulation. Biopharmaceutical properties of antiretroviral drugs relevant to bioavailability are discussed.

Expert opinion

Antiretroviral drug formulations and their excipients can significantly impact drug exposure. However, this is not yet fully recognized. It is important to realize that children use different formulations than adults. Effort should be made to ensure that adequate drug exposures are achieved to treat HIV-infected children. In addition, manipulation of drug formulations may lead to differences in pharmacokinetics.

5.1 Introduction

Globally, 35 million people were estimated to be HIV infected in 2012, of which 3.3 million were children under 15 years of age [1, 2]. The successful roll out of national and international antiretroviral treatment programs has dramatically reduced AIDS-related morbidity and mortality in many countries. Within these programs, a marketing application can be submitted for antiretroviral drugs even if they are still under patent or market protection. Despite the rapid scale up of antiretroviral treatment worldwide, only 34% of the children eligible for antiretroviral treatment were receiving it in 2012, compared to 64% for HIV-infected adults. The 2013 WHO treatment guidelines now recommend treating all HIV-infected children below the age of 5 years, independent of disease stage [3].

A combination of three different drugs from at least two different classes, often referred to as combination antiretroviral therapy, is generally used to treat HIV/ AIDS. Antiretroviral drugs are divided into different classes based on their mechanism of action. Current drug classes are: i) nucleoside reverse transcriptase inhibitors (NRTIs); ii) non-nucleoside reverse transcriptase inhibitors (NNRTIs); iii) protease inhibitors (PIs); iv) integrase inhibitors; and v) entry and fusion inhibitors. An overview of current (tentative) FDA-approved drug formulations for HIV treatment is shown in Table 5.1. In children, the pharmacological goal is to achieve similar drug exposure to adults [4]. The pharmacokinetics of some antiretroviral drugs in children is different to adults, primarily due to differences in the maturation of metabolic and renal pathways [5]. Patient characteristics, such as age, weight, renal function and host genetics, can explain a large portion of the interpatient variability observed, but drug formulation can also be a key component.

The low aqueous solubility and poor bioavailability of many antiretroviral drugs have been challenging for the development of antiretroviral drug products, particularly for pediatric formulations. Drug formulations are normally investigated in healthy adults, and it is generally assumed that any formulation effect would be the same in children. However, it is possible that a formulation can potentially behave differently in adults than in children, for example because of age-related differences in solubility and dissolution rate of a drug from a formulation [6]. Also, children are often not using the same formulation as adults and different exposures may be due to differences in bioavailability of the child-friendly formulation available compared to the adult formulation. In addition, excipients used to develop a (pediatric) formulation can influence the pharmacokinetics of drugs [7–9].

Sometimes, in order to administer drugs to children, adult formulations are manipulated, that is, crushed or split and this can affect the rate and extent of drug absorption.

This review aims to provide an overview of studies assessing the pharmacokinetics of different antiretroviral formulations that are currently marketed, including generic drugs. A literature search was performed using the PubMed, EMBASE and Cochrane databases (until March 2014). Combinations of the following words and variations on these words were used in the search strategy (title, abstract, mesh): 'pharmacokinetics' and 'antiretroviral therapy' and 'bioequivalence' or 'child.' Studies in both HIV-infected and -uninfected participants, published in English, were included. Conference abstracts and posters were not included. Latest drug label information was accessed through the US FDA website.

5.2 Bioavailability of drug formulations

It is important to determine whether a different type of formulation that is, tablet versus liquid, or a new generic drug formulation provides similar exposure compared to the innovator product. Drug formulations containing the same active pharmaceutical ingredient are considered 'bioequivalent' if the ratio of the bioavailability (rate and extent of absorption) after administration of the two formulations in the same molar dose falls within preset limits [10, 11]. Pharmacokinetic parameters analyzed to assess bioequivalence are the area under the concentration time curve (AUC), as a measure of the extent of exposure, and the maximum plasma concentration ($C_{\max}$), which is influenced by the rate of absorption. For both pharmacokinetic parameters, the 90%CI for the ratio of the test and reference products should be within the acceptance interval of 80–125%.

The rate and extent of absorption from the gastrointestinal tract can be influenced by the dissolution rate, solubility and permeability of the drug. Many physiological factors are also important for absorption, such as pH and gastrointestinal transit time. A drug must be dissolved before it can be absorbed. Physicochemical characteristics, such as pKa and lipid solubility, determine the solubility and permeability of a drug [12]. The biopharmaceutics classification system (BCS) is used by regulators to determine whether in vivo bioequivalence studies are necessary for the approval of new generic drug products of solid immediate release dosage forms [10,11,13,14]. According to this system, the active pharmaceutical ingredient of a formulation can be classified into one of four categories depending on its aqueous

solubility and intestinal permeability, where solubility is based on the highest dose strength of the innovator product (to be) approved. The BCS class of antiretroviral drugs is shown in Table 5.2 [14–19]. Theoretically, it is expected that the influence of the formulation on pharmacokinetics will be largest for drugs with low solubility.

Excipients can affect the pharmacokinetics of drugs [7–9]. For example, bioavailability can be influenced by an altered gastrointestinal transit time, for example, by polyethylene glycol, sugars and sweeteners such as sorbitol, possibly related to their osmotic and/or viscosity-enhancing effect [7,9,20]. Excipients can also change transporter-mediated uptake and efflux of drugs [8]. Antiretroviral drugs, even within the same antiretroviral drug class, can be substrates for different drug transporters [21]. The impact of drug transporters on antiretroviral drug disposition is reflected in the Biopharmaceutics Drug Disposition Classification System (BDDCS), as suggested by Wu and Benet (Table 5.2) [22,23].

5.3   Antiretroviral drug formulations

5.3.1   NRTI formulations

NRTIs are categorized in BCS class 1 (high solubility, high permeability) or class 3 (high solubility, low permeability). Most of the NRTIs have both solid and liquid formulations licensed. Despite the good solubility in water, abacavir, emtricitabine and lamivudine liquid formulations use propylene glycol as an organic co-solvent. Propylene glycol is an unfavorable excipient for children because of its potential toxic effects, especially for infants [24]. A maximum allowed daily intake of 25 mg/kg is recommended by the FDA, which is exceeded when abacavir is dosed according to the label.

5.3.2   NNRTI formulations

All NNRTIs have a very low solubility in water and are categorized as either BCS class 2 (low solubility, high permeability) or class 4 (low solubility, low permeability). The low solubility of NNRTIs is also reflected in the composition of the liquid formulations of efavirenz and nevirapine: efavirenz liquid formulation contains medium chain triglycerides as a solvent and nevirapine is formulated as a suspension.

5.3.3 Protease inhibitor formulations

All PIs are either BCS class 2 or 4 drugs. The low aqueous solubility is reflected in the available liquid formulations being either a suspension (darunavir), powder for suspension (nelfinavir) or solution containing organic co-solvents, such as propylene glycol (fosamprenavir, tipranavir), or propylene glycol combined with ethanol (lopinavir/ritonavir, ritonavir). Most PIs are licensed in combination with low doses of ritonavir due to the more favorable pharmacokinetic profile. Administration of the ritonavir liquid formulation is challenging, particularly in children, because of its poor palatability. The licensed doses of fosamprenavir and tipranavir do not exceed the maximum recommended daily intake of propylene glycol, but combined with ritonavir these limits are exceeded. Lopinavir is co-formulated with ritonavir. The innovator lopinavir/ritonavir oral solution contains 15.3% propylene glycol and 42.2% alcohol. Using the dosing recommendations in the label, the amount of propylene glycol exceeds the recommended maximum daily intake. The risk of toxicity may also be increased because of the inhibition of propylene glycol metabolism by the ethanol present in the solution. Significant clinical toxicity has been observed in neonates after use of lopinavir/ritonavir liquid and has been related to the high concentrations of these excipients [25].

5.3.4 Integrase inhibitor formulations

All integrase inhibitors are classified as BCS class 2 or 4. Next to tablets, only for raltegravir a granule formulation for oral suspension is currently available.

5.3.5 Entry and fusion inhibitor formulations

Together with the integrase inhibitors, the entry and fusion inhibitors are the newest classes of antiretroviral drugs. The only entry inhibitor approved is maraviroc, which is categorized as a BCS class 3 drug. Only tablet formulations of maraviroc are available. The fusion inhibitor enfuvirtide is administered subcutaneously, and no BCS class has been determined.

5.3.6 Fixed-dose combination formulations

Antiretroviral therapy is currently a lifelong treatment. Combining different antiretroviral drugs into a single formulation, termed fixed-dose combinations (FDC), has been one of the strategies to reduce pill burden and

improve long-term adherence. To stimulate the development and availability of antiretroviral drug products, a guidance on FDC was released by the FDA in 2006 [26]. To date, both dual and triple innovator and/or generic FDC have been approved (Table 5.1). Adult and pediatric FDC formulations have been developed. Many of these formulations have been developed by generic pharmaceutical companies specifically for resource-limited settings.

5.4   Comparing the bioavailability of antiretroviral drug formulations

5.4.1 Studies comparing liquid and solid antiretroviral drug formulations

Intestinal transit time of liquid and solid formulations can differ, and it takes time for solid dosage forms to disintegrate into smaller particles, from which the drug can dissolve easier. For this reason, it is generally expected that the absorption rate of a liquid will be higher compared to a solid formulation. However, it is easier to manipulate solid dosage forms to aid in vivo solubility, for example by reducing particle size, changing the salt or crystal form of the drug and use of excipients. Thus, in some instances bioavailability can be higher for solid compared to liquid formulations, particularly for BCS class 2 and 4 drugs.

Liquids are commonly used for pediatric formulations but have several disadvantages over solid formulations [27]. For example, it can be challenging to develop liquid formulations that are palatable and have adequate stability. Subsequently, more excipients have to be used, for example, for preservation, to mask bad taste and sometimes increase solubility. As described in Section 5.2, excipients can influence the pharmacokinetics of drugs.

Several studies have evaluated the pharmacokinetics of solid versus liquid formulations, and most of the studies published were performed in HIV-infected children. An overview of these studies is shown in Table 5.2. Studies on efavirenz, emtricitabine, lamivudine and raltegravir show differences in bioavailability between solid and liquid formulations and will be further discussed below.

5.4.1.1 Efavirenz

The oral efavirenz solution is less bioavailable than the hard capsule on an mg per mg basis (the liquid formulation is approved by the EMA but

not the US FDA) [28]. In healthy adults, the efavirenz AUC and $C_{\max}$ following a 240 mg dose of the liquid were 97 and 78%, respectively, of the values following a 200 mg dose with the hard capsules. Therefore, 20–35% higher doses are recommended when efavirenz is administered with the liquid formulation. A population pharmacokinetic study in HIV-infected children reported that the bioavailability of the liquid formulation was more than 50% lower than the capsule formulation [29]. A pharmacometric model also suggested a lower relative bioavailability of two liquid formulations compared to the capsule [30]. In addition, this model estimated a different relative bioavailability for children of different ages. For children 1 year of age, the relative bioavailability was estimated 42%, for 3 years of age 61% and reaching 90% of the mature value at the age 8 years.

5.4.1.2 Emtricitabine

The pharmacokinetics of a liquid and capsule formulation of emtricitabine has been assessed in HIV-infected adults and children 6–17 years old [31, 32]. In children, the AUC and $C_{\max}$ were higher after administration of the capsule compared to the solution. The relative bioavailability of the capsule was 120% compared to the liquid formulation. A shorter gastrointestinal transit time of the solution compared to the capsule, resulting in a reduced mucosal contact time, was proposed as possible explanation for the observed difference. Excipients used in the liquid formulation might also be able to influence absorption of emtricitabine. The authors noted no meaningful statistical comparison was possible between the formulations due to the small sample size and lack of randomization. A higher maximum dose for emtricitabine solution (240 vs. 200 mg with the tablet) is approved [4, 32].

5.4.1.3 Lamivudine

Numerous studies have assessed the pharmacokinetics of the lamivudine oral solution and/or tablet formulations in adults and children [33–40]. Comparable bioavailability of the oral formulations has been reported in adults [40]. In contrast, several recent studies in HIV-infected children have not found the same result. One study evaluated the bioequivalence of the innovator liquid solutions of zidovudine, lamivudine and abacavir to the innovator tablets in HIV-infected children (ARROW trial) [35]. Exposure to lamivudine was significantly higher with the co-formulated lamivudine/zidovudine tablet compared to the liquid formulation. The dose-normalized lamivudine AUC, $C_{\max}$ and minimum plasma concentra-

tion (C_{min}) were 58, 55 and 29% higher, respectively. Of note, lamivudine was administered alone in the adult study while in the ARROW trial the liquid formulation of lamivudine was administered together with zidovudine and abacavir liquid formulations. Therefore, it is possible that excipients from the different liquid formulations interact and influence drug absorption. For example, all three liquids contain sugars, sweeteners and/or sugar alcohols, which could possibly impact gastrointestinal transit time. No significant differences in bioavailability of zidovudine or abacavir were observed. Using the BDDCS classification, lamivudine is classified as class 3 and both abacavir and zidovudine are classified as class 1 (Table 5.2) [22]. Theoretically, the influence of transporters on absorption processes is important for lamivudine, but only minimal for abacavir and zidovudine [23]. Thus, another possible explanation could be an influence of excipients on transporter-mediated absorption of lamivudine.

Lower lamivudine exposure was also found with the innovator liquid formulation compared to generic pediatric FDC tablets containing lamivudine in children [33, 34]. These pediatric generic FDC tablets containing lamivudine were found to be an independent predictor of lamivudine bioavailability in a pooled population pharmacokinetic model, including both innovator solid and liquid formulations [39].

In contrast to these findings, a study showed bioequivalence of a pediatric generic FDC tablet (lamivudine, stavudine, nevirapine) compared to the individual innovator and generic liquid formulations in HIV-infected children [41]. Three studies also showed bioequivalence of different pediatric FDC granules/tablet for oral suspension containing lamivudine, compared to the individual liquid formulations in healthy adults [42–44]. For one study, the excipients of the granules were described, which included xylitol and sucralose [42]. Population pediatric pharmacokinetic models developed using data from HIV-infected children receiving the liquid or solid formulation of lamivudine also did not find formulation to be a predictor of lamivudine concentrations [36, 37, 45]. No information on co-medication was provided, and it was not known how many children used either the tablet or liquid formulation.

5.4.1.4 Raltegravir

The pharmacokinetics of raltegravir after administration of the different pediatric formulations has been studied in healthy adults [46]. Compared to the film-coated tablet, the granules for suspension had a 2.6-fold higher $AUC_{0-\infty}$ and a 4.6-fold higher C_{max}.

5.4.2 Studies comparing solid antiretroviral drug formulations

Several of the available antiretroviral drugs have different solid formulations available, for example, both a capsule and a tablet. The number of generic antiretroviral drugs available has rapidly increased over the last five years: over 160 generic formulations were (tentatively) approved by the US FDA at the beginning of 2014. Studies in which bioequivalence or comparable pharmacokinetics has been shown of solid antiretroviral drug formulations are summarized in Table 5.3. Studies showing differences in bioavailability between solid formulations are discussed below.

5.4.2.1 Didanosine

Didanosine is unstable in acidic solutions. During the drug development phase, the pharmacokinetics of buffered tablets, enteric-coated tablets, sachets and an intravenous solution were assessed [47]. Large differences in bioavailability between formulations were observed. The first approved didanosine formulations contained a buffer that increases the risk of drug-drug interactions. Encapsulated enteric-coated beads were developed to try and overcome this problem, but were not bioequivalent to the buffered tablet formulation in healthy adults and HIV-infected subjects ($C_{\max}$ of the enteric-coated bead formulation was 42% lower in healthy subjects and 36% lower in HIV-infected individuals) [48, 49].

5.4.2.2 Lopinavir and ritonavir

Lopinavir co-formulated with ritonavir (lopinavir/r) is available as tablets, soft gel capsules and oral solution. A qualitative and quantitative analysis was performed on a generic tablet, which was not prequalified by WHO [50]. The tablets contained comparable amounts of both lopinavir and ritonavir as the innovator tablet. However, a small study in four healthy adults showed that the median lopinavir trough level of the generic tablets was substantially lower compared to the innovator tablets: 158 ng/ml compared to 3884 ng/ml.

5.4.2.3 Nelfinavir-mesylate

A 625 mg tablet of nelfinavir mesylate was developed to reduce the pill-burden of the 250 mg tablet. Bioequivalence of these two tablets in healthy adults was shown under fed conditions but not under fasting conditions, with the 625 mg tablet having a 27% lower AUC [51]. It is unclear whether a different tablet formulation has eventually been

marketed, since the data presented in the label information report a 34 and 24% higher AUC for the 625 mg tablet compared to the 250 mg tablet under fasting and fed conditions, respectively, in healthy adults.

5.4.2.4 Raltegravir

The film-coated and chewable tablet formulation of raltegravir are not bioequivalent [46, 52]. Compared to the film-coated tablet, the chewable tablet had a 1.8-fold higher $AUC_{0-\infty}$ and a 3.2-fold higher $C_{\max}$ in healthy adults [46].

5.5 Manipulation of antiretroviral drug formulations

If no pediatric formulations are available, adult formulations are sometimes manipulated and administered to children, that is, tablets are cut or split to achieve a lower dose, and sometimes also crushed to ease administration. Deviations from the optimal dose can occur when splitting or crushing tablets, which can significantly impact drug efficacy and/or toxicity. Only five studies were found in which the effect on the pharmacokinetics of antiretroviral drugs of manipulation of the formulation was investigated. These studies are summarized in Table 5.4, and studies in which bioequivalence was not shown are further discussed below [53–57].

5.5.1 Efavirenz, emtricitabine and tenofovir DF

In an attempt to facilitate the oral administration of the single FDC tablet of efavirenz, emtricitabine, tenofovir DF a liquid compounded out of the crushed innovator tablet formulation was tested [54]. In healthy adults, only emtricitabine was found to be bioequivalent. Exposure parameters for tenofovir were higher after administration of this liquid formulation: AUC 20% higher and $C_{\max} \sim 40\%$ higher. Exposure to efavirenz was highly variable, and the 90%CI criteria were not met for this drug. These findings are in line with the need to develop a bilayer film-coated FDC tablet to achieve bioequivalence to the separate innovator formulations [58].

5.5.2 Lopinavir and ritonavir

Although not recommended, the co-formulated lopinavir/ritonavir tablets are sometimes broken or crushed. Lopinavir exposure was decreased in HIV-infected children after crushing 200/50 mg tablets: AUC decreased by 45% and $C_{\min}$ by 33% [56]. To attempt to reduce the pill count and

overcome the need of refrigeration of the soft gel capsules, melt-extrusion technology has been used to obtain a solid dispersion of lopinavir and ritonavir in a polymer, which is further processed into tablets [12,59]. This solid dispersion is a system in which a solubilizing polymer gives rise to an optimal transfer of the drug from the formulation into the aqueous environment of the gastrointestinal tract. Crushing the tablets results in disruption of this system and bioavailability can thus be decreased.

5.6 Expert opinion

The composition of a drug formulation can be indicative for some of the biopharmaceutical properties of the active pharmaceutical ingredient. The influence of formulation due to drug-excipient interactions is not yet fully understood nor recognized and described for only a small number of excipients [7–9]. The possible influence of excipients is even more complicated when different formulations are used together. The exact impact the formulation can have on the pharmacokinetics of an individual antiretroviral drug is sometimes difficult to predict, even for drugs within the same antiretroviral drug class.

Bioequivalence studies for regulatory purposes are generally performed in healthy adults and not in HIV-infected subjects, or children. Developmental changes affecting the drug absorption process could result in differences in the rate and extent of drug absorption. More research is needed to fully understand the ontogeny of drug absorption [6,60]. Whether bioequivalence criteria are met for different formulations can also depend on the meal conditions under which it is studied. The influence of food on the absorption of drugs is of extra importance for children for several reasons: food can be used to ease the administration of drugs, for example to mask bad taste of the drug and intake with food can reduce side effects.

Variability in exposure has been found after administration of liquid and solid formulations of drugs in several BCS classes. The majority of published studies comparing solid and liquid formulations have been performed in children. The BCS classification is based on adult dosing and physiological data, and extrapolation to children needs to be done with caution. In this light, the role of the dose/solubility ratio is of extra importance, since children receive different doses than adults. Drugs that show a certain dose/solubility ratio in adults cannot be assumed to show the same ratio in pediatric patients [61]. It is debated how the BCS should be adjusted to be applied to children [6]. A 'BCS-shift' to a lower solubility class, because of higher dose/solubility ratio in children, might

partly explain observed differences in exposure between adults and children. A dose-dependent BCS has been proposed, which could be applied to drugs used in children [62, 63].

The observed lower exposure to lamivudine after administration of the liquid is concerning, and more research is warranted in children. These results have not yet been included in (pediatric) guidelines [3, 4, 64].

Almost all published results of studies in healthy adults where a generic single-drug formulation was compared to the innovator product showed that formulations were bioequivalent. More problems, although mostly minor, are seen with generic FDC formulations. Unfortunately, only a small proportion of data from in vivo bioequivalence trials are published and could thus be found.

Only a few studies were found in which the effect of manipulating solid formulations was investigated. It is important to know whether exposure is still adequate, which might be a problem when for example lopinavir/r tablets are crushed, or exposure is too high, which could lead to more adverse events. This is relevant for children, but also for HIV-infected patients who (temporarily) are not able to swallow solid drug formulations and crushed formulations need to be administered via a feeding tube.

Several bioequivalence studies included only a small number of subjects, herewith limiting the power of the results found. For some of the studies, this was also reflected in the large variability of the calculated pharmacokinetic parameters. Studies in which population pharmacokinetic models are developed not always include formulation as possible covariate. When formulation has been investigated and included as covariate in a population pharmacokinetic model, this needs to be confirmed in a bioequivalence study.

The role of formulation is also important on the adherence to antiretroviral treatment. Long-term compliance is essential to reduce the risk of selecting drug-resistant viruses. Important issues to improve adherence are related to the formulation, such as palatability, pill size, liquid volume, conspicuousness and use of FDC formulations [3, 65]. Effort must be made to facilitate the administration of antiretroviral treatment, to enhance treatment compliance and thus treatment outcome.

It is recognized that there still is a need for more and better antiretroviral formulations, especially for children, as reflected in the most recent WHO guideline and pediatric HIV as one of the focuses of the Drugs for Neglected Disease initiative. Developments in the use of nanotechnology, which can help to improve the dissolution and bioavailability of drugs, are promising

to develop better formulations of antiretroviral drugs.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Table 5.1: Antiretroviral drug formulations (tentatively) approved by the US FDA

	Formulation	Strength
Single-drug formulations		
Nucleoside reverse transcriptase inhibitors		
Abacavir	Tablets	300 mg (scored)
		60, 300 mg
	Solution	20 mg/ml
	Tablets for oral suspension	60 mg
Didanosine	Capsules, delayed release	125, 200, 250, 400 mg
	Powder for oral solution	2 and 4 g, to obtain 10 mg/ml suspension
	Tablets for oral suspension	100, 150, 200 mg
Emtricitabine	Capsules	200 mg
	Solution	10 mg/ml
Lamivudine	Tablets	150 (scored), 300 mg
	Solution	10 mg/ml
Stavudine	Capsules	15, 20, 30, 40 mg
	Powder for oral solution	1 mg/ml
Tenofovir	Tablets	150, 200, 250, 300 mg
(disoproxil fumarate)		300 mg
	Oral powder	40 mg/g
Zidovudine	Tablets	300 mg
		60, 100, 300 mg
	Capsules	100 mg
	Syrup	10 mg/ml
Non-nucleoside reverse transcriptase inhibitors		
Efavirenz	Tablets	600 mg
		50, 100, 200 (scored), 600 mg
	Capsules	50, 200 mg
		50, 100, 200 mg
	Liquid (only EMA approval)	30 mg/ml
Etravirine	Tablets	25 (scored), 100, 200 mg

Table 5.1: Antiretroviral drug formulations (tentatively) approved by the US FDA (continued)

	Name	Age approved from (FDA)
Single-drug formulations		
Nucleoside reverse transcriptase inhibitors		
Abacavir	Ziagen	3 months
	Generic	
	Ziagen	3 months
	Generic	
	Generic	
Didanosine	Videx	6 years
	Generic	
	Videx	2 weeks
	Generic	
	Generic	
	Generic	
Emtricitabine	Emtriva	3 months
	Generic	
	Emtriva	0 month
Lamivudine	Epivir	3 months
	Generic	
	Epivir	3 months
	Generic	
Stavudine	Zerit	Birth
	Generic	
	Zerit	Birth
	Generic	
Tenofovir	Viread	2 years
(disoproxil fumarate)	Generic	
	Viread	2 years
Zidovudine	Retrovir	4 weeks (birth, prophylactic)
	Generic	
	Retrovir	4 weeks (birth, prophylactic)
	Generic	
	Retrovir	4 weeks (birth, prophylactic)
	Generic	
Non-nucleoside reverse transcriptase inhibitors		
Efavirenz	Sustiva	3 months
	Generic	
	Sustiva	3 months
	Generic	
	Stocrin/Sustiva	3 years
Etravirine	Intelence	6 years

Table 5.1: Antiretroviral drug formulations (tentatively) approved by the US FDA (continued)

	Formulation	Strength
Nevirapine	Tablets	200 mg
	Tablet extended release	100, 400 mg
		400 mg
	Suspension	10 mg/ml
	Tablets for oral suspension	50, 100 mg
Rilpivirine	Tablets	25 mg
Protease inhibitors		
Atazanavir	Capsules	150, 200, 300 mg
		100, 150, 200, 300 mg
Atazanavir/ritonavir	Tablets	300/100 mg
Darunavir	Tablets	75, 150, 600, 800 mg
		75, 150, 300,
		400, 600 mg
	Oral suspension	100 mg/ml
Fosamprenavir	Tablets	700 mg
	Oral suspension	50 mg/ml
Indinavir	Capsules	200, 400 mg
Lopinavir/ritonavir	Tablets	100/25, 200/50 mg
	Capsules	133.3/33.3 mg
	Solution	80/20 mg/ml
Nelfinavir	Tablets	250, 625 mg
Ritonavir	Tablets	100 mg
	Capsules	100 mg
	Solution	80 mg/ml
Saquinavir	Capsules	200 mg
	Tablets	500 mg
Tipranavir	Capsules	250 mg
	Solution	100 mg/ml
Entry inhibitor		
Enfuvirtide	Powder for subcutaneous injection	108 mg, to obtain 90 mg/ml
CCR5 co-receptor antagonist		
Maraviroc	Tablets	150, 300 mg

[1] PNA: Postnatal age
[2] PMA: Post-menstrual age

Table 5.1: Antiretroviral drug formulations (tentatively) approved by the US FDA (continued)

	Name	Age approved from (FDA)
Nevirapine	Viramune	15 days
	Generic	
	Viramune XR	6 years
	Generic	
	Viramune	15 days
	Generic	
	Generic	
Rilpivirine	Edurant	18 years
Protease inhibitors		
Atazanavir	Reyataz	6 years
	Generic	
Atazanavir/ritonavir	Generic	
Darunavir	Prezista	3 years
	Generic	
	Prezista	3 years
Fosamprenavir	Lexiva	4 weeks
	Lexiva	4 weeks
Indinavir	Crixivan	18 years
Lopinavir/ritonavir	Kaletra	14 days
	Generic	
	Kaletra	14 days
	Kaletra	14 days (PNA[1]) and 42 weeks (PMA[2])
	Generic	
Nelfinavir	Viracept	2 years
Ritonavir	Norvir	1 month
	Generic	
	Norvir	1 month
	Norvir	1 month (PMA[2] 44 weeks)
Saquinavir	Invirase	16 years
	Invirase	16 years
Tipranavir	Aptivus	2 years
	Aptivus	2 years
Entry inhibitor		
Enfuvirtide	Fuzeon	6 years
CCR5 co-receptor antagonist		
Maraviroc	Selzentry	18 years

Table 5.1: Antiretroviral drug formulations (tentatively) approved by the US FDA (continued)

	Formulation	Strength
Integrase inhibitors		
Dolutegravir	Tablet	50 mg
Raltegravir	Tablets	400 mg
	Chewable tablet	25, 100 mg (scored)
	Powder for suspension	100 mg/packet
Fixed-dose combination formulations		
Abacavir/lamivudine	Tablets	600/300 mg
	Tablets	60/30 mg
	Tablets for oral suspension	60/30 mg
Abacavir/lamivudine/ zidovudine	Tablets	300/150/300 mg
Efavirenz/emtricitabine/ tenofovir DF	Tablets	600/200/300 mg
Efavirenz/lamivudine/ tenofovir DF	Tablets	600/300/300 mg
Elvitegravir/cobicistat/ emtricitabine/ tenofovir DF	Tablets	150/150/200/300 mg
Emtricitabine/ tenofovir DF	Tablets	200/300 mg
Emtricitabine/rilpivirine/ tenofovir DF	Tablets	200/25/300 mg
Lamivudine/stavudine	Tablets	150/30, 150/40, 60/12, 30/6 mg
Lamivudine/zidovudine	Tablets	150/300 mg (scored)
	Tablets (for oral suspension)	30/60 mg
Lamivudine/tenofovir DF	Tablets	300/300 mg
Lamivudine/stavudine/ nevirapine	Tablets	150/30/200, 150/40/200 mg
	Tablets, dispersible	30/6/50, 60/12/100 mg
Lamivudine/zidovudine/ nevirapine	Tablets	150/300/200 mg
	Tablets for oral suspension	30/60/50 mg

Table 5.1: Antiretroviral drug formulations (tentatively) approved by the US FDA (continued)

Name	Age approved from (FDA)	
Integrase inhibitors		
Dolutegravir	Tivicay	12 years
Raltegravir	Isentress	4 weeks
	Isentress	4 weeks
	Isentress	4 weeks
Fixed-dose combination formulations		
Abacavir/lamivudine	Epzicom	18 years
	Generic	
	Generic	
	Generic	
Abacavir/lamivudine/ zidovudine	Trizivir	Adolescents
	Generic	
Efavirenz/emtricitabine/ tenofovir DF	Atripla	12 years
	Generic	
Efavirenz/lamivudine/ tenofovir DF	Generic	
Elvitegravir/cobicistat/ emtricitabine/ tenofovir DF	Stribild	18 years
Emtricitabine/ tenofovir DF	Truvada	12 years
	Generic	
Emtricitabine/rilpivirine/ tenofovir DF	Complera	18 years
Lamivudine/stavudine	Generic	
Lamivudine/zidovudine	Combivir	bodyweight of 30 kg
	Generic	
	Generic	
Lamivudine/tenofovirDF	Generic	
Lamivudine/stavudine/ nevirapine	Generic	
	Generic	
Lamivudine/zidovudine/ nevirapine	Generic	
	Generic	

Table 5.2: Studies comparing solid and liquid formulations of antiretroviral drugs. Studies were performed in HIV-infected subjects, unless specified otherwise.

	BCS [14–19]	BDDCS [22]	Compared formulations
Nucleoside reverse transcriptase inhibitors			
Abacavir (ABC)	3	1	Tablet and solution
			Innovator scored tablet and solution
			Tablet and solution
			Innovator (scored) tablet and solution
Didanosine	3	3	*See section 5.4.2.1*
Emtricitabine	1	3	Tablet and solution
Lamivudine (3TC)	1/3	3	Tablet and solution
			Innovator scored FDC tablet (3TC/ZDV) and liquids
			Generic pediatric FDC granules for suspension (3TC/ZDV/NVP) and innovator liquids
			Generic pediatric FDC tablets (3TC/ZDV/NVP) and innovator liquids
			Generic pediatric FDC tablet (3TC/ZDV/NVP) and innovator liquids
			Generic pediatric FDC tablet for suspension (3TC/d4T/NVP) and liquids
			Generic pediatric FDC tablet (3TC/d4T/NVP) and innovator liquids
			Generic FDC tablet (3TC/d4T/NVP) and innovator liquids

Table 5.2: Studies comparing solid and liquid formulations of antiretroviral drugs. Studies were performed in HIV-infected subjects, unless specified otherwise (continued)

Subjects (years, range)	Outcome	Ref.
Adult	GMR (90%CI): $AUC_{0-\infty}$: 1.01 (0.93, 1.10) C_{max}: 0.89 (0.77, 1.02)	[66]
Child (1.8 − 4)	GMR (90%CI): AUC_{0-12}: 0.96 (0.83, 1.12) C_{max}: 1.02 (0.89, 1.17)	[35]
Child (0 − 16)	Formulation not retained as covariate in final population pharmacokinetic model	[67]
Child (0.4 − 13)	Formulation not retained as covariate in final population pharmacokinetic model	[68]
		[47–49]
Child (6 − 17)	Relative bioavailability of capsule formulation was 120% compared to the solution	[31]
Adult	GMR (90%CI): $AUC_{0-\infty}$: 0.98 (0.98, 1.00) C_{max}: 0.98 (0.98, 1.01)	[40]
Child (1.8 − 4)	GMR (90%CI): AUC_{0-12}: 1.58 (1.37, 1.81) C_{max}: 1.55 (1.33, 1.81)	[35]
Healthy adults	GMR (90%CI): $AUC_{0-\tau}$: 1.06 (0.96, 1.18) C_{max}: 0.94 (0.86, 1.03)	[42]
Healthy adults	Comparable pharmacokinetic profile	[69]
Child (0.5 − 12)	GMR (90%CI): AUC: 1.79 (1.68, 1.90)	[34]
Healthy adults	GMR (90%CI): $AUC_{0-\tau}$: 1.08 (1.01, 1.15) C_{max}: 1.15 (1.07, 1.24)	[44]
Child (0.5 − 11)	GMR (90%CI): AUC: 1.41 (1.30, 1.53) C_{max}: 1.59 (1.39, 1.82)	[33]
Child (1.3 − 14)	GMR (90%CI): AUC_{0-6}: 0.80 (0.64, 1.01) C_{max}: 0.76 (0.58, 0.99)	[41]

Table 5.2: Studies comparing solid and liquid formulations of antiretroviral drugs. Studies were performed in HIV-infected subjects, unless specified otherwise (continued)

	BCS [14–19]	BDDCS [22]	Compared formulations
			Tablet and solution
			Generic FDC tablet (3TC/ZDV/NVP or 3TC/d4T/NVP) and innovator liquid
Stavudine (d4T)	1/3	3	Tablet and solution
			Generic pediatric FDC tablets (3TC/ZDV/NVP) and innovator liquids Generic pediatric FDC tablet for suspension (3TC/d4T/NVP) and liquids
			Generic pediatric FDC tablet (3TC/d4T/NVP) and innovator liquids
			Generic FDC tablet (3TC/d4T/NVP) and innovator liquids
Tenofovir DF	3	3	No liquid formulation approved
Zidovudine (ZDV)	1/3	1	Innovator capsule and solution (syrup)
			Innovator scored FDC tablet (3TC/ZDV) and liquids
			Generic pediatric FDC tablet (3TC/ZDV/NVP) and innovator liquids Generic pediatric FDC granules for suspension (3TC/ZDV/NVP) and innovator liquids Tablets and liquid

Table 5.2: Studies comparing solid and liquid formulations of antiretroviral drugs. Studies were performed in HIV-infected subjects, unless specified otherwise (continued)

Subjects (years, range)	Outcome	Ref.
Child (several age groups)	Formulation not retained as covariate in final population pharmacokinetic model	[36, 37, 45]
Child (0.1 − 14)	Formulation was found to be an independent predictor for bioavailability and was retained in the final population pharmacokinetic model	[39]
Child (0 − 16)	Formulation not retained as covariate in final population pharmacokinetic model	[70]
Healthy adults	Comparable pharmacokinetic profile	[69]
Healthy adults	GMR (90%CI): $AUC_{0-\tau}$: 0.92 (0.89, 0.95) $C_{\max}$: 0.89 (0.83, 0.94)	[44]
Child (0.5 − 11)	GMR (90%CI): AUC: 0.97 (0.92, 1.02) $C_{\max}$: 1.08 (0.97, 1.20)	[33]
Child (1.3 − 14)	GMR (90%CI): AUC_{0-6}: 0.96 (0.69, 1.34) $C_{\max}$: 0.88 (0.60, 1.28)	[41]
Adult	GMR (syrup:capsule) (90%CI): AUC: 0.95 (0.83, 1.00)	[71]
Child (1.8 − 4)	GMR (90%CI): AUC_{0-12}: 1.01 (0.87, 1.18) $C_{\max}$: 1.07 (0.92, 1.25)	[35]
Child (0.5 − 12)	GMR (90%CI): AUC: 0.99 (0.92, 1.06)	[34]
Healthy adults	GMR (90%CI): $AUC_{0-\tau}$: 1.12 (1.00, 1.25) $C_{\max}$: 0.95 (0.83, 1.09)	[42]
Child (0.2 − 18)	Formulation not retained as covariate in final population pharmacokinetic model	[72]

Table 5.2: Studies comparing solid and liquid formulations of antiretroviral drugs. Studies were performed in HIV-infected subjects, unless specified otherwise (continued)

	BCS [14–19]	BDDCS [22]	Compared formulations
Non-nucleoside reverse transcriptase inhibitors			
Efavirenz	2/4	2	Tablet, capsule and liquid
			Capsule and liquids
Etravirine	4	2	No liquid formulation available
Nevirapine (NVP)	2	2	Generic pediatric FDC tablet for suspension (3TC/d4T/NVP) and liquids
			Generic pediatric FDC tablets (3TC/ZDV/NVP) and innovator liquids
			Generic pediatric FDC tablet (3TC/d4T/NVP) and innovator liquids
			Generic FDC tablet (3TC/d4T/NVP) and innovator liquids
			Generic pediatric FDC granules for suspension (3TC/ZDV/NVP) and innovator liquids
			Generic pediatric FDC tablet (3TC/ZDV/NVP) and innovator liquids
Rilpivirine	2	–	No liquid formulation approved
Protease inhibitors			
Atazanavir	2	2	No liquid formulation approved
Darunavir	2	2	No comparison data published
Fosamprenavir	2	2	No comparison data published
Indinavir	2/4	2	No liquid formulation approved
			Capsule and extemporaneous suspension

Table 5.2: Studies comparing solid and liquid formulations of antiretroviral drugs. Studies were performed in HIV-infected subjects, unless specified otherwise (continued)

Subjects (years, range)	Outcome	Ref.
Child (0.9 − 19)	The population pharmacokinetic model estimated a relative bioavailability of the liquid compared with the capsule and tablet formulations of 46.6%	[29]
Child (0.2 − 17)	The pharmacometric model estimated a lower and age-dependent relative bioavailability of the liquid formulations compared to the capsule formulation	[30]
Healthy adults	GMR (90%CI): $AUC_{0-\tau}$: 1.00 (0.96, 1.04) $C_{\max}$: 1.05 (0.99, 1.12)	[44]
Healthy adults	Comparable pharmacokinetic profile	[69]
Child (0.5 − 11)	GMR (90%CI): AUC: 1.08 (1.04, 1.13) $C_{\max}$: 1.14 (1.08, 1.19)	[33]
Child (1.3 − 14)	GMR (90%CI): AUC_{0-6}: 0.99 (0.84, 1.15) $C_{\max}$: 0.97 (0.82, 1.15)	[41]
Healthy adults	GMR (90%CI): $AUC_{0-\tau}$: 0.97 (0.89, 1.06) $C_{\max}$: 0.98 (0.88, 1.09)	[42]
Child (0.5 − 12)	GMR (90%CI): AUC: 0.85 (0.81, 0.88)	[34]
Healthy adults	GMR (liquid:capsule) (90%CI): AUC_{0-8}: 0.95 (0.92, 0.99) $C_{\max}$: 1.00 (0.95, 1.06)	[73]

Table 5.2: Studies comparing solid and liquid formulations of antiretroviral drugs. Studies were performed in HIV-infected subjects, unless specified otherwise (continued)

	BCS [14–19]	BDDCS [22]	Compared formulations
Lopinavir	2/4	2	Solid and liquid formulation
			Soft gel capsule and liquid
Nelfinavir	2/4	2	No liquid formulation approved Tablets and extemporaneous suspension
Ritonavir	2/4	2	Not included, since no longer used as protease inhibitor
Saquinavir mesylate	4	2	No liquid formulation approved
Tipranavir	2	2	No comparison data published
Entry, fusion and integrase inhibitors			
Enfuvirtide	–	–	No oral formulation approved
Maraviroc	3	1	No liquid formulation approved
Dolutegravir	2	–	No liquid formulation approved
Elvitegravir	2	–	No liquid formulation approved
Raltegravir	2/4	2	Film-coated tablet and granules for suspension

AUC: Area under the concentration time curve
BCS class: Biopharmaceutics classification system:
  class 1 (high solubility, high permeability)
  class 2 (low solubility, high permeability)
  class 3 (high solubility, low permeability)
  class 4 (low solubility, low permeability)
BDDCS class: Biopharmaceutics drug disposition classification system:
  class 1 (high solubility, extensive metabolism)
  class 2 (low solubility, extensive metabolism)
  class 3 (high solubility, poor metabolism)
  class 4 (low solubility, poor metabolism)
FDC: Fixed-dose combination formulations
GMR (90%CI): Geometric mean ratio with 90%CI (tablet:liquid, unless specified otherwise)

Table 5.2: Studies comparing solid and liquid formulations of antiretroviral drugs. Studies were performed in HIV-infected subjects, unless specified otherwise (continued)

Subjects (years, range)	Outcome	Ref.
Child (0 − 18)	Formulation not retained as covariate in final population pharmacokinetic model	[74]
Child (5 − 18)	Formulation was significantly associated with absorption lag time: median lag time was twofold shorter for liquid compared to capsules	[75]
Healthy adults	GMR (suspension: tablets) (90% CI): $AUC_{0-\infty}$: 0.9 (0.90, 1.24) C_{max}: 1.0 (0.92, 1.08)	[76]
Healthy adults	GMR (granules:tablet) (90%CI): $AUC_{0-\infty}$: 2.62 (2.17, 3.17) C_{max}: 4.64 (3.41, 6.30)	[46]

Table 5.3: Studies showing bioequivalence or comparable pharmacokinetics of solid antiretroviral drug formulations

Antiretroviral	Compared formulations
Nucleoside reverse transcriptase inhibitors	
Abacavir	Generic and innovator tablet[‡]
Didanosine	Generic and innovator chewable tablet[‡]
	Generic and innovator powder for oral solution[‡]
Lamivudine	Generic and innovator tablet[‡]
Stavudine	Generic and innovator capsule[‡]
Tenofovir	Generic and innovator tablet[‡]
Zidovudine	Generic and innovator capsule[‡]
	Generic and innovator tablet[‡]
Non-nucleoside reverse transcriptase inhibitors	
Efavirenz	Generic and innovator capsule[‡]
Etravirine	Innovator tablets[§]
	Innovator tablets[‡]
Nevirapine	Generic and innovator tablet[‡]
	Generic and innovator tablet[‡]
Protease inhibitors	
Atazanavir	Generic and innovator capsule[‡]
Darunavir	Generic and innovator tablet[‡]
Indinavir	Generic and innovator capsule[‡]
	Generic and innovator capsule[§]
Lopinavir/ritonavir	Generic and innovator capsule[‡]
	Generic and innovator tablet[‡]
	Generic and innovator tablet[‡]
	Innovator capsule and innovator tablet[§]
	Innovator capsule and innovator tablet[§]
	Innovator capsule and generic tablet[§]
	Innovator capsule and (cut) generic tablet[§]
	Innovator capsule and generic tablet[§]
	Generic tablets[§]
Nelfinavir	Generic and innovator tablet[‡]
Saquinavir mesylate	Innovator capsule and tablet[‡]
	Innovator capsule and innovator tablet[§]
	Generic and innovator tablet[‡]

Table 5.3: Studies showing bioequivalence or comparable pharmacokinetics of solid antiretroviral drug formulations (continued)

Strength	Subjects	Meal condition	Ref.
300 mg	Healthy adults	Fasting and fed	[77]
100 mg	Healthy adults	Fasting	[78]
4 g	Healthy adults	Fasting	[79]
150 mg	Healthy adults	Fasting	[80–83]
40 mg	Healthy adults	Fasting	[84–86]
300 mg	Healthy adults	Fasting and fed	[87, 88]
100 mg	Healthy adults	Fasting	[89, 90]
300 mg	Healthy adults	Fed	[91]
200 mg	Healthy adults	Fasting	[92]
100 and 25 mg	Healthy adults	Fed	[55]
100 and 200 mg	Healthy adults	Fed	[55]
200 mg	Healthy adults	Fasting	[93–95]
200 mg	HIV-infected adults	Fasting	[96]
300 mg	Healthy adults	Fasting	[97]
600 mg	Healthy adults	Fasting and fed	[98]
400 mg	Healthy adults	Fasting	[99]
400 mg*	HIV-infected adults	Fasting	[100]
133/33 mg	Healthy adults	Fed	[101]
200/50 mg	Healthy adults	Fasting	[102, 103]
200/50 mg compared to two 100/25 innovator tablets	HIV-infected adults	Not described	[104]
133/33 and 200/50 mg	Healthy adults	Various meal conditions	[59]
133/33 and 200/50 mg	HIV-infected adults	Not described	[105]
133/33 and 200/50 mg	HIV-infected adults	Variable	[106]
133/33 and 200/50 mg	HIV-infected children	Not described	[107]
133/33 and 200/50 mg	HIV-infected adults	Not described	[108]
200/50 mg	HIV-infected adults	Not described	[108]
250 mg	Healthy adults	Not described	[109]
200 and 500 mg	Healthy adults	Fed	[110]
200 and 500 mg	HIV-infected adults	Not described	[111]
500 mg	Healthy adults	Fed	[112]

Table 5.3: Studies showing bioequivalence or comparable pharmacokinetics of solid antiretroviral drug formulations (continued)

Antiretroviral	Compared formulations
Fixed-dose combination formulations	
Abacavir/lamivudine/zidovudine	Innovator tablet and separate innovator tablets[‡]
	Innovator tablet and innovator abacavir + FDC lamivudine/zidovudine tablets[‡]
Efavirenz/emtricitabine/tenofovir DF	Innovator tablet and separate innovator tablets[‡]
Emtricitabine/rilpivirine/tenofovir DF	Innovator tablet and separate innovator capsule and tablets[‡]
Lamivudine/nevirapine/stavudine	Generic tablet and separate innovator tablets[‡]
	Generic tablet and separate innovator tablets[§]
	Generic tablet and separate innovator tablets and capsule[‡]
	Generic tablet and nevirapine tablet[§]
Lamivudine/nevirapine/zidovudine	Generic tablet and separate innovator tablets[‡]
	Generic tablet and innovator nevirapine + FDC lamivudine/zidovudine tablet[§]
Lamivudine/stavudine	Generic tablet and separate standard tablet and capsule[‡]
	Generic tablet and separate innovator tablet and capsule[§]
Lamivudine/tenofovir DF	Generic tablet and separate innovator tablets[‡]
Lamivudine/zidovudine	Innovator tablet and separate innovator tablets[‡]
	Generic tablets and innovator FDC[‡]
	Eight subunit pediatric tablet and generic FDC tablet[‡]

[*] The formulation was not specifically described, but as these were adult patients on antiretroviral treatment with indinavir, it is expected that the 400 mg capsule formulation was used.

[‡] Bioequivalence criteria were met.

[§] Bioequivalence criteria not determined or not met, but comparable pharmacokinetic parameters.

Table 5.3: Studies showing bioequivalence or comparable pharmacokinetics of solid antiretroviral drug formulations (continued)

Strength	Subjects	Meal condition	Ref.
300/150/300 mg	Healthy adults	Fasting and fed[¶]	[113]
300/150/300 mg	HIV-infected adults	Fasting	[114]
600/200/300 mg	Healthy adults	Fasting	[58]
200/25/300 mg	Healthy adults	Fed	[19]
150/200/40 mg	Healthy adults	Fasting	[115]
150/200/40 mg	HIV-infected adults	Fasting and fed	[116–118]
150/200/30 mg	Healthy adults	Fasting	[119]
30/6/50 and 60/12/100 mg	Healthy adults	Fasting	[69]
150/200/300 mg	Healthy adults	Fasting	[120]
150/200/300 mg	Healthy adult women	Fasting	[121]
150/40 mg	Healthy adults	Fasting	[122]
150/30 mg	Healthy adults	Unknown	[123]
300/300 mg	Healthy adults	Fasting	[124]
150/300 mg	Healthy adults	Fasting and fed	[125]
150/300 mg	Healthy adults	Fasting	[126]
160/300 mg	Healthy adults	Fasting	[127]

[¶] The GMR (90%CI) of the FDC tablet under fed compared to fasting conditions was 0.68 (0.62, 0.76).

Table 5.4: Studies comparing the influence of manipulation of antiretroviral drug formulations on pharmacokinetics

Antiretroviral	Compared formulations	Subjects
Efavirenz	Intact innovator capsules and opened capsules mixed with food	Healthy adults
Efavirenz, emtricitabine, tenofovir DF	Intact innovator tablet and extemporaneously compounded liquid out of tablets	Healthy adults
Etravirine	Innovator 100 mg tablet as a whole and dispersed in 100 ml water	Healthy adults
Lopinavir/ritonavir	Innovator 200/50 mg tablet as a whole and crushed	Children (10-16 years)
Stavudine	Whole compared to opened generic capsules	Healthy adults

Table 5.4: Studies comparing the influence of manipulation of antiretroviral drug formulations on pharmacokinetics (continued)

Outcome	Ref.	Antiretroviral
Bioequivalence criteria met	[53]	Efavirenz

GMR (liquid:tablet) (90%CI):
   Efavirenz: $AUC_{0-\infty}$ 0.97 (0.82, 1.26)
     and $C_{\max}$ 0.86 (0.75, 1.04)
   Emtricitabine: $AUC_{0-\infty}$ 0.99 (0.91, 1.05)
     and $C_{\max}$ 1.15 (0.97, 1.25)
   Tenofovir: $AUC_{0-\infty}$: 1.21 (1.07, 1.40)
     and $C_{\max}$ 1.38 (1.12, 1.70)
	[54]	Efavirenz, emtricitabine, tenofovir DF

Bioequivalence criteria met	[55]	Etravirine

GMR (crushed:whole) (90%CI):
   Lopinavir: AUC: 0.55 (0.45, 0.69)
     and $C_{\max}$ 0.75 (0.61, 0.92)
   Ritonavir: AUC: 0.53 (0.4, 0.71)
     and $C_{\max}$ 0.7 (0.51, 0.97)
	[56]	Lopinavir/ritonavir

Bioequivalence criteria met	[57]	Stavudine

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Making things easier

6 Pharmacokinetics of pediatric lopinavir/ritonavir tablets in children when administered twice daily according to FDA weight bands

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Abstract

Background

Lopinavir/ritonavir (LPV/r) pediatric tablets (100/25 mg) are approved by the United States Food and Drug Administration (FDA) and European Medicines Agency (EMA) as part of combination antiretroviral therapy. Dosing is based on body weight bands or body surface area under FDA approval and only body surface area by the EMA. This can lead to a different recommended dose. In addition, weight band-based dosing has not been formally studied in the target population. We evaluated the pharmacokinetics (PK) of LPV/r in children, administered twice daily according to the FDA weight bands, using pediatric tablets.

Methods

Fifty-three HIV-infected children were included in the PK substudy of the Paediatric European Network for the Treatment of AIDS 18 trial (KONCERT). In this study, children were randomized to receive LPV/r twice or once daily, according to FDA weight bands. A PK assessment was performed in 17, 16 and 20 children in the 15–25 kg, 25–35 kg and > 35 kg weight band, respectively, while children took the tablets twice daily. Rich sampling was performed, and PK parameters were calculated by noncompartmental analysis. Given the high percentage of Asian children, it was also tested whether there was a difference in PK parameters between Asian and non-Asian children.

Results

For the total group, LPV geometric mean AUC_{0-12}, C_{max} and C_{12} were 106.9 h×mg/l, 12.0 mg/l and 4.9 mg/l, respectively. There were no significant differences in LPV PK parameters between the weight bands. In addition, weight was not found to be associated with variability in C_{max}, C_{12} or AUC_{0-12} for the LPV PK parameters.

Conclusion

FDA weight band-based dosing recommendations provide adequate exposure to LPV when using LPV/r pediatric tablets.

Introduction

HIV-infected children require lifelong treatment with combination antiretroviral therapy (cART). Currently, the preferred cART regimen recommended for antiretroviral treatment-naive children with no resistance to antiretroviral drugs comprises a backbone of two nucleoside reverse transcriptase inhibitors plus either a nonnucleoside reverse transcriptase inhibitor (NNRTI) or a ritonavir (RTV)-boosted protease inhibitor [1–3].

Lopinavir boosted with low-dose ritonavir (LPV/r) is used worldwide in HIV-infected children as part of first- and second-line treatments [1, 2]. It is licensed to be taken twice daily for children from the age of 2 years by the European Medicines Agency (EMA) and from the age of 14 days by the United States Food and Drug Administration (FDA). There are several LPV/r formulations available: a liquid formulation (LPV/r 80/20 mg/ml), adult tablets (LPV/r 200/50 mg) and pediatric tablets (LPV/r 100/25 mg). The pediatric tablet (100/25 mg) was approved by the FDA in 2007 and by the EMA in 2008; however, the posology in the product labels approved by these two agencies is not the same. The dose for an individual is based on body surface area only as recommended by the EMA, while it is based on body weight bands or body surface area as recommended by the FDA. Dosing based on body weight bands versus body surface area may lead to differences in the number of tablets recommended for a child with a certain weight, which is confusing and undesirable from a global perspective. The FDA-recommended weight band dosing has been derived from pharmacokinetic (PK) modeling but has not been formally studied in the target population. To validate the FDA weight band-based dosing recommendations, we evaluated the PK of LPV/r administered twice daily, using the pediatric 100/25 mg tablet, in HIV-infected children.

6.1   Methods

This PK study is part of the ongoing Paediatric European Network for the Treatment of AIDS (PENTA)-18 trial or KONCERT trial: A Kaletra ONCE daily Randomized Trial of the pharmacokinetics, safety and efficacy of twice-daily versus once-daily lopinavir/ritonavir tablets dosed by weight as part of combination antiretroviral therapy in HIV-1 infected children (NCT01196195). KONCERT is a phase II/III, prospective, randomized, open-label, international, multicenter trial in virologically suppressed HIV-1-infected children. Children were eligible when they were younger than 18 years, 15 kg in weight, receiving cART that includes LPV/r and had

an HIV-1 RNA < 50 copies/ml for at least 24 weeks and were able to swallow tablets. Children receiving an NNRTI or a PI other than LPV/r were excluded. The use of concomitant drugs, except for prophylaxis, was not allowed unless permission was granted by the trial team. Children were randomized (1 : 1) to either continue the same ART regimen with LPV/r tablets taken twice daily ($n = 80$) or to switch to LPV/r tablets dosed once daily ($n = 80$). The KONCERT trial has been approved by the regulatory bodies and ethics committees for all participating countries and sites. KONCERT is being conducted in full conformance with the principles of the current version of the Declaration of Helsinki and with the local laws and regulations concerning clinical trials.

6.1.1 Population and treatment

Children were enrolled in KONCERT in one of three weight bands: ≥ 15 to ≤ 25 kg (low), > 25 to ≤ 35 kg (middle) and > 35 kg body weight (high). In the first phase of the trial, children who consented were selected to participate in a PK substudy, until a minimum of 16 children in each weight band had evaluable PK data. If needed, the LPV/r formulation was changed to the pediatric tablet at the screening visit and the dose adjusted to follow the recommended FDA dosing plan based on body weight bands (see Table 6.1). It was required that tablets be swallowed whole and could be taken with or without food. Adherence was assessed by pill counts and a questionnaire at screening and on the day of the PK assessment. During the trial, an amendment to the protocol was made to ensure ethnic representativeness within the PK study. At that time, the highest weight band already had 12 children from Thailand and further inclusion into the PK study from this country was stopped. Enrollment of subsequent participants continued to have 8 children from countries other than Thailand in each of the weight bands.

6.1.2 Sample size

Based on plasma LPV PK data from an adult study on tablet formulation, the estimated variance of $\log_{10}$ area under the curve (AUC) for pediatric tablets was approximately 0.2 (internal Abbott data). Forty-eight children (16 in each weight band) providing plasma LPV PK data on twice-daily tablet regimens was estimated to provide at least 80% power for the width of the 90% confidence interval for the mean $\log_{10}$ AUC on twice-daily dosing to be < 0.230 on the $\log_{10}$ scale. Therefore, to confirm FDA body weight-

Table 6.1: FDA recommended dosing plan based on body weight band for lopinavir/ritonavir 100/25 mg tablets twice-daily (not given with non-nucleoside reverse transcriptase inhibitors, fosamprenavir or nelfinavir)

Weight (kg)	Number of tablets twice daily	Exposure twice daily (mg/kg)	Approximate equivalent BSA (m^2)	Exposure twice daily (mg/m^2)
$\geq$ 15 to $\leq$ 25	2	8 to 13	$\geq$ 0.65 to $\leq$ 0.92	217 to 308
> 25 to $\leq$ 35	3	9 to 12	> 0.92 to $\leq$ 1.2	250 to 326
> 35	4	< 11	> 1.2	< 333

based dosing recommendations of twice-daily LPV/r 100/25 mg tablets, 48 children was considered to be sufficient for the estimation of interpatient variability.

6.1.3 Pharmacokinetic assessment

Full 12-hour PK assessment of LPV/r was conducted prior to randomization. Children must have been receiving the pediatric tablets twice daily at the FDA-recommended weight band-based dose for at least 7 days prior to the PK assessment. On the PK day, the child's medications were intended to be administered before taking breakfast. Depending on the country and hospital, breakfast according to local custom was served (e.g., pork/chicken/fish soup, rice and a cup of milk; cereal, toast, cooked eggs and scones; or bread roll with butter, ham, cheese, jam or hazelnut spread). Blood samples (2 ml/time point) were taken in ethylenediaminetetraacetic acid (EDTA) tubes at 0 (predose, before morning dose), 2, 4, 6, 8 and 12 hours after an observed intake.

6.1.4 Pharmacokinetics

Lopinavir (LPV) and RTV PK parameters were determined using noncompartmental analysis (WinNonlin®, version 5.3., Pharsight® Corporation, Mountain View, CA): AUC$_{0-12}$ (area under the plasma concentration-time curve calculated (linear-log trapezoidal method) over a dosing interval from time 0 to 12 hours after dosing), $C_{\max}$ (maximum observed plasma concentration) and C_{12} (drug concentration 12 hours postdose).

6.1.5 Measurement of plasma drug concentration

Samples were processed within 3 days of collection, and plasma was stored immediately at $-80°C$. Plasma concentrations of LPV and RTV were determined using a validated ultrahigh performance liquid chromatography assay with ultraviolet detection derived from the previously published assay [4]. The analysis was performed at the Department of Pharmacy, Radboud University Nijmegen Medical Centre, and this laboratory participates in an international interlaboratory quality control program for therapeutic drug monitoring of antiretroviral drugs [5]. The analytical range of the assay for LPV was 0.109–31.2 mg/l and for RTV 0.044–29.4 mg/l. The intraday and interday precision for both assays ranged from 0.6% to 4.2% (coefficient of variation) and 0.3% to 1.8%, respectively. The percentage accuracy of the assay ranged from 98.2% to 105.6%.

6.1.6 Statistical analysis

To validate dosing by the FDA weight bands, linear regression of the PK parameters against dose (mg/kg body weight) was performed. Analysis of variance (ANOVA) was used to compare the geometric means of each PK parameter between the three different weight bands. This was further tested by regression of logtransformed parameters against weight as a continuous variable. Comparison of the PK parameters between different ethnic groups (Asian vs. non-Asian children) was performed using an independent t test on logtransformed values. Statistical analysis was performed using SPSS software version 20.0.0.1 (IBM® SPSS® 1989, 2011).

6.2 Results

6.2.1 Population

Fifty-three children were enrolled in the PK study between August 2010 and December 2011: 17, 16 and 20 children in the 15 to ≤ 25 kg, > 25 to ≤ 35 kg and > 35 kg weight bands, respectively. Median (range) age of the children was 11.0 (4.4, 17.7) years. Twenty-two (42%) were male children. Further demographic data of the patients are presented in Table 6.2.

The use of concomitant medication was reported for 5 children. According to the literature, none of the concomitant medication used is known to influence the metabolism of LPV or RTV. No use of natural products or herbs was reported. None of the children vomited either the day before or on the day of the PK assessment. The median (range) LPV

Table 6.2: Demographic data of children in KONCERT pharmacokinetic substudy

	All children (n = 53)	15–25 kg (n = 17)	25–35 kg (n = 16)	> 35 kg (n = 20)
Male	22 (42%)	5	10	7
Age (y) median, (IQR)	11.0 (8.8, 14.7)	7.4 (6.8, 8.8)	10.9 (10.1, 13.9)	15.0 (13.7, 15.7)
Weight (kg) median, (IQR)	31.0 (23.6, 40.0)	20.5 (19.3, 23.5)	30.2 (29.1, 32.1)	41 (38.3, 49.5)
Ethnicity				
Asian	29 (55%)	8	8	13
Black African	14 (26%)	6	6	2
White	6 (11%)	2	1	3
Mixed black/white	2 (4%)	1	1	0
Other	2 (4%)	0	0	2

dose taken by the children was 9.8 (5.5, 13.3) mg/kg or 270 (215, 329) mg/m^2.

6.2.2 Lopinavir pharmacokinetics

For the total group, the LPV geometric mean (95%CI) AUC_{0-12} was 106.9 (97.8, 116.9) h×mg/l, C_{max} 12.0 (11.1, 12.9) mg/l and C_{12} was 4.9 (4.1, 5.8) mg/l. The geometric mean plasma LPV concentration-time profile for the total group and the profiles per weight band are shown in Figure 6.1. The geometric means of the PK parameters for each weight band and the total group are presented in Table 6.3.

Because the PK parameters of LPV were not normally distributed, statistical analysis was performed on the logtransformed data. Linear regression showed no correlation of the LPV PK parameters AUC_{0-12} ($P = 0.062$), C_{12} ($P = 0.87$) and CL/F ($P = 0.20$) against the weight-based dose (mg/kg body weight). Linear regression did show a correlation between the weight-based dose of LPV and C_{max} ($P = 0.039$).

There were no significant differences when comparing LPV PK parameters between the weight bands (AUC_{0-12}, $P = 0.42$; C_{max}, $P = 0.17$; C_{12}, $P = 0.46$ and CL/F, $P = 0.75$). Weight was also not associated with variability in the LPV PK parameters (AUC_{0-12}, $P = 0.44$; C_{max}, $P = 0.12$; C_{12}, $P = 0.33$ and CL/F, $P = 0.25$).

One of the 53 children (1.9%) had an LPV $C_{12} < 1.0$ mg/l (0.56 mg/l). This child received an LPV dose of 11.5 mg/kg, which was higher than the
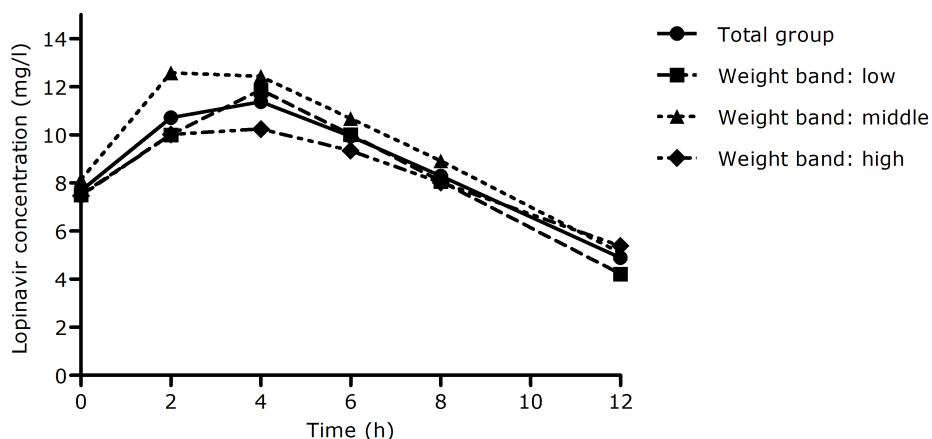
Figure 6.1: Plasma lopinavir concentration versus time for the total group and for each weight band (data presented as geometric means).

Table 6.3: Pharmacokinetic parameters of lopinavir and ritonavir

Geometric mean (95%CI)	n	Lopinavir	Ritonavir
AUC_{0-12} (h×mg/l) total	53	106.9 (97.8, 116.9)	5.9 (5.28, 6.65)
Weight (kg)			
$\geq$ 15 to $\leq$ 25	17	104.1 (84.9, 127.5)	5.7 (4.71, 6.84)
> 25 to $\leq$ 35	16	116.9 (100.6, 135.8)	6.8 (5.27, 8.65)
> 35	20	101.9 (89.1, 116.6)	5.5 (4.53, 6.76)
$C_{\max}$ (mg/l) total	53	12.0 (11.1, 12.9)	0.88 (0.78, 1.00)
Weight (kg)			
$\geq$ 15 to $\leq$ 25	17	12.2 (10.4, 14.5)	0.87 (0.71, 1.06)
> 25 to $\leq$ 35	16	13.0 (11.4, 14.8)	1.02 (0.76, 1.35)
> 35	20	11.0 (10.0, 12.2)	0.80 (0.67, 0.95)
C_{12} (mg/l) total	53	4.9 (4.14, 5.80)	0.18 (0.15, 0.21)
Weight (kg)			
$\geq$ 15 to $\leq$ 25	17	4.2 (3.07, 5.78)	0.16 (0.12, 0.20)
> 25 to $\leq$ 35	16	5.1 (3.53, 7.36)	0.18 (0.14, 0.24)
> 35	20	5.4 (4.16, 6.97)	0.19 (0.13, 0.28)
Clearance (l/(h×kg)) total	53	0.089 (0.081, 0.097)	0.400 (0.356, 0.450)
Weight (kg)			
$\geq$ 15 to $\leq$ 25	17	0.092 (0.078, 0.109)	0.422 (0.358, 0.497)
> 25 to $\leq$ 35	16	0.085 (0.071, 0.100)	0.366 (0.281, 0.477)
> 35	20	0.089 (0.077, 0.104)	0.411 (0.333, 0.508)

Clearance (CL/F/kg) = dose (mg)/[AUC_{0-12} (h×mg/l) × body weight (kg)].

median value in this study (9.8 mg/kg). Remarkably, the predose morning level of this child was 8.1 mg/l. The last dose was reported to be taken approximately 12 hours prior to the observed intake on the day of PK assessment. No explanation could be found for this difference, other than the possible diurnal variation in absorption, distribution, metabolism and excretion [6].

Given the high percentage of Asian children within the PK study population, it was tested whether there was a difference in PK parameters between Asian and non-Asian children. Significant influence of ethnicity was found on CL/F ($P = 0.021$), with Asian children having a higher clearance. There were no significant differences in the other PK parameters between Asian and non-Asian children (AUC$_{0-12}$, $P = 0.17$; C_{max}, $P = 0.089$ and C_{12}, $P = 0.18$). The geometric mean plasma LPV concentration-time profiles for the Asian children and non-Asian children are shown in Figure 6.2.
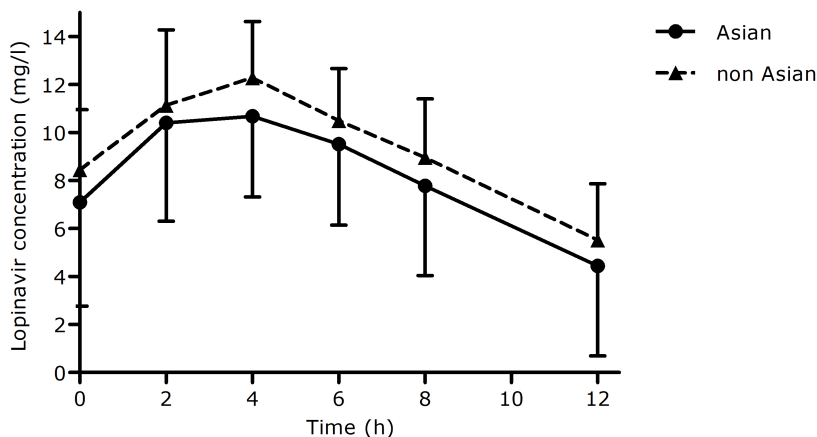
Figure 6.2: Plasma lopinavir concentration versus time of Asian and non-Asian children (data are presented as geometric means and arrow bars show standard error of the mean).

6.3 Discussion

This study conducted in 53 HIV-infected children found adequate LPV exposure when LPV/r 100/25 mg tablets were administered according to the FDA-recommended weight band-based dose. No significant differences of PK parameters for LPV were observed between the three weight bands.

The current recommendation for the LPV trough concentration in HIV-infected patients with no evidence of resistance is 1.0 mg/l [3]. Fifty-two (98%) of the children achieved a trough concentration of 1.0 mg/l or higher. Therefore, the weight band-based dosing described by the FDA can be used to treat HIV-infected children with the LPV/r pediatric tablets.

In this study, the geometric mean LPV AUC_{0-12} was 106.9 h×mg/l, C_{max} 12.0 mg/l and C_{12} 4.9 mg/l. The LPV AUC_{0-12}, C_{max} and C_{12} were all higher than that observed in children receiving 230/57.5 mg/m^2 of the LPV/r solution described in the summary of product characteristics (AUC_{0-12} 72.6 h×mg/l, C_{max} 8.2 mg/l and C_{12} 3.4 mg/l) [7]. This can mostly be explained by the higher dose that is used in our population (median dose 270 mg/m^2). The clinical relevance of this higher exposure (i.e., side effects) is being assessed in the ongoing main trial. A summary of LPV/r PK parameters reported from 10 studies in HIV-infected children ($n > 10$) is shown in Table 6.4 [7–15]. Studies in which some of the children used an NNRTI are included, but unfortunately results were not always specified for concomitant use of an NNRTI. When comparing results of these PK studies, it is important to realize that different formulations of LPV/r may have been used, which could lead to differences in PK parameters. The AUC_{τ} and AUC_{∞} of the soft gel capsule and oral solution are 18% lower compared with the 200/50 mg tablet formulation in healthy volunteers, although the 90%CI of the geometric mean ratio is within the bioequivalence range of 80–125% [16]. A study in HIV-infected adults showed that the mean C_{last} was higher with a generic tablet compared with the soft gel capsule formulation [17]. A 45% decrease in LPV AUC_{0-12} was observed in HIV-infected children when LPV/r tablets were crushed compared with administration of whole tablets [8]. The formulation used is also significantly associated with the absorption lag time: the lag time in HIV-infected children using the solution was half the lag time in children using capsules [13]. PK parameters found in a study evaluating the PK of LPV/r in Thai adolescents using the 200/50 mg tablet formulation were comparable with our results [11].

Diet can also influence the PK of LPV. Differences in LPV AUC_{τ} and C_{max} have been shown when the LPV/r tablet formulation is administered under fasting conditions or with a moderate or high-fat meal. Compared with administration of LPV/r under fasting conditions, the LPV AUC_{τ} and C_{max} increased, respectively, by 26.9% and 17.6%, with a moderate-fat meal. When administered with a high-fat meal, the LPV AUC_{τ} increased by 18.9% and $C_{max}$was unchanged [16].

This is the first PK study in which the PK parameters of LPV in a large group of Asian children can be directly compared with the results of children of other ethnic origins. However, this should be done with caution because the baseline characteristics of both groups are different. Mean age of the Asian patients is significantly higher than that of non-Asian patients. Furthermore, the weight for age is significantly lower in the Asian children. Regarding the PK parameters, a significant difference in LPV clearance (CL/F) was observed between Asian and non-Asian children. The CL/F of LPV in Asian children was on average 21% higher than in non-Asian children: 0.097 versus 0.080 l/h×kg ($P = 0.021$). This result was unexpected, as usually lower clearance is expected, and therefore, a lower LPV/r dose has previously been investigated in Thai children [11,12]. However, plasma half-life of LPV of Asian and non-Asian children was the same for both groups (6.5 hours). A comparison of CL/F of Thai children and a group of mainly (78%) African-American children showed similar clearance in both groups: median (IQR) CL/F 1.7 (1.0, 3.5) and 1.8 (1.0, 2.6) l/h, respectively [12, 13, 18]. Despite the significantly higher CL/F in Asian children in our study, there was no significant difference in AUC_{0-12} between Asian and non-Asian children, which can be explained by the relatively higher dose in the Asian children on a mg/kg basis [9.8 vs. 9.1 mg/kg (median dose)]. A possible explanation for these observations could be a difference in diet and more Asian children in our study taking their medication under fasting conditions at the day of PK assessment: 52% versus 13% of the non-Asian children. Differences between ethnic groups in expression of cytochrome P450A (CYP3A) isoenzymes could explain interpatient variability in the PK of LPV [14, 19–21]. For instance, wild-type CYP3A, which is associated with more rapid metabolism, is more frequently expressed in Southeast Asians compared with Caucasians [22–24]. Another mechanism possibly involved in the PK of LPV relates to transporter proteins, such as the permeability glycoprotein (P-gp; MDR1, ABCB1), the multidrug resistance protein 2 (MRP2; ABCC2) and the organic anion transporters (OATP; SLCO1B1) [14, 20, 21, 25–28]. Genotype frequencies of these transporter proteins differ between ethnic groups [29, 30], but data from Asian patients are lacking. Unfortunately, no genetic testing was performed in our patients, and the influence of these polymorphisms on the PK of LPV cannot be explored. It must be noted that despite potential ethnic influences on LPV PK, exposure in all subgroups was adequate.

Table 6.4: Pharmacokinetic studies of twice-daily lopinavir in HIV-infected children

Author	n	Age (year,range)	LPV dose (mean, mg/m^2)	AUC$_{0-12}$ (mg×h/l)	$C_{\max}$ (mg/l)
Current study[2]	53	4.4–17.7	270	106.9	12.0
Best 2011[3] [8]	12	10–16	275	144	11.3
Foissac 2011[4] [9]	45	0.5–19	266	116	–
Jullien 2006[4] [10]	157	0–18	281	108	9.6
Klinklom 2012[3] [11]	23	IQR: 12–15	208	86.7	10.5
	24	IQR: 12–15	290	102.7	11.4
Puthanakit 2009[3] [12]	11	IQR: 6.7–11.8	194	83.8	10.1
	11	IQR: 8.4–14.7	279	117.6	11.9
Rakhmanina 2009[3] [13]	50	5.3–17.5	275	96.1	10.3
Rakhmanina 2011[3] [14]	50	4.3–17.2	275	96.9	–
Rosso 2006[3] [15]	21	3.5–13.5	230	–	14.6
Saez Lloren 2003[4,5] [7]	12		230	72.6	8.2
	15		300	116.4	12.5

[1] tablet: 200/50 mg lopinavir/ritonavir, pediatric tablet: 100/25 mg lopinavir/ ritonavir, pediatric tablet: 100/25 mg lopinavir/ritonavir, SGC = soft gel capsule
[2] geometric mean pharmacokinetic parameters are shown
[3] median pharmacokinetic parameters are shown
[4] mean pharmacokinetic parameters are shown
[5] Study also with non-nucleoside reverse transcriptase inhibitors (NNRTI) (efavirenz or nevirapine), data shown are from the children using no NNRTI

In conclusion, FDA weight band-based dosing recommendations provide adequate exposure when using the pediatric LPV/r tablets. Hence, the FDA-recommended dose for the weight bands of ≥ 15 to ≤ 25 kg, > 25 to ≤ 35 kg and > 35 kg body weight can be used to achieve adequate exposure of LPV in HIV-infected children.

Acknowledgments

Table 6.4: Pharmacokinetic studies of twice-daily lopinavir in HIV-infected children (continued)

C_{12} (mg/l)	NNRTI use	Formulation[1]	Pharmacokinetic modeling method
4.9	no	pediatric tablet	noncompartmental
6.8	no	tablet	noncompartmental
8.5	part	SGC, solution, (pediatric) tablet	population
7.9	part	SGC, solution	population
3	no	tablet	noncompartmental
4.1	no	tablet	noncompartmental
3.4	no	solution	noncompartmental
4.9	no	solution	noncompartmental
5.9	unknown	SGC, solution	population
–	part	SGC, solution, tablet	noncompartmental
7.9	no	SGC, solution	–
3.4	no	solution	noncompartmental
6.5	no	solution	noncompartmental

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7 A new paediatric formulation of valaciclovir: development and bioequivalence assessment

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Abstract

Background

The aim of this study was to develop a new paediatric formulation of valaciclovir and to assess the bioequivalence of the new formulation compared to valaciclovir tablets.

Methods

A new paediatric formulation was developed with good pharmaceutical quality and stability, and with the possibility of flexible dosing. Bioequivalence of the new formulation compared to the innovator product (tablet) was tested in a randomized, single-dose (500 mg), open label, two-period crossover, phase-I trial in fasting healthy adult volunteers.

Results

A solution with 20 mg/ml valaciclovir with a shelf life of at least nine months was developed. The geometric mean ratio of the test versus reference formulation was 106% (90%CI 100 to 112%) for AUC_{0-12} and 122% (90%CI 110 to 133%) for $C_{\max}$. The 90% confidence interval for the ratio of the AUC_{0-12} was within the acceptance range for bioequivalence. The upper limit of the ratio for the $C_{\max}$ was above the limit of 125%.

Conclusion

The newly developed valaciclovir solution has a comparable exposure to the innovator product and is therefore an alternative formulation for (paediatric) patients who experience difficulties with the intake of valaciclovir tablets.

7.1 Introduction

Valaciclovir is an oral prodrug of aciclovir and is used for the treatment and prophylaxis of herpes simplex virus (HSV) and varicella zoster virus (VZV) infections [1]. Valaciclovir has a higher and more reliable bioavailability than aciclovir with at least equal efficacy, a similar safety profile and the advantage of decreased dosing frequency [2–4]. In adults, valaciclovir has replaced aciclovir in many clinical scenarios. Valaciclovir is approved by the European medicines agency (EMA) to be used in children from the age of 12 years and above for the treatment of certain herpes infections. Pharmacokinetic studies performed in children from 0 to 18 years of age, have shown that bioavailability is 2- to 5-fold higher compared to oral aciclovir and comparable plasma levels can be achieved as in adults [5–8]. Although these studies were performed with various formulations (adult-dose tablets, crushed tablets and an extemporaneous solution of valaciclovir), the results support the use of oral valaciclovir instead of aciclovir in children.

The extemporaneous formulation made from crushed tablets as described in the FDA label information is in our opinion a suboptimal formulation for several reasons [9]. First, crushing tablets can result in heterogeneous particle size associated with differences in sedimentation of the drug compound in a suspension, with a resulting difficulty in redispersing the suspension upon agitation. This may lead to dosing errors and practical problems such as obstruction of feeding tubes. Secondly, the valaciclovir formulation from crushed tablets has to be discarded after 28 days, which for practical reasons is too short. A paediatric formulation with acceptable palatability, good pharmaceutical quality and stability, and with the possibility of flexible dosing is currently not available.

The aim of this study was to develop a paediatric formulation of valaciclovir and to assess the bioequivalence of the new formulation compared to the brand named valaciclovir tablets.

7.2 Methods

7.2.1 Formulation development

We chose to develop an oral liquid due to the need of flexible dosing in the paediatric population and because this is generally considered acceptable for use in both young infants and children [10]. A liquid can also be used in children and adults dependent on feeding tubes. Next to this, most pharmacies are adequately equipped to prepare oral liquids. The formula-

tion had to meet the following criteria: accurate dosing from first to last dose, non-toxic excipients, acceptable palatability, and good pharmaceutical stability for an acceptable period of time. Appropriateness for administration to neonates was also taken into account during development of the formulation.

Valaciclovir HCl.1 H_2O (Duchefa Farma, Haarlem, the Netherlands) was used as active pharmaceutical ingredient. Stability testing included: inspection of clarity and colour of the solution, pH-measurement, and determination of the concentration of valaciclovir using a stability indicating validated HPLC method. The valaciclovir solution was stored in brown polyethylene terephthalate (PET) bottles. For primary stability testing the valaciclovir solution was stored both at room temperature and at 4°C. At each predefined time point two previously unopened bottles were analysed. For definitive stability testing, six previously unopened bottles per time point were analysed. The new paediatric valaciclovir formulation was prepared in accordance with Good Manufacturing Practice (GMP).

7.2.2 Bioequivalence assessment

Bioequivalence of the new paediatric formulation compared to the innovator product was tested in a randomized, single-dose, open label, two-period crossover, phase-I trial in fasting healthy adult volunteers, according to the EMA guideline for bioequivalence [11]. Valaciclovir is a highly soluble and low permeable drug (biopharmaceutical class 3) and bioequivalence between the two formulations needs therefore to be assessed in vivo [11,12]. The trial was approved by the medical ethical committee of the Radboud University Medical Center (Radboudumc, CMO Arnhem-Nijmegen, NCT01689285). The trial was conducted in conformance with the principles of the current version of the Declaration of Helsinki and with regulations concerning clinical trials. All subjects provided written informed consent.

A total of 16 healthy adult volunteers were randomly assigned to one of two treatment groups. Treatment order was randomly allocated using random values created with SPSS software version 18.0.2 [SPSS Inc., 1993–2007]. Randomisation was stratified for sex. Group A received the reference formulation (valaciclovir tablet 500 mg (Zelitrex®)) on day 1 and after a wash-out of seven days the same dose as the new paediatric formulation (valaciclovir 500 mg (=25 ml) test formulation). Group B received the test formulation at day 1 and the reference on day 8. Food was restricted from 8 h before and 2 h after intake of the study medication. Tablets were swallowed intact and consumed with 200 ml water, while the new paedi-

atric formulation (25 ml) was consumed with 175 ml water. Because of the difference in appearance of the test and reference formulation, subjects and investigators could not be blinded for the treatment. After observed intake blood samples were drawn at the following time points: t=0 (pre-dose), 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 3, 4, 6, 8, 10, 12 hours. Indwelling venous catheters were used for blood collection during the stay at the clinical research centre.

7.2.3 Safety

To determine the safety profile of a single dose of the new valaciclovir formulation, adverse events were collected. Next to this, blood samples were drawn at screening and 12 hours post dosing to determine possible changes in biochemistry and haematology (liver and kidney function parameters and blood cell counts).

7.3 Pharmacokinetic and statistical analysis

Valaciclovir is rapidly hydrolysed to aciclovir, and $C_{\max}$ of valaciclovir is only 4% of $C_{\max}$ of aciclovir. Therefore analysis of exclusively aciclovir is justified to investigate bioequivalence. Aciclovir plasma concentrations were determined using a validated ultra performance liquid chromatography assay with ultraviolet detection, with a lower limit of quantification of 0.03 mg/l and a higher limit of quantification of 30 mg/l. The coefficient of variation for intraday precision ranged from 0.6% to 3.8% and for interday precision from 0.0% to 2.0%. The percentage accuracy of the assay ranged from 91.7% to 105.7%. The analysis was performed by the laboratory of the department of pharmacy of the Radboud University Medical Center, Nijmegen, the Netherlands.

Bioavailability (rate and extent of absorption) was determined by three pharmacokinetic parameters: the area under the plasma concentration time curve (AUC), the maximum plasma concentration ($C_{\max}$), and the time to maximum plasma concentration ($T_{\max}$).

Pharmacokinetic parameters were calculated using non-compartmental analysis with the linear up-log down calculation method (WinNonlin/Phoenix v6.4. Pharsight Corporation, Mountain View, CA, USA). Actual sampling times were used for pharmacokinetic analysis.

The primary comparison was made between aciclovir AUC_{0-12} and $C_{\max}$ values after intake of the valaciclovir new paediatric formulation (test) versus intake of the valaciclovir tablets (reference). AUC_{0-12} is used,

because with the sampling schedule and known pharmacokinetics in adults it is expected to cover at least 80% of $AUC_{0-\infty}$ [1, 11]. The geometric mean ratio for each pharmacokinetic parameter was calculated. Within the WinNonLin software package 90% confidence intervals (90%CI) were calculated using the bioequivalence crossover design tool approach with fixed effects in the model specification. Both regimens were considered bioequivalent if for AUC_{0-12} and C_{max} the 90%CI for the ratio of the test and reference product were contained within the 80–125% acceptance interval [13].

Based on an inter-subject coefficient of variation in AUC of 15.8%, after administration of 500 mg valaciclovir, and equal expected mean values of AUC in the test and reference formulation, a sample size of 16 would be sufficient to investigate bioequivalence with a desired power of 80% and to account for possible dropouts [1, 11, 14].

Comparison of pharmacokinetic parameters between the two treatment groups was performed using an independent t test on log-transformed values. Statistical analysis was carried out using SPSS software version 18.0.2 [SPSS Inc., 1993–2007].

7.4 Results

7.4.1 Formulation

A solution with 20 mg/ml valaciclovir was developed using glycerol (42.5%), citric acid, disodium hydrogenphosphate and water as excipients, with pH 3.5 as target. Electronic tongue testing of the solution indicated that the assumed bitter taste of valaciclovir could partly be masked with the used excipients [15]. Primary stability testing showed that storage at room temperature resulted in loss of almost 10% of the initial concentration of valaciclovir in 3 months. This storage condition was therefore considered not adequate and was no longer investigated. When the solution was stored at 4°C, a shelf life of at least nine months was observed (see Table 7.1).

7.4.2 Bioequivalence assessment

A total of 16 healthy volunteers were included in the study in June and July 2015, of which 9 were male. Median age of the subjects was 31.5 years (range 19, 55) and median body mass index was 23.7 (range 18.8, 29.9). The two treatment groups consisted of each 8 subjects and had similar demographic characteristics (equality of means, $P > 0.10$). The geometric mean AUC_{0-12} of aciclovir after administration of the valaciclovir solution

Table 7.1: Stability of valaciclovir 20 mg/ml solution (mean ($\pm$ sd))

Primary stability testing (50 ml bottles)					
	week 0	week 4	week 8	week 12	–
Room temperature					
pH	3.6	3.6	3.6	3.5	
content (%)	99.5 (0.7)	97.2 (0.6)	94.2 (0.7)	92.8 (0.0)	
Refrigerated (4°C)					
pH	3.6	3.6	3.6	3.6	
content (%)	100.3 (0.1)	99.2 (0.2)	99.0 (0.1)	99.6 (0.1)	
Definitive stability testing (200 ml bottles)					
	week 0	week 6	week 12	week 26	week 39
Refrigerated (4°C)					
pH	3.6	3.6	3.6	3.6	3.6
content (%)	102.5 (0.4)	102.1 (0.5)	100.3 (0.5)	99.6 (0.7)	99.0 (1.0)

was 10.6 (95%CI 9.65 to 11.7) h×mg/l compared to 9.96 (95%CI 8.71 to 11.4) h×mg/l for the reference formulation, and for C_{max} these were 3.63 (95%CI 3.27 to 4.02) mg/l and 2.98 (95%CI 2.59 to 3.44) mg/l, respectively. The geometric mean ratio of the test versus reference formulation was 106 (90%CI 100 to 112)% for AUC_{0-12} and 122 (90%CI 110 to 133)% for C_{max}. The 90%CI for the AUC ratio was within the 80 to 125% acceptance range. The upper limit of the ratio for the C_{max} (133%) was above the limit of 125%. (see also Table 7.2)

Table 7.2: Geometric mean values of aciclovir pharmacokinetic parameters after administration of 500 mg valaciclovir

Parameter	Test (solution) (95%CI)	Reference (tablet) (95%CI)	Ratio T/R (%) (90%CI)
C_{max} (mg/l)	3.63 (3.27, 4.02)	2.98 (2.59, 3.44)	121 (110, 133)
AUC_{0-12} (h×mg/l)	10.6 (9.56, 11.7)	9.96 (8.71, 11.4)	106 (100, 112)
$AUC_{0-\infty}$ (h×mg/l)	11.0 (9.95, 12.2)	10.4 (9.07, 12.0)	–
Residual area (%)	3.9 (2.9, 5.1)	3.5 (2.3, 5.3)	–
$t_{1/2}$ (h)	2.75 (2.50, 3.02)	2.58 (2.23, 2.98)	–
T_{max} (h)[†]	0.75 (0.75, 1.44)	1.25 (1.00, 1.94)	–

[†] T_{max}: median value and interquartile range

The mean plasma concentration-time profiles of aciclovir after administration of the test and reference formulation are depicted in Figure 7.1.

Adverse events reported by the subjects which were possibly related to
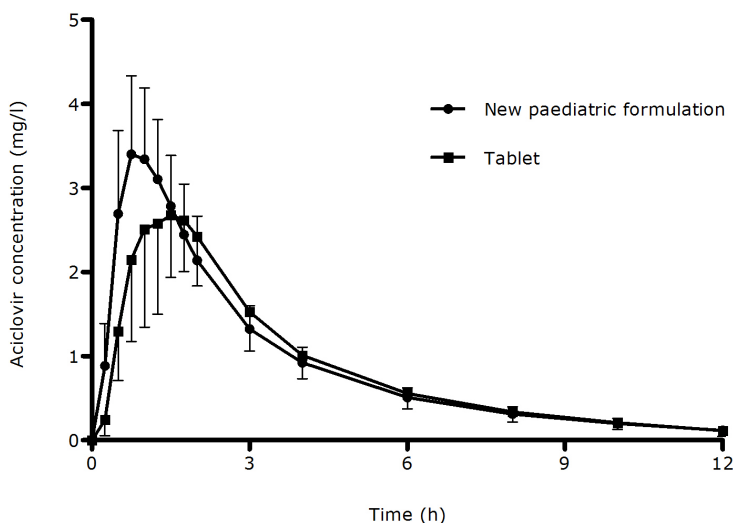
Figure 7.1: Mean ($\pm$ sd) aciclovir plasma concentration-time profile of the new valaciclovir solution and the reference formulation (tablet) after administration of 500 mg valaciclovir.

the study drug were dizziness and fainting ($n = 1$, solution), and nausea, lethargy and headache ($n = 1$, tablet). Both subjects recovered from the adverse events within 24 hours after onset of the symptoms.

7.5 Discussion

The newly developed valaciclovir solution met the criteria for bioequivalence regarding the AUC_{0-12}. However, the upper limit of the 90%CI of the ratio for C_{max} was just above the 125% criterion. The higher mean C_{max} and shorter T_{max} can be explained by a faster absorption rate of valaciclovir from the new paediatric liquid formulation compared to the tablet. The tablet needs to disintegrate first before the valaciclovir is solved and available for absorption. Because of the mild toxicity profile of (val)aciclovir, it is not expected that the slightly higher C_{max} will result in more adverse events [1].

Bioequivalence studies in adults instead of paediatric populations are preferred by regulatory agencies for bridging between adult and paediatric formulations, when it can be assumed that absorption will be comparable in children [16]. Extrapolation of adult biopharmaceutical properties of a drug, such as solubility and permeability, to children should be done with

caution. Biopharmaceutical methods applicable for paediatrics are being developed [17–19]. Following Batchelor et al. it can be estimated that valaciclovir needs to be solved in a relatively lower gastric volume in children compared to adults, but will still be classified as a highly soluble drug. Valaciclovir is absorbed through uptake by dipeptide transporters [20, 21]. It is not expected that the excipients used will influence uptake through these transporters [22]. A possible influence of glycerol on the intestinal transit time did not result in a lower absorption of valaciclovir from the new paediatric formulation.

The preparation of a valaciclovir liquid as described in the FDA label information was found not to be adequate for use in daily practice, because of the need to use crushed tablets and the short shelf life [9, 23]. The pharmacokinetics of an extemporaneously prepared suspension following the FDA label information compared to the brand named tablet has been investigated in a small paediatric study ($n = 8$) [24]. Comparison was not made following the regulatory guidance for the assessment of bioequivalence. The mean relative bioavailability of the suspension compared to the tablets was 91.1% (sd, 33.1%). The newly developed valaciclovir formulation reported here is a solution instead of a suspension, has a longer shelf life (at least nine months) and has a bioavailability comparable to the brand named tablets.

We acknowledge that the relatively low concentration of the developed solution (20 mg/ml) might be a problem when higher doses have to be administered. The EMA recommends a maximum volume of intake per dosing time point depending on the age of a child: < 5 ml for children < 5 years of age and < 10 ml for older children, corresponding to a valaciclovir dose of 100 mg and 200 mg, respectively. At the early stage of development we tested a valaciclovir solution with a higher concentration (50 mg/ml), but this formulation showed inferior stability and was not investigated further. Available tablet strengths for valaciclovir are 250 mg and 500 mg, and the new paediatric formulation has been developed specifically to administer low dosages, or through a feeding tube. Whether higher dose volumes are acceptable, depends on the palatability of the formulation [10]. Palatability influences the acceptability and compliance substantially. Taste of the new paediatric formulation has been tested in vitro [15]. Palatability testing of this new formulation in children has been performed and results and full details will be reported separately.

When an extemporaneous preparation is made by a pharmacist, either by adapting an approved formulation or preparation from raw materials

(unlicensed preparations), the compounding pharmacist is responsible for the pharmaceutical quality of the formulation [25]. Standardization of the formulation is recommended, if possible at an (inter)national level [26, 27]. The preparation method and developmental aspects of the currently developed paediatric formulation have been included in the Formulary of the Dutch Pharmacists (FNA) and are freely available upon request.

With the results of this study it can be concluded that the newly developed valaciclovir solution is an alternative formulation for (paediatric) patients who experience difficulties with the administration of valaciclovir tablets. The new formulation will facilitate to administer targeted dosages to young children and patients depending on a feeding tube.

Acknowledgments

Financial disclosure

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8 In vivo and in vitro palatability testing of a new paediatric formulation of valaciclovir

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Abstract

Background

Palatability testing of oral drug formulations in children is challenging, although palatability is highly relevant for the acceptability of drug formulations.

Methods

The palatability of a new paediatric formulation of valaciclovir in children (aged 4 to 12 years) and their parents was assessed in a randomized, two-period, multicentre, cross-over study. To support formulation development and palatability testing electronic tongue measurements were applied. The liking of the new valaciclovir formulation and the reference formulation was scored on a 100 mm visual analogue scale (VAS). The mean difference in VAS score of the test and reference formulation as indicated by the children was the primary outcome measure.

Results

The electronic tongue measurement indicated taste-masking capabilities for the three different formulations in the developmental phase. A glycerol-based formulation was further tested and compared to the reference formulation prepared out of crushed and suspended tablets. The mean (95%CI) VAS scores as indicated by the children ($n = 20$) were 26 mm $(18, 34)$ for the test formulation and 24 mm $(16, 32)$ for the reference formulation with a mean difference (95%CI) of 2.4 $(-8.5, 13)$ mm, in favour of the test formulation. The mean (95%CI) VAS scores indicated by the parents ($n = 20$) were 45 $(36, 54)$ mm for the test formulation and 46 $(37, 55)$ mm for the reference formulation, and a mean difference (95%CI) of -0.9 $(-12, 9.8)$ mm.

Conclusion

The palatability of the new paediatric valaciclovir formulation was considered non-inferior to the reference formulation prepared out of crushed tablets. We were able to optimize the study design and number of children to be included in the palatability testing by using electronic tongue measurements.

8.1 Introduction

The palatability of oral drug formulations is a key characteristic for the acceptability and compliance to drug therapy, especially for children [1–3]. This aspect was emphasized by the regulatory authorities in the 'Guideline on pharmaceutical development of medicines for paediatric use', in which a verification of the acceptability of a new paediatric formulation prior to its approval is demanded [3]. Next to this, the Paediatric Regulation of 2006 requests the development of appropriate formulations for children, but without performance of unnecessary clinical trials in children [4]. Palatability testing should best be performed in the paediatric target population due to high differences in taste preferences between adults and children, and also between healthy and sick children [2]. However, the experience to test the acceptability of drug formulations in children is still limited. In general, palatability testing of drug formulations with human taste panels is reluctantly chosen and hampered by ethical concerns, toxicological aspects, high costs and poor reproducibility [5]. Taking all regulatory, ethical and statistical requirements into account, in vitro methods for the taste assessment of oral drug formulations might be a favourable alternative.

The electronic tongue is a promising tool used for in vitro taste assessments [6]. These instruments are commonly equipped with a sensor array and based on electrochemical measurement principles including potentiometry, voltammetry and amperometry [7–9]. Most of the used electronic tongue sensors are potentiometric membrane electrodes following the Nernst law and their membrane potentials are correlated to at least one reference electrode [5, 7, 8]. The sensor responses are caused by interactions of the sample molecules with incorporated components of the electrode membrane. Currently, two commercially available electronic tongue systems are employed for the taste assessment of drug formulations, the α-Astree (AlphaMOS, Toulouse, France) and the taste sensing systems TS-5000Z and SB402B (Insent Inc., Atsugi-Shi, Japan) [8–10]. In case of the TS-5000Z and SB402B, different sensors are dedicated to different tastes, such as bitterness and sourness. The α-Astree and non-commercially available electronic tongues work cross-selective, meaning that one sensor is dedicated to a combination of different tastes [10]. Even though electronic tongues are commonly used tools in the development of properly taste-masked drug formulations, the obtained results are only a relative interpretation of taste [6]. A relationship between electronic tongue measurements with human taste has been shown to some extent in adults [7, 9, 11–13].

The aim of this study was to investigate the palatability of a new paedi-

atric oral liquid formulation containing valaciclovir, compared with a formulation of crushed and suspended tablets. Valaciclovir is used for the treatment and prophylaxis of herpes simplex virus and varicella zoster virus infections [14]. However, in Europe, its use is off-label in children below the age of 12 years. The FDA label information describes the preparation of an oral liquid formulation from crushed tablets [15]. To overcome practical problems associated with the formulation prepared out of crushed tablets, such as a short shelf life and obstruction of feeding tubes, we developed a new paediatric valaciclovir formulation [16]. In vivo palatability testing was performed in children and their parents. The electronic tongue was applied for the formulation development as well as for the in vitro taste assessment of the new formulation.

8.2 Materials and Methods

8.2.1 Materials

8.2.1.1 Electronic tongue

For the preparation of the standard and washing solutions for the electronic tongue, potassium chloride (Grussing, Filsum, Germany), tartaric acid (AppliChem, Darmstadt, Germany), potassium hydroxide (Gruessing, Filsum, Germany), hydrochloric acid (Merck, Germany), and absolute ethanol (VWR international, Darmstadt, Germany) were used. The measurements were performed with a sensor array consisting of 8 commercially available sensors (Insent Inc., Atsugi-Shi, Japan), each dedicated to a defined taste: SB2AAE: umami, SB2CT0: saltiness, SB2AE1: astringency, SB2CA0: sourness SB2AC0: bitterness (cationic substances), SB2AN0: bitterness (cationic substances), SB2BT0: bitterness (cationic substances), SB2C00: bitterness (anionic substances).

8.2.1.2 Valaciclovir formulations

Valaciclovir formulations were prepared based on valaciclovir hydrochloride monohydrate (Duchefa Farma, Haarlem, the Netherlands), glycerol (Spruyt Hillen, IJsselstein, the Netherlands), maltodextrin (Kleptose® Linecaps, Roquette, France), citric acid (Duchefa Farma, Haarlem, the Netherlands), disodium hydrogenphosphate (Spruyt Hillen, IJsselstein, the Netherlands), OraSweet® SF (Paddock Laboratories LLC, Minneapolis, US) and purified water. Samples for the formulation development comprised valaciclovir in three different dosing vehicles, named liquid A, B and C. Liquid A contained

glycerol as main excipient, liquid B maltodextrin and liquid C contained both glycerol and maltodextrin as excipients (see Table 8.1).

Table 8.1: Composition of liquids tested by the electronic tongue A) in the developmental phase and B) to support the in vivo palatability testing

Composition of the formulation	Abbreviation	Valaciclovir concentration (mg/ml)
A) Liquids developmental phase		
Liquid A: water, glycerol (42.5%), citric acid,	A1/A2	0
disodium hydrogenphosphate	Val20_A	20
	Val50_A	50
Liquid B: water, maltodextrin (0.5:1 mol/mol	B1/B2	0
valaciclovir), citric acid, disodium hydrogen-	Val20_B	20
phosphate, sorbic acid	Val50_B	50
Liquid C: water, glycerol (25.5%), maltodextrin	C1/C2	0
(0.5:1 mol/mol valaciclovir), citric acid,	Val20_C	20
disodium hydrogenphosphate	Val50_C	50
water	Val20	20
	Val50	50
B) Liquids for in vivo palatability assessment		
Liquid A: water, glycerol (42.5%), citric acid,	Test	0
disodium hydrogenphosphate	Val20_test	20
	Val50_test	50
OraSweet®	Reference	0
	Val25_ref	25
	Val50_ref	50
water	Val20	20

The test formulation for the in vivo palatability assessment was chosen based on the results of the first electronic tongue measurement combined with the results of stability testing. The reference formulation was derived from the extemporaneous liquid made from crushed innovator tablets as described in the FDA label information with OraSweet® SF as suspension vehicle [15, 17]. The mixed valaciclovir formulation was a 1 : 1 mixture of the test and reference formulation.

8.2.2 Methods

8.2.2.1 Electronic tongue

Electronic tongue measurements were performed using the taste sensing system SA402B (Insent Inc.) and the measurement protocol according to Woertz et al. [18]. Two electronic tongue measurements were performed: A) one in the developmental phase and B) one to support the in vivo palatability testing. Most reliable use of electronic tongues measurements for drug formulation development requires a concentration dependent behaviour of the applied sensors: if for example one of the bitter sensors detects a reduced sensor signal for a drug formulation compared to the pure drug, a taste-masking effect of the excipients can be assumed [6,8,19,20]. Therefore, the behaviour of the sensors to different concentrations of pure valaciclovir was determined prior to evaluating the drug formulations. For this purpose, calibration samples containing 0, 0.2, 2, 20 and 50 mg/ml valaciclovir in only water were analysed.

Samples with 0, 20 and 50 mg/ml valaciclovir in liquid A, B and C were tested for the formulation development. The samples for the taste assessment of the newly developed drug formulation comprised 20 mg/ml and 50 mg/ml valaciclovir in the chosen liquid or 25 mg/ml and 50 mg/ml in OraSweet® SF (Table 8.1). Each sample was measured four times, and the last three measurements were used for the data evaluation.

8.2.2.2 In vivo palatability testing

8.2.2.2.1 Study population

Children were eligible if aged at least 4 and less than 12 years and having received (val)aciclovir in the past, or using valaciclovir as prophylaxis at that time, or had a high probability of future use, such as children with primary immune deficiency or recipients of haematopoietic stem cell transplantation. Children and one of their parents attending the outpatient clinic of the Leiden University Medical Centre, Radboud University Medical Centre or the University Medical Centre Utrecht in the Netherlands, were asked for their participation. Children with a sensitivity or idiosyncrasy to medicinal products or excipients were excluded, as were children with any condition that influences taste sensation (such as upper respiratory infection, mucositis or use of medication that influences taste perception).

The Central Committee on Research Involving Human Subjects (CCMO) provided ethical approval for performance of the assessment (NCT01682109). The trial was conducted conform the principles of the

Declaration of Helsinki and regulations concerning clinical trials.

8.2.2.2.2 Palatability testing

The palatability assessment was a randomized, two-period, multicentre, cross-over study. The design of the study was based on the EMA Reflection Paper and description of conducting taste assessment trials in children [2, 21–24]. The main outcome was based on liking indicated by the subjects on a 100 mm visual analogue scale (VAS) combined with five smiley faces (Figure 8.1). Upon signed informed consent, the child and parent were together taken to a private area. First, the 100 mm VAS-smiley scale was explained and practiced by the child. To have the same 'taste starting point' all subjects tasted 4 ml (children 4–8 years) or 8 ml (children 8–12 years and the parent) of the same mixed valaciclovir formulation. After this, 4 ml or 8 ml of the test and reference formulation were presented to the subject in a plastic medication cup in randomized order. To neutralize taste before and between tastings, subjects ate a cracker and rinsed their mouth with water. After tasting each of the three formulations the subject rated their liking on the VAS-smiley scale. Parents were also asked to record which formulation they thought their child would favour.
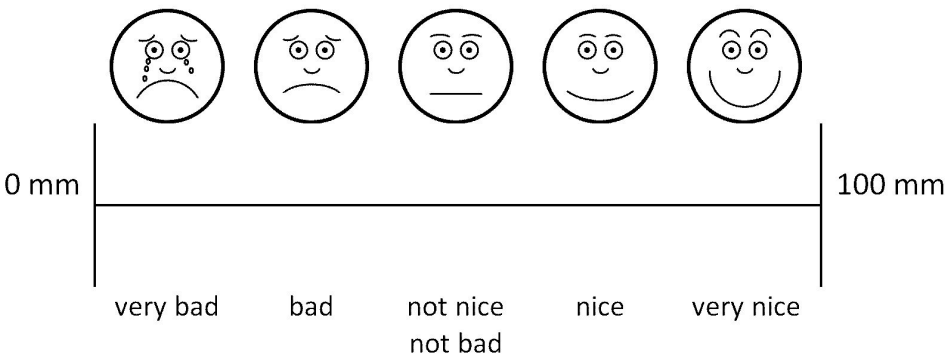
Figure 8.1: Applied combined 100 mm visual analogue/facial hedonic scale.

Treatment order was randomly allocated using random values created with SPSS® software version 18.0.2 (SPSS Inc., 1993–2007). Randomisation was stratified for sex and age in blocks of four. After the mixed valaciclovir formulation, group A first received the test formulation followed by the reference formulation, group B received the reference formulation first.

Correlating the results of an electronic tongue measurement with results from a human taste panel increases the value of the information from both

measurements. Since the ability to describe basic tastes develops during the first decade of a child's life, not the children but the parents were asked to describe the basic tastes of the different valaciclovir formulations [25,26]. Parents could report all the tastes that were applicable: bitter, sweet, salt, sour or other, regardless of the intensity of taste.

With an expected difference between VAS scores of the formulations of 10 mm, a standard deviation of the within subject differences of about 30 mm and a non-inferiority margin of 10 mm, a total of 20 children was needed to reach a power of 80% to demonstrate non-inferiority of the test formulation compared to the reference formulation.

8.2.2.3 Data analysis

8.2.2.3.1 Electronic tongue measurement

Data obtained by electronic tongue measurements were evaluated using Microsoft Excel®, OriginPro 9.0G and Simca 13.0.2 (Umetrics, Sweden) for univariate and multivariate data analysis. The sensor responses were corrected with an external standard solution of 0.5 mM quinine hydrochloride dihydrate (Buchler GmbH, Germany) and the mean values and standard deviations were calculated. The results of the measurements of the dosing vehicles without valaciclovir were used as a positive reference for taste-masking efficiency. The pure drug solutions with 20 mg/ml and 50 mg/ml valaciclovir in water were used as a negative taste reference.

8.2.2.3.2 In vivo palatability testing

The mean difference in palatability of the test and reference formulation as indicated by the children on the 100 mm VAS-smiley scale was used as primary outcome measure. A difference of 10 mm or less was considered negligible. Non-inferiority was shown when the lower limit of the 95% two-sided confidence interval (95%CI) for the difference in VAS scores of the formulations was above -10 mm. The primary analysis was a model without carry-over, with the formulation and period as fixed effects. A model with carry-over effects (interaction period by formulation) was used to verify whether an identical trend in the ordering of the formulations was found and is regarded as a measure of the robustness of our findings. The scores given by the parents were analysed in a similar way. To determine whether there was a correlation for the rating of the child and the parent, the correlation coefficient with repeated observations within families was calculated [27]. Subjects not able to evaluate all three liquids were excluded from the data analysis. Statistical analysis was performed in

SPSS® (software version 18.0.2, SPSS Inc., 1993–2007).

8.3 Results

8.3.1 Electronic tongue measurement

8.3.1.1 Electronic tongue supported formulation development

Concentration dependent sensor responses towards valaciclovir were observed for all applied sensors. Sensors SB2CT0 (saltiness), SB2AE1 (astringency) and SB2BT0 (bitterness of cationic substances) were found to be most sensitive, showing the best response towards the pure drug substance.

The three different formulations (liquid A, B and C, Table 8.1), each with different concentrations of valaciclovir, were analysed by the employed sensor array. With respect to the resulting sensors response pattern, the drug free and drug containing formulations were compared to each other to derive differences in taste properties. Differences within the sensors response pattern were also used to compare the three different formulations regarding their capability to taste-mask valaciclovir. For this purpose, a principal component analysis (PCA) was performed where the formulations liquid A, B and C, the pure drug solutions (20 mg/ml and 50 mg/ml valaciclovir in water) and the dosing vehicles (formulations without valaciclovir) were included (Figure 8.2). The PCA was built based on the first two principal components explaining 92% of the information given by the sensor responses (principal component 1: 71.1%, principal component 2: 20.9%, Figure 8.2). In this case, the first principal component defines the bitterness of the investigated sample: samples located on the left side of the map (dosing vehicles) are less bitter than those located on the right side of the map (drug containing formulations). Merging sensorial information in such a map helps assessing how different samples are detected by the sensors. For example, it indicates a similar taste for the drug formulations 'Val20_A' and 'Val20_C' (Figure 8.2). In general, taste-masking of valaciclovir was observed in all investigated drug formulations. As the formulations containing maltodextrin had an inferior physical stability, liquid A was selected for further investigations.

8.3.1.2 Electronic tongue evaluation of formulations for in vivo palatability testing

The in vitro taste-masking capability of the test formulation (liquid A) was compared to the reference formulation (Table 8.1). The obtained
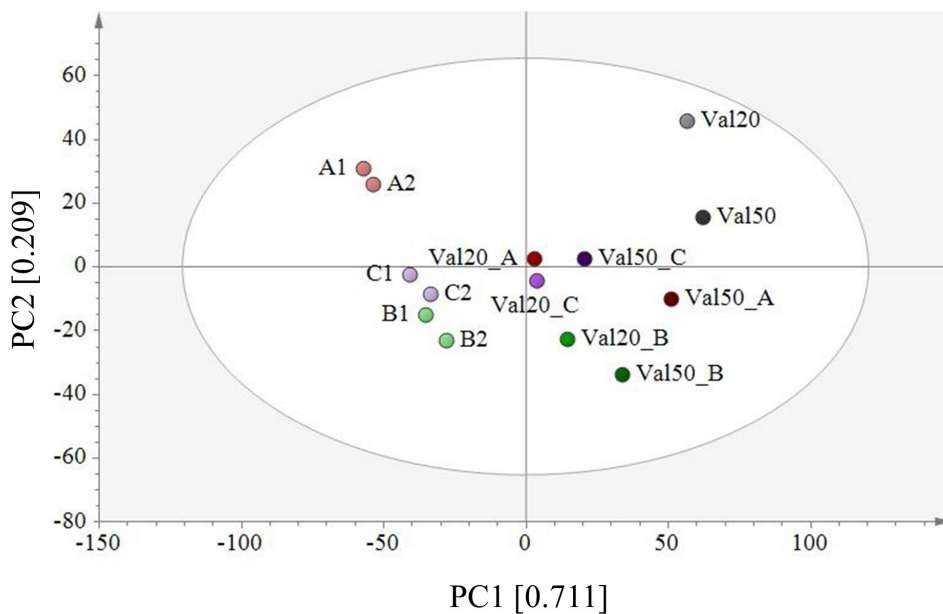
Figure 8.2: PCA map for the formulation development of valaciclovir (mean, $n = 3$, $R^2 = 0.920$, $Q^2 = 0.508$): Val20/50: valaciclovir in water (20 and 50 mg/ml); A1/2, B1/2 and C1/2: dosing vehicles; Val20/50_A/B/C: valaciclovir in dosing vehicles A, B, and C (20 and 50 mg/ml)

sensor responses were initially evaluated by multivariate data analysis by performing a PCA with 2 principal components (Figure 8.3). The first principal component described 88.2% of the sensor information. No differentiation as aforementioned was possible (right side: bitter, left side: less bitter). In this case main information along the x-axis differentiates the two vehicles, which is most probably due to the different viscosity (viscous located on the left side, less viscous on the right side), while bitterness might be differentiated along the y-axis. Thus, when assessing taste masking based on the PCA map, both formulations have to be evaluated individually.
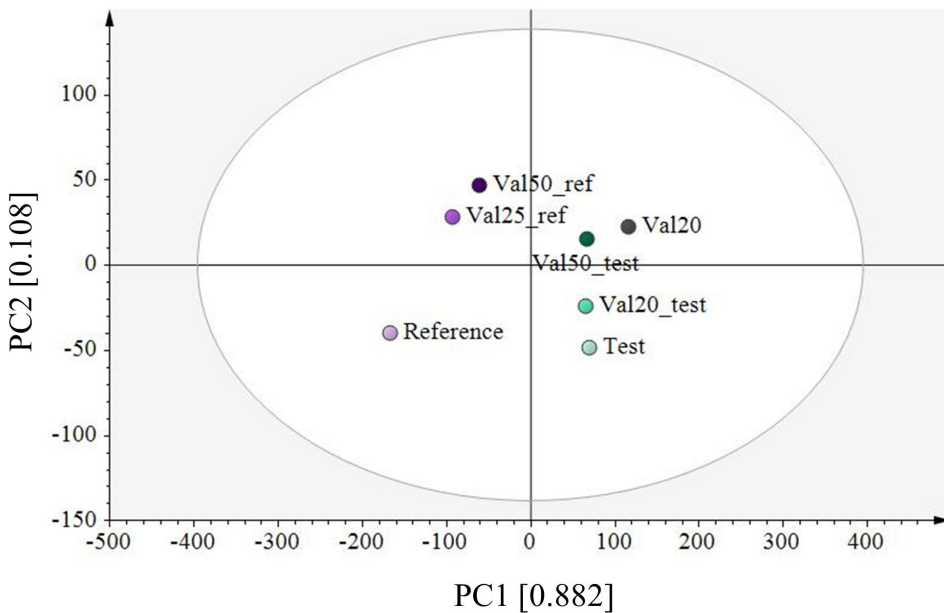
Figure 8.3: PCA map comparing the test and reference formulation containing valaciclovir in different concentrations (mean, $n = 3$, $R^2 = 0.990$, $Q^2 = 0.932$): Val20: valaciclovir in water (20 mg/ml); Test: dosing vehicle of test formulation; Reference: dosing vehicle of reference formulation; Val20/50_test: valaciclovir test formulation (20 and 50 mg/ml); Val25/50_ref: valaciclovir reference formulation (25 and 50 mg/ml)

The small distances between samples 'Val20_test' and 'test' demonstrate a good taste-masking effect of the dosing vehicle. On the contrary, the test sample containing 50 mg/ml valaciclovir (Val50_test) was located close

to the pure drug solution (Val20), indicating only a minor taste-masking effect. The reference formulations (Val25_ref and Val50_ref) were located close to each other but further away from their corresponding dosing vehicle (reference). This indicated differences in taste sensation.

To individually quantify the differences in the taste pattern of the tested samples, the Euclidean distances

$$d(p, q) = \sqrt{\sum_{i=1}^{n} (q_i - p_i)^2}$$

were calculated (Figure 8.4 and Table 8.2). For the test and reference formulations, the samples containing 20 mg/ml and 25 mg/ml valaciclovir demonstrated a high taste-masking efficiency indicated by high distances between the drug formulations to the pure drug solution and low distances to their corresponding dosing vehicles. Both calculated Euclidean distances of the reference formulations were higher than of the test formulations (Table 8.2), meaning that the reference formulations were less similar in the taste pattern to either the pure drug solution or the reference dosing vehicle. Due to this contradictory outcome, the test formulation was assumed to better taste mask valaciclovir than the reference formulation. As a result a difference between liking of the test and reference formulation of approximately 10 mm on the 100 mm VAS score in favour of the new paediatric formulation was expected for the palatability assessment in children.

Table 8.2: Euclidean distances (mV) between formulations for the test and reference formulations ($n = 3$, mean $\pm$ sd)

Formulation	Dosing vehicle vs formulation	Pure drug solution vs formulation
Test (liquid A)		
20 mg/ml	0.94 $\pm$ 0.05	2.48 $\pm$ 0.08
50 mg/ml	2.18 $\pm$ 0.04	1.62 $\pm$ 0.05
Reference (OraSweet®) SF		
25 mg/ml	3.36 $\pm$ 0.06	5.51 $\pm$ 0.04
50 mg/ml	4.63 $\pm$ 0.04	4.53 $\pm$ 0.03

8.3.2 In vivo palatability testing

Twenty-one children and 20 parents were included in the taste assessment. One child tasted only two formulations, and the parents of another child
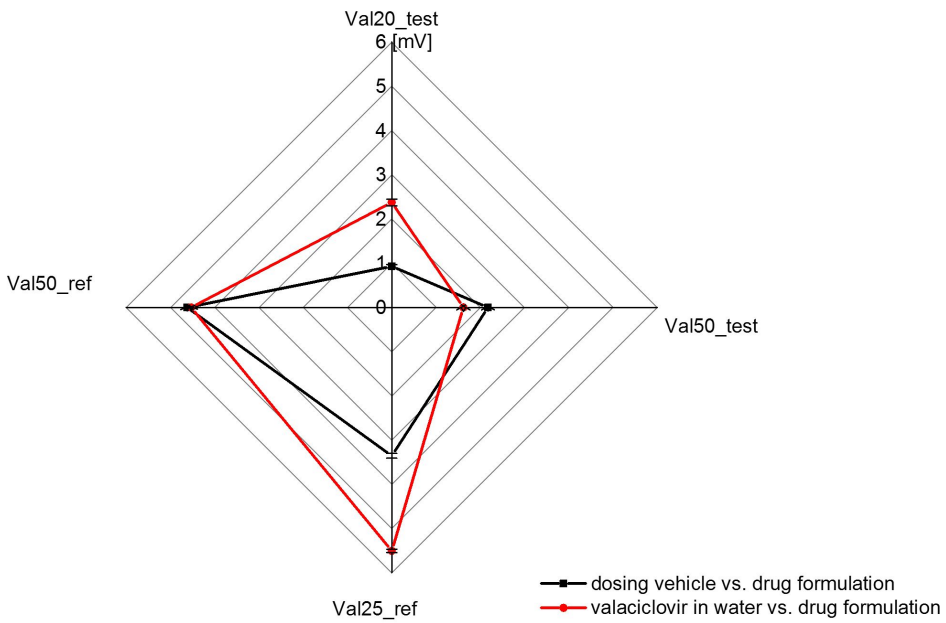
Figure 8.4: Euclidean distances of the drug formulations compared either to the corresponding dosing vehicle (black) or the corresponding samples of valaciclovir in water (red) (mean $\pm$ sd, $n = 3$): Val20/50_test: valaciclovir in dosing vehicle of test formulation (20 and 50 mg/ml); Val25/50_ref: valaciclovir in dosing vehicles of reference formulation (25 and 50 mg/ml)

were not able to attend. Therefore, VAS scores of all three liquids from 20 children and 20 parents (19 child-parent pairs) could be included in the analysis. Characteristics of the subjects and VAS scores are displayed in Table 8.3. For the children, mean (95%CI) VAS scores were 26 mm $(18, 34)$ for the test formulation and 24 mm $(16, 32)$ for the reference formulation with a mean difference (95%CI) of 2.4 $(-8.5, 13)$ mm, in favour of the test formulation. The test formulation can herewith be considered non-inferior to the reference formulation. Based on the VAS scores of the test and reference formulation, 7 children (35%) preferred the test formulation, 4 (20%) the reference formulation and 9 (45%) children indicated a difference of 10 mm or less between the formulations. Inclusion of the interaction of period by formulation as a fixed effect did not have a significant effect $(P = 0.871)$ and therefore no carry-over effects were observed.

Table 8.3: Baseline characteristics and results from scores on the visual analogue scale (VAS score)

Baseline characteristics (median, range)	Children	Parents
	$n = 20$	$n = 20$
age,years	8.7 (6.3, 11.9)	41 (34, 54)
sex (female/male)	6/14	11/9
underlying condition		
primary immune deficiency	18	
stem cell transplantation	2	
VAS scores (mm) (mean, 95%CI)		
test formulation	26 (18, 34)	45 (36, 54)
reference formulation	24 (16, 32)	46 (37, 55)
test − reference formulation	2.4 (–8.5, 13)	–0.9 (–12, 9.8)

For the parents, mean (95%CI) VAS scores were 45 $(36, 54)$ mm for the test formulation and 46 $(37, 55)$ mm for the reference formulation and a mean difference (95%CI) of -0.9 $(-12, 9.8)$ mm, in disadvantage of the test formulation. Based on the VAS scores of the formulations, 6 parents (30%) preferred the test formulation, 7 (35%) the reference formulation and 7 (35%) indicated a difference of 10 mm or less between formulations. The test for the interaction of period by formulation showed a P-value of 0.074 and therefore carry-over effects cannot be ruled out for the parents. When only the results from the first period are analysed: 11 parents tasted the test formulation and 9 the reference formulation. In this way, the mean (95%CI)

difference between the VAS scores of the formulations was 12 $(-4.5, 28.6)$ in favour of the test formulation.

VAS scores of the children and the parents did not show a correlation (correlation coefficient = $-0.03, P = 0.91$). Predictions made by 18 parents which formulation would be preferred by their child showed 6 (33%) correctly predicting their child's preference and 10 (56%) choosing a different formulation. Two children were not able to show a preference.

Nineteen parents assigned basic tastes to the formulations (Table 8.4). The predominantly chosen basic tastes by the parents for both the reference and the test formulation were bitterness and sweetness. Twelve parents (63%) thought the reference formulation had a bitter taste and 8 parents (42%) thought the test formulation had a bitter taste. This indicates that the bitter taste was better masked with the test formulation. The high percentage of parents tasting bitter corresponds to the results found by the electronic tongue, in which the bitter sensor (SB2BT0) was most distinctive for valaciclovir, together with sensor signals of the saltiness and astringency receptor. The parents did not predominantly choose the saltiness taste. Twelve parents (63%) indicated the reference formulation as being sweet and eleven parents (58%) for the test formulation.

Table 8.4: Basic tastes assigned to the tested valaciclovir formulations by the parents $(n = 19)$

Taste	Reference $(n, \%)$	Test $(n, \%)$
Bitter	12 (63%)	8 (42%)
Sweet	12 (63%)	11 (58%)
Salt	1 (5%)	2 (11%)
Sour	3 (16%)	8 (42%)
Other	0 (0%)	1 (5%)

8.4 Discussion

The palatability of the newly developed paediatric valaciclovir formulation was non-inferior to the reference formulation in children. No significant differences were found between the liking of the new paediatric formulation of valaciclovir, compared to crushed and suspended tablets. Non-inferiority was shown for the children, but not for the parents, since the lower margin of the 95% confidence interval was just below -10% (-11.6%). This can be explained by the higher mean VAS score of the reference formulation compared to the test formulation, and the somewhat higher variance in VAS

scores of the parents, compared with the children. Variability in preference of the test or reference formulation was also observed between the subjects: 35% of the children and 30% of the parents preferred the test formulation and 20% of the children and 35% of the parents preferred the reference formulation. No carry-over effects were observed for the children, but carry-over effects for the parents could not be ruled out. When only the results from the first period are analysed, non-inferiority of the reference formulation was observed, also for the parents. When carry-over effects are observed, an alternative for an AB cross-over design should be chosen [28]. However, this would imply a possible larger burden of the assessment such as a longer assessment, more visits or more subjects, which are undesirable in studies involving paediatric patients.

No correlation was found between the liking of the formulations by the children and their parents. Only a minority of the parents was able to correctly predict the preferred formulation for their child. This confirms that taste assessments of (new) paediatric formulations should be performed in children [2].

This is the first study in which results from palatability testing in children and adults as well as results from an electronic tongue assessment are simultaneously available. Based on the results from the electronic tongue measurement, it was expected that the test formulation would have a slightly better palatability than the reference formulation. Without the use of the electronic tongue in the palatability testing, no difference in VAS scores would be expected and the number of children to be included would have been more than 3.5 fold higher. The expected difference of 10 mm in VAS score between the formulations could not be confirmed, nor ruled out. An explanation could be the complexity of tastes of both formulations. The pure active pharmaceutical ingredient valaciclovir has a bitter taste. The test and reference formulation both contain citric acid, which has a sour taste, and both contain sweet tasting substances. Next to this, sensors to capture the complexity of sweetness were not available. Sweetness could thus not be measured with the electronic tongue. Especially children mostly like sweetness, but it is generated by substances with a wide molecular diversity. Sweetness sensors for selected sweet tasting substances have been developed and development is extended to more complicated molecules [7, 29, 30]. Validated sensors to measure sweetness are needed for optimal in vitro evaluation of paediatric drug formulations.

Besides the taste also texture, smell and appearance of a formulation can influence acceptability [2, 3]. These aspects were not scored nor measured

during the assessment. However, for acceptability, solutions are generally preferred over suspensions [31]. The new formulation of valaciclovir is a solution, which would for that reason be preferred over the suspension out of crushed tablets.

One of the strengths of this study is that we were able to perform palatability testing in a paediatric target population using valaciclovir, or with a high probability of future use. A different paediatric population who could benefit from the new paediatric formulation of valaciclovir are (premature) neonates. Because of the possible burden of the trial assessments and the lack of experience with methods for acceptability testing of drug formulations in neonates, they were not included in the study.

Debatable is the difference in valaciclovir concentration between the test and reference formulation (20 vs. 25 mg/ml, respectively). As was shown with the electronic tongue measurement a higher concentration of valaciclovir in water results in a higher sensor signal and an assumed more bitter tasting formulation, which would be unfavourable for the reference formulation. However, it was chosen to use concentrations that would be used in clinical practice. The concentration for the test formulation was based on pharmaceutical stability testing and expected ease of calculation of the volume to be administered. The concentration of the reference formulation was as included in the FDA label information, and would thus be used in daily practice.

In conclusion, we found that in children the palatability of the new paediatric valaciclovir formulation was non-inferior to the reference formulation prepared out of crushed tablets. Next to a longer shelf life than crushed and suspended tablets combined with a comparable exposure as tablets, the results of this palatability study further support the use of the new paediatric formulation as an alternative formulation for (crushed and suspended) valaciclovir tablets. By applying an electronic tongue measurement during the development and as screening for the in vivo palatability testing, we were able to optimize effort and number of children to be included in a clinical trial.

Acknowledgments

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9 General discussion

9.1 Introduction

The results of the studies described in this thesis show that it is possible to make antiviral drug treatment easier for children. The focus was on orally administered drugs. Two main topics were investigated. At first, the possibility to simplify antiretroviral dosing regimens was investigated and secondly, a new paediatric formulation of valaciclovir was developed. Different aspects were taken into account when searching for more child friendly treatments. These included dosing frequency, appropriateness and acceptability of a formulation, and the biopharmaceutical properties of a drug.

9.2 Research in children

9.2.1 Clinical trials

Treatment options for children are often limited because of the lack of evidence on safe and effective dosing regimens, and the availability of appropriate formulations. One of the ways to collect more information on the appropriate use of medicines in paediatrics is to include children in clinical trials. However, to include children as subjects in (medical) scientific research has long been deemed unethical which consequently resulted in the lack of information on safe and effective use of medicines in children. Slowly this changed to the opinion that it is unethical to exclude children from medical research, and thus denying the opportunity to search for better treatment options [1]. The Dutch law on medical research involving human subjects states it is not allowed to include children in medical-scientific research, unless the research will directly benefit the child, or unless the burden of the trial assessments is negligible for research needed to be performed in a specific population. For example for new drugs, strict application of the law results in the nearly impossible situation to perform clinical trials with children. In 2009 the committee 'Doek' investigated the Dutch situation and came forward with recommendations [2]. One of the major recommendations was to change the law, from the 'no, unless' principle, into a 'yes, if' principle, where researchers have to meet several criteria before they are allowed to perform medical research in children, such as a clear description in the study protocol of the burden and risk of participation. The protocol should also describe whether the subject might benefit from participation. When the research is not directly of benefit

for the subject, the risk and burden should be minimal compared to the standard treatment. Based on the recommendations from the committee, the House of Representatives agreed in 2015 to change the Dutch law on medical research, extending the possibilities for performing research in children. The new law will also encompass that children from an earlier age on (16 instead of 18 years) have to give informed consent for participation in medical scientific research. However, children below this age should also be involved in deciding about participation in research. As agreed in the United Nations Convention on the Rights of the Child, all children capable of expressing a view, have the right to be heard in decisions affecting them. Therefore, they should also be included as much as possible when they participate in research and understandable information should be available for them. From a broader perspective, children, and not only their parents, can be part of research networks focussing on certain research questions within specific paediatric patient populations. Involvement of children and their parents in the earliest stages when setting up research will lead to additional research questions and improved acceptance and willingness to participate.

The results of a large international clinical trial investigating the safety and efficacy of a once-daily lopinavir/ritonavir containing antiretroviral drug regimen in a large group of children (KONCERT, $n = 173$) is described in **chapter 1** [3]. In adults a once-daily dosing regimen with lopinavir was already approved by the European Medicines Agency (EMA) in 2009. Early pharmacokinetic studies in children indicated an adequate exposure when children use lopinavir once daily [4–7]. To investigate the efficacy in HIV-infected children of a once-daily lopinavir/ritonavir containing regimen, results from a clinical trial were needed, but setting up clinical trials are mostly very expensive and more difficult in paediatric than in adult populations. It took until 2014 for the results of the KONCERT trial to be presented at an international conference [8]. More children treated with once-daily lopinavir/ritonavir experienced viral load rebound within the first 48 weeks: 14% compared to 8% in children treated with twice daily lopinavir. The estimated difference between the two groups was 6%, with a 90% confidence interval of −2 to 14%. The upper limit was above the predetermined non-inferiority bound of 12%. Even after adjustment for the difference in viral rebound between the two groups at baseline, the upper bound of the confidence interval was 11%, only just within the predefined 12% margin of non-inferiority. Based on these results the once-daily dosing regimen has not been included as approved dosing regimen in

the summary of product characteristics [9]. In the 2015 guideline of the PENTA-network (Paediatric European Network for Treatment of AIDS), other boosted protease inhibitors with proven efficacy when given once daily, are preferred over lopinavir combined with ritonavir [10].

9.2.2 Real-world evidence

Next to clinical trials, alternative ways to collect information on safe and effective use of medicines in children can be found. For example, there is growing interest in the use of real-world evidence as part of adaptive licensing, or adaptive pathways [11]. In real-world research, efficiency rather than efficacy of a treatment is investigated. Applying adaptive pathways is intended to result in more timely access to new medicines.

The observational cohort study reported in **chapter 2** is an example of investigating a once-daily lopinavir antiretroviral regimen using over ten years of clinical experience in real-life setting. While awaiting performance and results of a randomised controlled trial on lopinavir once daily in children, several paediatricians already treated selected children with this regimen. Since safety and efficacy were not yet investigated in a clinical trial, children who switched to once-daily lopinavir were closely monitored on clinical outcome and exposure to lopinavir. While on treatment 82–100% of the children ($n = 40$) had an undetectable viral load during yearly follow-up visits. The response rate is comparable to what was found in the KONCERT trial and it supports PENTA's statement that selected, adherent HIV-infected children can be treated with once-daily lopinavir/ritonavir when properly monitored. Although done with caution, a comparison of the results from both studies supports the use of real-life research as possible valuable alternative for the performance of a clinical trial.

9.2.3 Off-label and on-label use in clinical practice

Studies show that off-label and unlicensed prescribing in children ranges from 3 to 56% of the prescriptions in community practice, and from 36 to 100% in hospital settings [12]. Ethical issues arise when deciding to treat children with an off-label regimen, especially when it is not part of a clinical study. Current Dutch legislation obliges the clinician to discuss with the patient (and/or its carers) the off-label use. Also, the intended use should be described in authorised protocols, otherwise it should be discussed with a pharmacist. The Dutch Knowledge Centre for Paediatric

Pharmacotherapy (NKFK) was founded in 2005 with the main goal to develop an evidence-based paediatric formulary. At the end of 2015 over 650 drugs have been reviewed by NKFK and are now included in the Dutch paediatric formulary. From every drug listed in the formulary, the level and kind of evidence for the dosing advice is given (summary of product characteristics, published literature and/or expert opinion). The Dutch Paediatric Association (NVK), Dutch Association of Hospital Pharmacists (NVZA) and Royal Dutch Pharmacists Association (KNMP) recognize the Dutch paediatric formulary as professional standard. On-label and off-label use is thus supported for the most commonly used drugs in children in the Netherlands. It is important to evaluate safety and efficacy or efficiency when drugs are used off-label and to report the results found, as has been done in chapter 1 and 2 for the once-daily use of lopinavir in children.

For darunavir a once-daily regimen has been approved for a subgroup of HIV-infected children from the age of three years, first in January 2013 by the US FDA and at the end of 2014 by the EMA. Despite its approval for once-daily use, guidelines at the end of 2015 advised against the use of a once-daily regimen in children < 12 years of age, because of the lack of data with respect to efficacy, safety and exposure. An odd situation thus exists where an expected more convenient dosing regimen is approved, but not yet recommended by professional guidelines. To support the approved dosing regimen, exposure was measured in the first twelve children with the age of 6–12 years in the Netherlands who switched to the once-daily dosing regimen as described in the summary of the product characteristics. The results are described in **chapter 3**. Exposure was significantly lower than predicted based on population pharmacokinetics, but found to be adequate. These findings highlight the need to validate suggested dosing regimes in daily practice. In **chapter 4**, a population pharmacokinetic model of lamivudine was used to suggest a new dosing regimen for a subgroup of children in which the target exposure (area under the curve) is not reached with the current dosing regimen. Both noncompartmental analysis and population pharmacokinetic analysis can be used to evaluate dosing regimens. However, depending on the research question to be answered and the data available, either one or a combination of the two methods will be more suitable to evaluate and determine a well-founded dosing regimen.

9.3 Paediatric formulations

9.3.1 Regulation

The lack of appropriate formulations for children and information on dosing, efficacy and safety has been recognized by regulators already decades ago. This resulted in new regulation to stimulate the development of paediatric formulations in the US by the FDA and in Europe by the EMA. In Europe the Paediatric Regulation came into force at the end of 2006 [13]. Main objectives of the regulation are to facilitate the development and accessibility of medicinal products for use in children and to make sure that use of these products are properly investigated and authorised for children. These goals should be achieved without subjecting children to unnecessary trials [13]. The Paediatric Regulation covers medicinal products already authorised and products yet to be authorised. Certain obligations and incentives, such as extension of patent protection, were deemed necessary to stimulate the development and research on medicinal products for children. For products already off patent a paediatric use marketing authorisation (PUMA) can be granted, which rewards 10 years of data protection. The pharmaceutical company has to submit a paediatric investigation plan (PIP) to the EMA to obtain an incentive. A waiver can be granted, mainly when the disease for which the drug is intended to be approved does not occur in children.

In 2012, a report was published describing the achievements of the first five years after the paediatric regulation came into force [14]. The proportion of clinical trials in children and neonates increased, and more information relevant for the treatment of paediatric patients became available. Twenty-six new paediatric formulations were approved. Only one PUMA was granted. Although EMA and others count the successes on the first years of the paediatric regulation, others debate the success, also because of the gap between development and what is most needed to treat children properly [15–19]. The ten-year report is expected in 2017, for which research networks are also approached to give input in whether information stemming from PIPs is included in treatment guidelines. Despite all the efforts so far, there is still a lack of appropriate formulations and information on medicines for children in daily practice. Consequently, there is an on-going need of pharmacists and clinicians to search for a safe, effective and acceptable treatment for paediatric patients [20].

9.3.2 Appropriateness

As also mentioned in the introduction of this thesis, the appropriateness of a formulation is important for the success of the treatment with drugs in children. An important document on the appropriateness of paediatric formulations is the EMA Reflection paper: 'Formulations of choice for the paediatric population' [21]. In this report requirements for formulations are described, considering the different stages of growth and development, and the different routes of administration. Separately described are the age appropriateness of dosage forms, excipients and the importance of taste, smell and texture. Next to what is described, the environment in which the patient has to take the drug is important, as for example intravenous administration is usually restricted to hospital settings. Requirements for the development of medicines for paediatric use are described in a more recent guideline [22]. A separate chapter in this guideline describes the need to assess patient acceptability, highlighting the importance given by regulators to the acceptability of drugs for children. The Netherlands Organisation for Health Research and Development (ZonMW) has recognized the importance of the development of an appropriate paediatric formulation of valaciclovir resulting in 2010 in a grant within the 'Priority Medicines for Children' Programme for the VALID-project. **Chapter 7** and **chapter 8** describe the first results of the VALID-project, which are the development, bioequivalence assessment and palatability testing of a new paediatric formulation of valaciclovir.

9.3.3 Liquid paediatric formulations

Liquid formulations are generally considered appropriate for children from birth. It can be challenging to develop liquid formulations with good palatability and adequate stability. Excipients have to be used for preservation, to mask bad taste and sometimes to increase solubility. Excipients can give adverse effects and can also influence the pharmacokinetics of (co-administered) drugs. The STEP database is a large database collecting all information on the Safety and Toxicity of pharmaceutical Excipients for Paediatrics [23]. Detailed information is available of almost thirty excipients. Unfortunately, no such structured information is yet available of the influence of different excipients on the absorption of drugs. For the new valaciclovir paediatric formulation (**chapter 7** and **8**), it was chosen to develop a liquid, mainly because of the appropriateness of a liquid for young infants, children and patients dependent on feeding tubes, and the

possibility of flexible dosing. Moreover, most pharmacies are adequately equipped to prepare oral liquids, but not to prepare (mini) tablet formulations.

9.3.4 Solid paediatric formulations

For the antiretroviral drug lopinavir/ritonavir paediatric tablets were developed, as alternative for the unpalatable liquid formulation that has to be refrigerated. On approval by the FDA and EMA the posology in the product labels differed: the dose was based on body surface area only by the EMA, while it was based on body surface area or bodyweight by the FDA. Dosing based on bodyweight is usually considered easier than dosing on body surface area because the latter requires the use of a mathematical equation with a higher chance on miscalculations. The bodyweight based dosing recommendations with the new paediatric tablet, as described in the FDA label, were evaluated in a large pharmacokinetic substudy ($n = 53$) of the aforementioned KONCERT study. The results are described in **chapter 6**. It was shown that exposure was higher when using the paediatric tablets, compared to children receiving the liquid formulation, but exposure was found to be adequate. As a result of these data the European summary of product characteristics was updated by including the bodyweight based dosing recommendations for the paediatric tablets [9].

9.3.5 Liquid versus solid paediatric formulations

Liquid and solid formulations both have their own advantages and disadvantages. In general, less excipients have to be used for solid formulations and there are also less problems with stability of the active pharmaceutical ingredient. Techniques to increase stability and mask bad taste can easier be applied to solid compared to liquid formulations. On the other hand, easier use through feeding tubes and the possibility of flexible dosing are advantages of liquid formulations over solid formulations. Combining advantages of both formulations can be possible in flexible solid dosage forms. This type of dosage form has been recommended as most suitable for children, particularly for developing countries, by a World Health organisation expert forum in 2008 [24]. Examples are (orodispersible) minitablets and orodispersible films. Promising results have been shown for minitablets for which there is growing evidence that they are well accepted in the youngest children including premature infants [25–30]. Acceptability was equal, or even better, for uncoated minitablets compared to liquid placebo

formulations in neonates and children 0.5–6 years [27,28]. Older children in need of a higher dose can use more minitablets. Acceptability of different numbers of minitablets and the preferred size of the minitablets has been investigated in 2 and 3 year old children [30]. The ability to swallow 10 minitablets with a 3 mm diameter was very high: 75% and 93% in the 2 and 3 year olds, respectively. No significant differences were observed between the number (5 or 10) and the size (2 or 3 mm) of the minitablets.

As described in **chapter 5**, the pharmacokinetics of a drug can be different when different formulations, for example a liquid instead of a tablet, are administered. Pharmacokinetics (mainly absorption) can also be changed when formulations are manipulated. Pharmacists should know that they are fully responsible for the quality of a formulation when drugs are manipulated, but also for providing information on a possible change in exposure. Taking into account the biopharmaceutical properties of a drug, combined with the knowledge of pharmaceutical formulations and the function of the excipients used, (possible) differences in exposure can be explained or predicted. One needs to be aware that exposure can be different between formulations, and also when manipulating formulations, especially for drugs with a low aqueous solubility (biopharmaceutical classification system, class 2 and 4). Pharmacists are equipped with the knowledge of all these areas of interest. They should be able to combine the relevant information and translate it to a comprehensible advice for clinicians, patients and caregivers.

9.3.6 Extrapolation of adult data to children

In **chapter 7** the bioequivalence of the new paediatric valaciclovir formulation compared to the brand named tablet is described. For bridging between adult and paediatric formulations, bioequivalence studies of paediatric formulations are preferably performed in healthy adults, not in children, but only when it can be assumed that absorption will be comparable in children. It can be questioned whether we already know enough about the differences in biopharmaceutics in children compared to adults. We have to acknowledge that the same (general) biopharmaceutical principles might not be valid in children, since they are based on adult anatomy and physiology data. An example is lamivudine, which is classified as a highly water-soluble drug. In adults the administered dose will completely dissolve in the gastro-intestinal fluid. The estimated gastro-intestinal volume for adults to determine the solubility is 250 ml. Children have a lower gastro-intestinal volume, and although a lower dose is adminis-

tered, the dose is non-proportionally lower. The maximum solubility of lamivudine is approached in the youngest children. Only when the drug is dissolved, it will be available for absorption. In vitro models need to be developed to better predict solubility and permeability in children. Some groups have already proposed rules to determine a paediatric biopharmaceutical classification system, but consensus needs to be reached before it can be applied by regulators [31–33].

9.3.7 Acceptability of paediatric formulations

With the new EMA guideline on the development on paediatric formulations coming into force, acceptability has to be shown by applicants of a new drug formulation to be approved [22]. However, it is debatable when a drug formulation can be considered acceptable, and how acceptability should best be tested in children [34]. Future research is needed to determine which methods are best to test for acceptability of different formulations, for different diseases and for children with different ages and stages of development. The definition of good acceptability will depend on the patient population, the (severity of) disease, already available alternative treatments, and possibilities for formulating the drug. Experience with methods to test drug acceptability in children is limited [35–38].

For the new paediatric formulation of valaciclovir taste and acceptability were tested with an in vitro method (electronic tongue) combined with an in vivo method. Children and one of their parents performed the in vivo taste assessment, where the palatability score of the children was the primary outcome. Palatability testing of paediatric formulations can best be performed in children, because of differences in taste perception between adults and children [21]. Taste preferences are dependent on a lot of factors, such as age, health status, sex, environment and previous experiences. Taste preferences are already different between neonates, which can partly be explained by the food intake of the mother during the pregnancy and breastfeeding period [39]. Next to this, genetic differences determine for example whether humans are able to taste bitter [40]. The electronic tongue data suggested better taste-masking properties of the new paediatric formulation of valaciclovir, compared to the reference valaciclovir formulation. Based on these results the expected number of subjects needed to be included in the in vivo taste assessment could be more than 3.5 fold lower. To optimize the number of paediatric subjects needed for acceptability testing, more experience is needed with extrapolating results from in vitro and animal to in vivo data.

9.4 Future perspectives

Future developments in formulating drug products will be beneficial to optimize dosing regimens and to improve the acceptance of paediatric drug treatment. A large and fast developing area of research is nanotechnology in medicine (nanomedicine) [41–43]. Nanotechnology can have several aims, such as targeting drugs more specifically to the sites affected by the disease and increasing solubility of mainly low solubility class drugs. Solubility of drugs is a more critical factor for children and techniques to overcome this issue can be of high value for paediatric drug development and treatment. Improved pharmacokinetic profiles after oral administration of nanoformulations of different non-nucleotide reverse transcriptase inhibitors and protease inhibitors have been observed in preclinical studies [44]. More examples for the antiretroviral treatment can be found in the development of long acting drugs [45, 46]. One specific example is the development of an injectable formulation of a combination of two antiretroviral drugs (rilpivirine and cabotegravir) [47]. This combination is currently investigated in a clinical trial in HIV-1 infected adults, where this formulation is injected every 4 or 8 weeks (NCT02120352). Children mostly not favor parenteral administration, but it might be an alternative for the intake of a poor palatable drug every day.

When the new Dutch law on medical research involving human subjects comes into force, it is expected that more research with paediatric subjects can be performed in the Netherlands and that participation of Dutch research groups in international research can be strengthened. Close collaboration is important to be able to further optimize methodology in paediatric drug research. There will always be chances to intensify and broaden existing collaborations and to create new research networks. In case of research on acceptability of drug formulations in children, it can be beneficial when not only researchers within the field of paediatric drug research will cooperate, but also when we reap the benefits of the expertise developed by others in different areas of (paediatric) research. Examples are the area of research where acceptability of food products by children is investigated and the area where in vitro methods are developed. Taking into account differences in ethical and regulatory requirements, methods can be modified and used in paediatric drug research to optimize testing of drugs. The number of children needed to be included in clinical trials can thus be limited and safe and efficacious treatments can be made more timely available for children.

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Making things easier

Summary

Introduction

Paediatric patients cannot simply use the same drug formulations and dosages as adult patients. The appropriateness of a formulation is determined by the characteristics of the patient to be treated with the drug, the specific drug and the dose to be used, and the environment in which it has to be administered. Oral administration of drugs is generally considered the most convenient method to administer drugs, also for children. Since the dose might have to be changed while children grow and develop, drug formulations with the possibility of flexible dosing are preferred. Different aspects determine acceptability, such as food restrictions, size of tablets, amount of drug to be taken and the palatability. It can be expected that the lower the acceptability of the drug formulation, the more important also the dosing frequency is.

The aim of this thesis was to investigate how pharmacotherapy with antiviral drugs can be optimized, to ensure safe and effective treatment for children suffering from acute and chronic viral infections.

Part 1: Dosing regimen - pharmacokinetics

Children infected with human immunodeficiency virus (HIV) require antiviral treatment to prevent the development of acquired immune deficiency syndrome (AIDS). With current knowledge and treatment options, HIV-infected children can expect to have full adult life, but with the necessity to continue treatment lifelong. The need of combination therapy combined with the need of long-term adherence puts a challenge on the patient and its caregivers. First-line antiretroviral therapy as recommended by the PENTA network (Paediatric European Network for Treatment of AIDS) is a dual or triple nucleoside reverse transcriptase inhibitor (NRTI)-backbone together with either a ritonavir-boosted protease inhibitor or a non-nucleoside reverse transcriptase inhibitor (NNRTI).

The focus of the first part of this thesis is on how to simplify or optimize the dosing schedule of antiretroviral drugs as part of combination antiretroviral therapy.

Chapter 1

In this chapter the results of KONCERT are described. KONCERT is the acronym for "Kaletra ONCE daily Randomised Trial of the pharmacokinetics, safety and efficacy of twice-daily versus once-daily lopinavir/ritonavir (lopinavir/r) tablets dosed by weight as part of combination antiretroviral therapy in HIV-1 infected children (PENTA 18)." This large international clinical trial was performed within the PENTA-network. Children could participate when they were at least 15 kg, able to swallow intact tablets, treated with lopinavir/r twice daily, and were virologically suppressed for at least 24 weeks. They were randomized to continue lopinavir/r twice daily as part of their antiretroviral regimen, or to change to once-daily treatment. The main outcome measure was a confirmed viral load $\geq$ 50 copies/ml by 48 weeks (12% non-inferiority margin). The main outcome measures for the pharmacokinetic substudy were the pharmacokinetic parameters of lopinavir for once-daily compared with twice-daily dosing in the same children.

One hundred seventy-three children were randomized in the KONCERT trial, of which 86 were randomized to switch to once-daily treatment with lopinavir/r. The rate of virological rebound was low in both arms: 12 children on once-daily versus 7 on twice-daily lopinavir/r had a confirmed viral load within 48 weeks. The estimated difference in percentage with viral load rebound was 6% [90% confidence interval (–2, 14)]. Non-inferiority for viral load suppression on once-daily versus twice-daily lopinavir/r could therefore not be demonstrated. This difference was partially explained by the chance imbalance between arms in viral rebound, which occurred between screening and baseline. Among 26 children in the intra-subject lopinavir/r pharmacokinetic substudy, lower daily exposure (area under the plasma-concentration time curve (AUC_{0-24}) 161 h×mg/l vs. 224 h×mg/l) and lower C_{last} (1.03 mg/l vs. 5.69 mg/l) were observed with once-daily versus twice-daily dosing. Although these results do not support routine use of once-daily lopinavir/r, lack of safety concerns or resistance suggests that once-daily dosing remains an option in selected, adherent children, with close viral load monitoring.

Chapter 2

The effectiveness of the off-label use of once-daily lopinavir/r in a real-life setting is analysed and described in chapter 2. Several studies on the pharmacokinetics and clinical efficacy of lopinavir/r once daily in

HIV-infected children have been published in the past decade. Despite frequently reported subtherapeutic trough levels, most children in these studies showed good short-term outcomes (follow up 6–12 months). Two of the pharmacokinetic studies on lopinavir/r once daily were initiated in the Erasmus Medical Centre in 2002. After completion of these studies, patients on lopinavir/r once daily were allowed to continue this treatment as long as their viral loads were undetectable. Subsequently, selected children were also offered to switch to lopinavir/r once daily. To ensure that children had adequate exposure to lopinavir, therapeutic drug monitoring was used. The long-term effectiveness of the once-daily lopinavir/r-containing regimen was evaluated in HIV-1 infected children in clinical practice. The main outcome measures were the percentage of patients with an undetectable HIV-1 viral load each subsequent year after switch to lopinavir/r once daily (on treatment and last observation carried forward (LOCF)), and virological failure during follow-up (> 400 copies/ml twice within 6 months). Also the exposure to lopinavir on the initial once-daily dosing regimen was determined.

Forty children with a median follow-up of 6.3 years (range 1.0, 10.3) were included. During yearly follow-up, the percentage of children with an undetectable viral load varied between 82–100% (on treatment) and 80–93% (LOCF). Five children (12.5%) met the criteria for failure. Lopinavir geometric mean AUC_{0-24} was 169.3 h$\times$mg/l and C_{last} 1.35 mg/l. It was concluded that a once-daily lopinavir/r-containing regimen in HIV-1 infected children with intensive clinical and therapeutic drug monitoring is well tolerated and has good long-term clinical, virological, and immunological outcome.

Chapter 3

For HIV-infected children 3–12 years old, once-daily dosing of darunavir/r has been approved by the European medicines agency (EMA) at the end of 2014. Dosing recommendations for children 6–12 years old are based on a modelling and simulation procedure by the company. Despite its approval for once-daily use, guidelines at the end of 2015 advised against the use of a once-daily regimen in children < 12 years of age, because of the lack of data on efficacy, safety and exposure. This pharmacokinetic study was designed to validate the proposed dosing recommendation for once-daily darunavir/r in HIV-infected children 6–12 years of age.

Twelve children on a stable antiretroviral regimen with a viral load < 50 copies/ml were included. A 24h pharmacokinetic curve was collected after

observed intake. The geometric mean (%CV) AUC_{0-24} was 63.1 (33%) h×mg/l, C_{max} 5.6 (34%) mg/l and C_{last} was 1.5 (44%) mg/l. It was predefined that exposure would be adequate, when the lower limit of the 90% one sided confidence interval (90%CI) of the geometric mean of the AUC_{0-24} was higher than 0.8 of the value of adults (0.8×89.7 = 71.8 h×mg/l). The lower limit of the one sided 90%CI was 55.7 h×mg/l, which is 62% of the adult value. Therefore, the target was not reached. The geometric mean AUC_{0-24} was 70% of the AUC_{0-24} found in adults. Ten out of the 12 children had an AUC_{0-24} below the adult target value, of which seven had an AUC below 0.8 of the adult target value. C_{last} of all of the children was above 0.55 mg/l (range: 0.69, 2.38 mg/l), which is the target for patients treated with protease inhibitors before. The AUC of darunavir in children 6–12 years was substantially lower than predicted by the population pharmacokinetic model that was submitted for approval of the once-daily dosing regimen of darunavir/r in children. Since trough levels were above the target value, the treatment was considered adequate.

Chapter 4

Lamivudine is a nucleoside reverse transcriptase inhibitor widely used as part of antiretroviral treatment of children. Several issues have been raised concerning the treatment with lamivudine, such as differences in bioavailability between formulations and possible under dosing in the youngest age group. Dosing in children should be based on the understanding of the developmental changes in the pharmacokinetic and the pharmacodynamic relation of drugs instead of applying the adult mg/kg dose to children. The objectives of this study were to characterise age-related changes in lamivudine pharmacokinetics in children and to test how well this model can be generalized.

Based on the developed population pharmacokinetic model, lamivudine exposure upon currently used dosing recommendations was evaluated and a new dose was calculated for subgroups in which the target AUC_{0-24} was not reached. Bodyweight best predicted the developmental changes in apparent clearance and volume of distribution of lamivudine. For children with a bodyweight below 14 kg, the dose should be increased from 8 to 10 mg/kg/day if the adult target for AUC_{0-24} is aimed for.

Part 2: Paediatric formulations

Chapters 5 and 6

A multitude of antiretroviral drug formulations is available for the treatment of HIV-infected adults and children. These formulations include individual and co-formulated drugs, many of which are also supplied as generic drugs. It is important to know whether a different type of formulation such as a tablet and a liquid, or a new generic drug formulation provides similar exposure compared to the innovator product. The rate and extent of absorption from the gastrointestinal tract can be influenced by the dissolution rate, solubility and permeability of the drug. Many physiological factors are also important for absorption. A drug must be dissolved before it can be absorbed. Physicochemical characteristics determine the solubility and permeability of a drug. The biopharmaceutics classification system is used by regulators to determine whether in vivo bioequivalence studies are necessary for the approval of new generic drug products of solid immediate release dosage forms. According to this system, the active pharmaceutical ingredient of a formulation can be classified into one of four categories depending on its aqueous solubility and intestinal permeability. Theoretically, it is expected that the influence of the formulation on pharmacokinetics will be largest for drugs with low solubility. Many antiretroviral drugs have a poor bioavailability and a low aqueous solubility.

A review of studies assessing the pharmacokinetics of different antiretroviral drug formulations in adults and children is described in chapter 5. For some antiretroviral drugs, differences in pharmacokinetics have been described, with largest differences in exposure when a liquid formulation is compared to a solid formulation (tablet or capsule). It is important to realize that children sometimes use different formulations than adults.

If no appropriate formulations are available, existing formulations are sometimes manipulated, for example tablets are cut or split to achieve a different dose, and sometimes also crushed to ease administration. Deviations from the optimal dose can occur when splitting or crushing tablets, which can significantly impact drug efficacy and toxicity. Only a few studies were found in which the effect of manipulating solid formulations was investigated. It is important to know whether exposure is still adequate. This is relevant for children, but also for HIV-infected patients who (temporarily) are not able to swallow solid drug formulations. Given the increasing number of new formulations and drug combinations it is important to be

aware that the formulation and excipients can significantly influence the pharmacokinetics of antiretroviral drugs.

The results of the study investigating the pharmacokinetics of a new paediatric tablet of lopinavir combined with ritonavir are described in chapter 6. Lopinavir/r paediatric tablets (100 mg lopinavir and 25 mg ritonavir) are approved by the US food and drug administration (FDA) and EMA as part of combination antiretroviral therapy for children. However, at time of start of the KONCERT trial, dosing was based on body weight bands or body surface area under FDA approval, and only body surface area by the EMA. This could lead to a different recommended dose. Also, weight band based dosing was not yet formally studied in the target population. The pharmacokinetics of lopinavir in children who used the paediatric tablets twice daily was therefore studied in the first phase of the KONCERT trial. The first children who consented were selected to participate in a pharmacokinetic substudy, until a minimum of 16 children in each of three dosing weight bands had evaluable pharmacokinetic data.

A total of 53 HIV-infected children were included. For the total group, lopinavir geometric mean AUC_{0-12}, C_{max} and C_{12} were 106.9 h×mg/l, 12.0 mg/l and 4.9 mg/l, respectively. There were no significant differences in lopinavir pharmacokinetic parameters between the weight bands. Weight was not found to be associated with variability in C_{max}, C_{12} or AUC_{0-12} for the lopinavir pharmacokinetic parameters. Given the high percentage of Asian children, it was also tested whether there was a difference in pharmacokinetic parameters between Asian and non-Asian children. Significant influence of ethnicity was found on CL/F, with Asian children having on average a 21% higher CL/F than non-Asian children. Despite the significantly higher CL/F in Asian children there was no significant difference in AUC_{0-12}, which can be partly explained by the relatively higher dose in the Asian children on a mg/kg basis. A possible explanation for these observations could also be a difference in diet and more Asian children taking their medication under fasting conditions. Despite potential ethnic influences on lopinavir pharmacokinetics, exposure in all subgroups was adequate. In conclusion, the FDA weight band dosing recommendations provide adequate exposure in HIV-infected children when using the paediatric lopinavir/r tablets

Chapters 7 and 8

The final two chapters focus on the development of a new paediatric formulation of the antiviral drug valaciclovir. Valaciclovir is an oral prodrug of

aciclovir and is used for the treatment and prophylaxis of herpes simplex virus and varicella zoster virus infections. It is approved by the EMA to be used in children from the age of 12 years and above, but it is also used (off-label) in younger children. No appropriate formulation is available for young children. The extemporaneous formulation made from crushed tablets as described in the FDA label information was considered suboptimal. To overcome problems associated with that formulation, we aimed to develop a new paediatric formulation, and to assess the bioequivalence of this new formulation compared with the innovator valaciclovir tablets and to investigate its palatability. For the palatability assessment, both in vivo (children and parents) and in vitro (electronic tongue) methods were applied. These studies were performed within the so-called VALID-project, which has been granted by The Netherlands Organisation for Health Research and Development (ZonMW), within the 'Priority Medicines for Children' Programme.

A 20 mg/ml valaciclovir solution was developed with glycerol and water as main excipients. Stability of valaciclovir in this formulation has been shown for at least nine months. Stability of other formulations with a higher concentration and different excipients did not meet the stability requirements. An electronic tongue was applied during the formulation development and to support the in vivo palatability testing. The taste masking capability of the new formulation was compared with crushed tablets in OraSweet® SF as suspension vehicle. Both formulations demonstrated taste-masking efficiency. In vivo palatability was assessed during a two-period cross-over study in 21 children aged 4–11 years and 20 parents. The taste of each formulation was scored on a combined 100 mm visual analogue (VAS)/facial hedonic scale. The primary outcome was the difference between the VAS scores of the test and the reference formulation as rated by the children. For the children, mean (95%CI) VAS scores were 26 mm (18, 34) for the test formulation and 24 mm (16, 32) for the reference formulation with a mean (95%CI) difference of 2.4 (–8.5, 13) mm, in favour of the test formulation. The mean (95%CI) VAS scores indicated by the parents ($n = 20$) were 45 (36, 54) mm for the test formulation and 46 (37, 55) mm for the reference formulation, and a mean difference (95%CI) of –0.9 (–12, 9.8) mm. Predefined was that non-inferiority was shown when the lower limit of the two-sided 95%CI for the difference in VAS scores of the formulations given by the children was above –10 mm. Non-inferiority of the test compared with the reference formulation was therefore shown.

The bioequivalence of the new paediatric valaciclovir liquid formulation

was investigated in healthy adult volunteers. The pharmacokinetics of aciclovir was determined in 16 fasting volunteers after administration of a single dose of 500 mg of the new valaciclovir formulation and of the innovator valaciclovir tablet. The new valaciclovir formulation met the criteria for bioequivalence regarding the AUC_{0-12}. The upper limit of the 90%CI of the ratio for the maximum plasma concentration was 133%, which is just above the 125% criterion.

The results of the VALID-project support the use of the newly developed valaciclovir formulation as an alternative formulation for (paediatric) patients for whom valaciclovir tablets are not or less appropriate.

Discussion

Treatment options for children are often limited because of the lack of evidence on safe and effective dosing regimens, and the availability of appropriate formulations. There are several ways to collect more information on the appropriate use of medicines in paediatrics. Two studies in this thesis, a clinical trial and an observational cohort study, were performed to support once-daily use of lopinavir. Regulatory and practical issues are described of performing clinical trials in children compared to use of data from a real-life setting as an alternative approach to collect information. Although done with caution, a comparison of the results from both studies supports the use of real-life research as possible valuable alternative for the performance of a clinical trial.

It is important to evaluate safety and efficacy or efficiency when drugs are used off-label and to report the results found, as has been done in chapters 1 and 2 for the once-daily use of lopinavir/r in children. However, after approval, further research is sometimes needed to support the use of a drug, or to optimize dosing recommendations for all potential users.

The lack of appropriate formulations for children and information on dosing, efficacy and safety has been recognized by regulators already decades ago and resulted in new regulation to stimulate the development of paediatric formulations. Despite all efforts, there is still a lack of appropriate formulations and information on medicines for children in daily practice. Consequently, there is an on-going need of pharmacists and clinicians to search for safe, effective and acceptable treatments for paediatric patients. When administering alternative formulations or manipulating formulations attention is warranted. Taking into account the biopharmaceutical properties of a drug, combined with the knowledge of pharmaceutical formulations and the function of the excipients used, (possible)

differences in exposure can be explained or predicted. Pharmacists are equipped with the knowledge of these areas of interest and should be able to combine the relevant information and translate it to a comprehensible advice for patients and caregivers.

A more recent guideline of the EMA describes requirements for the development of medicines for paediatric use, also with regard to patient acceptability. It is debatable when a drug formulation can be considered acceptable, and how acceptability should best be tested in children. Combining expertise from different areas of (paediatric) research while taking into account differences in ethical and regulatory requirements, methods can be modified and used in paediatric drug research to optimize testing of drugs. The number of children needed to be included in clinical trials can thus be limited and safe and efficacious treatments can be made more timely available for children.

Making things easier

Nederlandse samenvatting

Inleiding

Kinderen kunnen niet simpelweg dezelfde geneesmiddelformuleringen en doseringen gebruiken als volwassenen. Of een formulering geschikt is hangt af van de patiënt die behandeld moet worden met geneesmiddelen, het geneesmiddel zelf en de dosering die moet worden toegediend, alsook de omgeving waarin de toediening plaatsvindt. Orale toediening van geneesmiddelen wordt in het algemeen als de meest geschikte manier van toedienen beschouwd, ook voor kinderen. Geneesmiddelformuleringen waarmee flexibel doseren mogelijk is hebben de voorkeur, omdat door de groei en ontwikkeling van kinderen regelmatig een aanpassing van de dosering nodig is. Verschillende aspecten bepalen in hoeverre een geneesmiddelformulering acceptabel is, zoals het al dan niet in moeten nemen met voedsel, de grootte van de tablet, de hoeveelheid en het aantal tabletten dat ingenomen moet worden, alsook de smaak. Het kan worden verwacht dat hoe minder acceptabel een formulering is, hoe belangrijker de doseerfrequentie is.

Het doel van dit proefschrift was om te onderzoeken hoe de behandeling van kinderen met geneesmiddelen kan worden geoptimaliseerd, om zo zorg te dragen voor een veilige en effectieve behandeling van kinderen met acute of chronische virusinfecties.

Deel 1: Doseerregime – farmacokinetiek

Kinderen die geïnfecteerd zijn met het humaan immunodeficiëntie virus (hiv) worden behandeld met antiretrovirale geneesmiddelen om te voorkomen dat zij het verworven immunodeficiëntiesyndroom (AIDS) ontwikkelen. Met de huidige kennis en behandelmogelijkheden hebben hiv-geïnfecteerde kinderen een normale levensverwachting, maar wel met de noodzaak de anti-hiv-behandeling de rest van hun leven vol te houden. De levenslange behandeling gecombineerd met het belang van therapietrouw is een uitdaging voor patiënten en zorgverleners. De eerstelijns behandeling van een hiv-infectie, zoals geadviseerd door PENTA (Paediatric European Network for Treatment of AIDS), bestaat uit twee of drie zogenaamde nucleoside reverse transcriptase remmers (NRTI) als basis, gecombineerd met of een proteaseremmer met ritonavir of een non-nucleoside reverse transcriptase remmer (NNRTI).

Het eerste deel van dit proefschrift richt zich op hoe we de dosering van antiretrovirale middelen kunnen vereenvoudigen en optimaliseren.

Hoofdstuk 1

In dit hoofdstuk worden de resultaten van de studie met de naam KONCERT besproken. KONCERT is het acroniem voor 'Kaletra ONCE daily Randomised Trial of the pharmacokinetics, safety and efficacy of twice-daily versus once-daily lopinavir/ritonavir tablets dosed by weight as part of combination antiretroviral therapy in HIV-1 infected children (PENTA 18).' In deze studie werd gekeken naar de farmacokinetiek, veiligheid en effectiviteit van eenmaal daags lopinavir met ritonavir (lopinavir/r), in vergelijking met het gebruik van deze middelen tweemaal daags bij hiv-geïnfecteerde kinderen. Deze grote en internationale studie werd uitgevoerd door het PENTA-netwerk. Kinderen konden meedoen met de studie als ze minimaal 15 kg wogen, tabletten in zijn geheel door konden slikken, behandeld werden met tweemaal daags lopinavir/r en als er geen virusdeeltjes meetbaar waren in het bloed voor minimaal 24 weken. De kinderen werden gerandomiseerd om door te gaan met hun tweemaal daagse behandeling of om lopinavir/r eenmaal daags te gaan gebruiken. Het belangrijkste waar naar werd gekeken was of het virus te meten was in het bloed ($>$ 50 virusdeeltjes/ml) gedurende 48 weken. De marge om noninferioriteit aan te tonen was vooraf vastgesteld op 12%. De belangrijkste uitkomstmaten voor de farmacokinetische substudie waren de farmacokinetische parameters van lopinavir na eenmaal daagse toediening in vergelijking met tweemaal daagse toediening bij dezelfde kinderen.

In totaal deden 173 kinderen mee met de studie, verdeeld over de twee behandelgroepen, waarvan 86 in de groep voor eenmaal daagse behandeling met lopinavir/r. Er waren maar weinig kinderen bij wie virusdeeltjes meetbaar waren in het bloed gedurende de studie: 12 kinderen (14%) in de eenmaal daagse groep en 7 (8%) in de tweemaal daagse groep. Het verschil in percentage van kinderen bij wie virusdeeltjes meetbaar waren was 6% met een 90% betrouwbaarheidsinterval (90%BI) van –2 tot 14%. Noninferioriteit van eenmaal daags in vergelijking met tweemaal daags lopinavir/r kon daarmee formeel niet aangetoond worden. Het verschil in percentages kon gedeeltelijk worden verklaard doordat bij de start van de studiebehandeling bij een aantal kinderen in de eenmaal daagse groep virusdeeltjes meetbaar waren. Van 26 kinderen die meededen aan de farmacokinetische substudie werden de farmacokinetische parameters vergeleken na eenmaal daags en na tweemaal daags lopinavir/r; dit waren met name de opper-

vlakte onder de plasmaconcentratie-tijd-curve (AUC) en de laatst gemeten plasmaconcentratie in het doseerinterval (dalspiegel, C_{last}). Na eenmaal daagse toediening was de blootstelling lager dan na tweemaal daagse toediening (AUC$_{0-24}$ 161 uur$\times$mg/l vs. 224 uur$\times$mg/l) en C_{last} (1,03 mg/l vs. 5,69 mg/l). Met de resultaten van deze studie kan eenmaal daags lopinavir/r bij kinderen niet standaard geadviseerd worden. Echter, omdat er geen problemen met veiligheid en resistentie werden gezien suggereert dit dat eenmaal daags een optie zou kunnen zijn voor geselecteerde kinderen waarvan verwacht wordt dat ze therapietrouw zijn en bij wie de behandeluitkomst goed vervolgd wordt.

Hoofdstuk 2

De effectiviteit van de off-label behandeling met eenmaal daags lopinavir/r in de praktijk wordt beschreven in hoofdstuk 2. Er zijn in de laatste decennia verschillende onderzoeken naar de farmacokinetiek en klinische effectiviteit van eenmaal daags lopinavr/r in hiv-geïnfecteerde kinderen gepubliceerd. Ondanks dat er in deze studies regelmatig subtherapeutische dalspiegels werden gemeten, werden op korte termijn (6–12 maanden) goede behandeluitkomsten gezien. Twee van de farmacokinetische studies werden uitgevoerd in het Erasmus medisch centrum (Rotterdam) in 2002. Na afloop van de studie mochten kinderen die met eenmaal daags lopinavir/r werden behandeld dit regime voortzetten, zolang er geen virusdeeltjes meetbaar waren in het bloed. Vervolgens werd een aantal geselecteerde kinderen aangeboden om ook lopinavir/r eenmaal daags te gaan gebruiken. Om te controleren of de blootstelling aan lopinavir voldoende was, werden regelmatig de concentratie van lopinavir in het bloed gemeten. De lange-termijn effectiviteit van de behandeling in de dagelijkse praktijk bij deze kinderen werd geëvalueerd. De belangrijkste uitkomstmaten waren het percentage kinderen waarbij geen virusdeeltjes meetbaar waren in het bloed elk jaar na start met het eenmaal daags regime en gedurende elk controle bezoek. Tevens werd de blootstelling aan lopinavir gemeten direct na start van het eenmaal daagse regime.

Veertig kinderen deden mee aan de studie, waarbij de mediane opvolgduur 6,3 jaar was, uiteenlopend van 1,0 tot 10,3 jaar. Bij de jaarlijkse controle waren geen virusdeeltjes meetbaar bij 82–100% van de kinderen die nog eenmaal daags lopinavir/r gebruikten. De farmacokinetische parameters van lopinavir waren een geometrisch gemiddelde AUC$_{0-24}$ van 169,3 uur$\times$mg/l en een C_{last} van 1,35 mg/l. De conclusie van deze studie was dat eenmaal daags lopinavr/r als onderdeel van antiretrovirale behandeling

van hiv-geïnfecteerde kinderen die intensief gevolgd worden, goed verdragen wordt en goede langetermijn behandelresultaten geeft.

Hoofdstuk 3

Voor hiv-geïnfecteerde kinderen van 3–12 jaar oud is het eenmaal daags gebruik van darunavir met ritonavir (darunavir/r) eind 2014 geregistreerd door het Europees geneesmiddelen agentschap (EMA). De doseeradviezen voor kinderen 6–12 jaar zijn door de farmaceutische industrie gebaseerd op extrapolatie van farmacokinetische gegevens met behulp van een farmacokinetisch populatiemodel. Ondanks dat het eenmaal daagse doseerregime is geregistreerd werd het voor kinderen jonger dan 12 jaar eind 2015 nog niet geadviseerd in de diverse richtlijnen. De reden hiervoor was het ontbreken van gegevens over effectiviteit, veiligheid en blootstelling. Deze farmacokinetische studie had als doel om de geregistreerde doseeradviezen voor eenmaal daags darunavir/r te valideren bij hiv-geïnfecteerde kinderen van 6 tot 12 jaar. Twaalf kinderen waarbij geen virusdeeltjes in het bloed meetbaar waren konden meedoen met de studie. Na inname van hun medicatie in het ziekenhuis werd verspreid over 24 uur bloed afgenomen om de concentratie darunavir in het bloed te meten.

De gemiddelde (%CV) AUC_{0-24} was 64,2 (34%) uur×mg/l, C_{max} 5,7 (35%) mg/l en C_{last} was 1,5 (46%) mg/l. Vooraf was bepaald dat de blootstelling adequaat zou zijn als de ondergrens van het 90%BI van de gemiddelde AUC_{0-24} boven 80% van de gemiddelde waarde bij volwassenen zou liggen (0,8×89,7 = 71,8 uur×mg/l). De ondergrens was 56,0 uur×mg/l, wat 62% is van de gemiddelde waarde bij volwassenen. De streefwaarde voor blootstelling (AUC) werd dus niet gehaald. De gemiddelde AUC_{0-24} was 72% van de gemiddelde waarde bij volwassenen. Negen van de 11 kinderen hadden een AUC_{0-24} die onder dit gemiddelde lag, en zeven kinderen hadden een AUC_{0-24} lager dan 80% van de gemiddelde waarde bij volwassenen. De gemeten C_{last} van alle kinderen was boven 0,55 mg/l, wat de streefwaarde is voor hiv-geïnfecteerde patiënten die reeds eerder behandeld zijn met proteaseremmers. De blootstelling aan darunavir, gemeten als AUC, bij kinderen van 6 tot 12 jaar was aanzienlijk lager dan voorspeld was met behulp van het farmacokinetisch populatiemodel, zoals dat overlegd is voor de registratie van het eenmaal daagse doseerregime voor kinderen. Aangezien de dalspiegels wel boven de streefwaarde waren, werd de behandeling toch als adequaat beschouwd.

Hoofdstuk 4

Lamivudine is een nucleoside reverse transcriptase remmer (NRTI) die veel wordt toegepast bij de antiretrovirale behandeling van kinderen. Over sommige punten is discussie bij de behandeling met lamivudine, zoals verschillen in biologische beschikbaarheid tussen formuleringen en een mogelijk te lage dosering bij jonge kinderen. Doseringen voor kinderen zouden gebaseerd moeten worden op kennis en begrip van de ontwikkeling in farmacokinetiek en farmacodynamiek bij kinderen van het geneesmiddel. Het doel van deze studie was om leeftijdsgebonden veranderingen in de farmacokinetiek van lamivudine te beschrijven in een populatiemodel en te testen of dit populatiemodel voor andere groepen kinderen kon worden gebruikt. Met behulp van het berekende populatiemodel werd gekeken of met de momenteel toegepaste dosering bij alle groepen kinderen een goede blootstelling te verwachten is.

Het lichaamsgewicht was een belangrijke factor om veranderingen in de farmacokinetische parameters klaring en verdelingsvolume te voorspellen. Voor kinderen met een lichaamsgewicht onder de 14 kg werd de streefwaarde voor de blootstelling (AUC) niet gehaald. Op basis van het model wordt voorgesteld om de dosering voor deze groep kinderen te verhogen van 8 naar 10 mg/kg.

Deel 2: Kinderformuleringen

Hoofdstukken 5 en 6

Er zijn vele antiretrovirale geneesmiddelformuleringen beschikbaar voor de behandeling van hiv-geïnfecteerde kinderen en volwassenen. Dit zijn formuleringen met maar één geneesmiddel of een combinatie van verschillende geneesmiddelen, waaronder ook merkloze formuleringen. Het is belangrijk om te weten of verschillende formuleringen, zoals een drank en een tablet, of een merkloos geneesmiddel en het oorspronkelijke geneesmiddel (specialité), dezelfde blootstelling geven. De mate en snelheid van opname vanuit het maagdarmkanaal wordt beïnvloed door onder andere de oplosnelheid, oplosbaarheid en permeabiliteit van het geneesmiddel. Diverse fysiologische factoren zijn ook belangrijk voor opname van een geneesmiddel. Voordat een geneesmiddel kan worden opgenomen in het lichaam moet het opgelost zijn. Fysisch-chemische eigenschappen bepalen de oplosbaarheid en permeabiliteit van het geneesmiddel. Volgens het biofarmaceutisch classificering systeem wordt een geneesmiddel ingedeeld in één van vier categorieën afhankelijk van de oplosbaarheid in water en de permeabiliteit. Theoretisch

kan worden verwacht dat de invloed van de formulering op de farmacokinetiek het grootste is bij geneesmiddelen met een slechte oplosbaarheid. Veel antiretrovirale middelen hebben een lage biologische beschikbaarheid en een slechte oplosbaarheid in water.

Een overzicht van studies waarbij is gekeken naar het verschil in farmacokinetiek van geneesmiddelen in verschillende formuleringen wordt beschreven in hoofdstuk 5. Voor een aantal antiretrovirale geneesmiddelen worden verschillen in farmacokinetiek beschreven, waarbij de grootste verschillen worden gevonden wanneer vloeibare en vaste (tablet, capsule) formuleringen worden vergeleken. Het is belangrijk om te realiseren dat kinderen soms andere formuleringen gebruiken dan volwassenen.

Wanneer er geen geschikte formulering beschikbaar is, worden bestaande formuleringen soms gemanipuleerd. Tabletten worden bijvoorbeeld gedeeld of verpulverd om de juiste dosering te krijgen of om de toediening te vergemakkelijken. Afwijkingen van de juiste dosering kunnen optreden wanneer tabletten gedeeld of verpulverd worden; ook kan dit invloed hebben op de effectiviteit en toxiciteit. Slechts enkele studies werden gevonden waarbij het effect van het manipuleren van vaste formuleringen was onderzocht. Het is belangrijk om te weten of the blootstelling nog steeds adequaat is. Dit is van belang voor hiv-geïnfecteerde kinderen, maar ook voor volwassen die (tijdelijk) niet goed in staat zijn om vaste toedieningsvormen in te nemen. Gezien het toenemende aantal nieuwe formuleringen en combinaties van geneesmiddelen is het belangrijk om bewust te zijn van het feit dat de formulering en hulpstoffen de farmacokinetiek van antiretrovirale geneesmiddelen significant kunnen beïnvloeden.

De resultaten van de studie waarin de farmacokinetiek van een nieuwe tablet voor kinderen met lopinavir en ritonavir werd onderzocht, wordt beschreven in hoofdstuk 6. De lopinavir/r kindertabletten (100 mg lopinavir en 25 mg ritonavir) zijn goedgekeurd door de Amerikaanse registratie autoriteit (FDA) en EMA als onderdeel van een behandeling van hiv-geïnfecteerde kinderen. Op het moment van start van de KONCERT studie was de dosering zoals goedgekeurd door de FDA gebaseerd op lichaamsgewicht of lichaamsoppervlakte en door de EMA alleen op lichaamsoppervlakte. Dit kan leiden tot verschillen in doseren. Verder was de op lichaamsgewicht gebaseerde dosering nog niet onderzocht in de doelgroep. De eerste kinderen die meededen aan de KONCERT studie, werden geselecteerd om deel te nemen aan een farmacokinetische substudie waarin deze dosering onderzocht werd.

In totaal deden 53 kinderen mee aan deze studie. Voor de totale

groep werden de volgende geometrisch gemiddelde waarden gevonden voor respectievelijk de AUC_{0-12}, C_{max} en C_{12}: 106,9 uur×mg/l, 12,0 mg/l en 4,9 mg/l. Er werden geen significante verschillen in farmacokinetische parameters gevonden tussen de verschillende gewichtscategorieën. Er was geen relatie tussen het lichaamsgewicht en variatie in C_{max}, C_{12} of AUC_{0-12}. Gezien het hoge percentage aan Aziatische kinderen dat meedeed, werd ook gekeken of er verschillen waren in de farmacokinetische parameters tussen Aziatische en niet Aziatische kinderen. Een significant verschil werd gevonden voor de klaring (CL/F), waarbij Aziatische kinderen gemiddeld een 21% hogere klaring hadden dan niet Aziatische kinderen. Ondanks de hogere klaring werd geen verschil gevonden in de AUC_{0-24}, wat deels kan worden verklaard door de relatief hogere dosering die de Aziatiche kinderen kregen. Een mogelijke verklaring kan verder het verschil in dieet zijn en het feit dat meer Aziatische kinderen de medicatie op nuchtere maag innamen. Ondanks mogelijke invloeden van de etniciteit, was de blootstelling aan lopinavir in alle subgroepen adequaat. Uit deze resultaten kan geconcludeerd worden dat met de op lichaamsgewicht gebaseerde doseringen een adequate blootstelling wordt bereikt bij hiv-geïnfecteerde kinderen die de lopinavir/r kindertabletten gebruiken.

Hoofdstukken 7 en 8

De laatste twee hoofdstukken beschrijven de ontwikkeling van een nieuwe kinderformulering van het antivirale middel valaciclovir. Valaciclovir wordt in het lichaam omgezet in de werkzame stof aciclovir en wordt gebruikt voor de behandeling en profylaxe van infecties met het herpes simplex virus en varicella zoster virus. Het is geregistreerd door de EMA voor gebruik bij kinderen vanaf 12 jaar, maar wordt ook (off-label) toegepast bij jongere kinderen. De magistrale bereiding vanuit verpulverde tabletten, zoals beschreven in de FDA bijsluiter, wordt gezien als suboptimale formulering. Om problemen met die formulering te vermijden werd een nieuwe kinderformulering ontwikkeld. Hiervan werd de bioequivalentie onderzocht in vergelijking met tabletten en werd gekeken naar de smaak. Voor de smaaktesten werd gebruik gemaakt van zowel in vivo (kinderen en hun ouders) als in vitro (elektronische tong) methoden. De onderzoeken werden uitgevoerd binnen het zogenaamde 'VALID-project' waarvoor een subsidie van de Nederlandse organisatie voor gezondheidsonderzoek en zorginnovatie (ZonMW) werd toegekend binnen het programma 'Priority Medicines for Children.'

Een oplossing met 20 mg/ml werd ontwikkeld met glycerol en water als

belangrijkste hulpstoffen. De stabiliteit van valaciclovir in deze formulering werd aangetoond voor minimaal 9 maanden. Gedurende de ontwikkelfase en ter ondersteuning van de in vivo smaaktesten werd gebruik gemaakt van een elektronische tong. De mate van smaakmaskering van de nieuwe formulering werd vergeleken met die van verpulverde tabletten in OraSweet® SF als referentie formulering. Beide formuleringen maskeerden gedeeltelijk de smaak van valaciclovir. De in vivo smaaktesten werden uitgevoerd door middel van een cross-over studie met twee perioden. Eenentwintig kinderen in de leeftijd van 4 tot 12 jaar en 20 ouders deden mee aan de smaaktest. Hoe lekker of hoe vies ze elke formulering vonden werd aangegeven op een gecombineerde visueel analoge-gezichtjes schaal (VAS) van 100 mm. De belangrijkste uitkomstmaat was het verschil in VAS scores tussen de nieuwe en referentie formulering, zoals aangegeven door de kinderen. De kinderen gaven een gemiddelde (95%BI) VAS score van 26 mm (18, 34) aan de nieuwe formulering en 24 mm (16, 32) aan de referentie formulering met een gemiddeld (95%BI) verschil van 2.4 (–8,5, 13) mm, ten gunste van de nieuwe formulering. De ouders gaven een gemiddelde (95%BI) VAS score van 45 (36, 54) mm aan de nieuwe formulering en 46 (37, 55) mm voor de referentie formulering met een gemiddelde (95%CI) verschil van –0,9 (–12, 9,8) mm). Vooraf was vastgesteld dat noninferioriteit kon worden aangetoond wanneer de ondergrens van het 95%BI van het verschil in VAS scores aangegeven door de kinderen boven de –10 zou liggen. Met de gevonden resultaten kon daarom noninferioriteit van de nieuwe formulering in vergelijking met de referentie formulering worden aangetoond.

De bioequivalentie van de nieuwe vloeibare kinderformulering van valaciclovir werd onderzocht in gezonde volwassen vrijwilligers. De farmacokinetiek van aciclovir werd bepaald bij 16 vrijwilligers die 500 mg van de nieuwe valaciclovir formulering en van de merktabletten nuchter innamen. De nieuwe formulering voldeed aan de bioequivalentie criteria voor wat betreft de AUC_{0-12}. De bovengrens van het 90%BI van de ratio van de maximale plasma concentraties was 133%, welke net boven het 125% criterium ligt.

De resultaten van het VALID-project ondersteunen de toepassing van de nieuw ontwikkelde valaciclovir formulering als alternatieve formulering voor (pediatrische) patiënten voor wie valaciclovir tabletten minder of niet geschikt zijn.

Discussie

Behandelopties voor kinderen worden vaak beperkt door een gebrek aan informatie over veilige en effectieve doseerregimes en de beperkte beschikbaarheid van geschikte formuleringen. Op verschillende manieren kunnen gegevens verzameld worden voor een betere toepassing van geneesmiddelen bij kinderen. Twee studies in dit proefschrift, een klinisch onderzoek en een observationeel cohort onderzoek, werden uitgevoerd om eenmaal daags gebruik van lopinavir/r te onderzoeken. Regulatorische en praktische aspecten worden besproken die bij de uitvoer van deze twee typen onderzoek van belang zijn. Wanneer, met enige voorzichtigheid, de resultaten van beide studies worden vergeleken, kan worden gesteld dat studies waarin gebruik wordt gemaakt van gegevens uit de dagelijkse praktijk een waardevol alternatief kunnen zijn voor de uitvoer van een klinische studie.

Het is belangrijk om de veiligheid en effectiviteit te bewaken wanneer geneesmiddelen off-label worden toegepast en om uitkomsten te rapporteren, zoals is gedaan in hoofdstuk 1 en 2, voor het gebruik van eenmaal daags lopinavir/r bij kinderen. Echter, ook na registratie is verder onderzoek soms nodig om beter gebruik van geneesmiddelen te ondersteunen voor alle (potentiële) gebruikers.

Het gebrek aan geschikte formuleringen voor kinderen en informatie over de juiste dosering, effectiviteit en veiligheid is decennia geleden reeds erkend door de registratie autoriteiten. Dit heeft geleid tot nieuwe regelgeving om de ontwikkeling van kinderformuleringen te stimuleren. Ondanks alle ontwikkelingen is er momenteel nog steeds een gebrek aan geschikte formuleringen en informatie over geneesmiddelen voor kinderen. Het is daarom nog regelmatig nodig dat door apothekers en dokters gezocht moet worden naar een veilige, effectieve en acceptabele behandeling voor kinderen. Bij het toedienen van alternatieve formuleringen of het manipuleren van formuleringen is oplettendheid geboden. Door de kennis van de biofarmaceutische eigenschappen van een geneesmiddel te combineren met de kennis van farmaceutische formuleringen en de functie van hulpstoffen, kunnen (mogelijke) verschillen in absorptie worden verklaard of voorspeld. Apothekers hebben kennis van deze gebieden en zouden de relevante informatie moeten kunnen combineren en vertalen in een begrijpelijk advies voor patiënten en zorgverleners.

Een recente EMA-richtlijn beschrijft de eisen voor de ontwikkeling van geneesmiddelen voor kinderen, waarin ook aandacht is voor acceptatie. Wanneer een formulering acceptabel is, is onderwerp van discussie, als ook hoe dit het beste getest zou moeten worden bij kinderen. Door exper-

tise te combineren vanuit verschillende (pediatrische) onderzoeksgebieden en rekening te houden met de ethische en regulatorische eisen, kunnen methoden aangepast en gebruikt worden om zo studies voor en bij kinderen te optimaliseren. Het aantal kinderen dat benodigd is voor medisch wetenschappelijk onderzoek kan zo beperkt worden en mede daardoor kunnen veilige en effectieve behandelingen eerder beschikbaar zijn voor kinderen.

List of abbreviations and acronyms

Acronym	Explanation
3TC	Lamivudine
ABC	Abacavir
AIDS	Acquired immune deficiency syndrome
ANOVA	Analysis of variance
ANRS	Agence nationale de recherches sur le sida et les hépatites virales
ARIEL	Darunavir/ritonavir in treatment-experienced pediatric patients aged 3 to < 6 years
ARROW	Anti-retroviral research for Watoto
ART	Antiretroviral therapy
ARV	Antiretroviral
AUC	Area under the plasma concentration time curve
AZT	Zidovudine
b.i.d.	Twice daily (bis in die)
BCS	Biopharmaceutics classification system
BDDCS	Biopharmaceutics drug disposition classification system
BSA	Body surface area
BW	Bodyweight
cART	Combination antiretroviral therapy
CCMO	Centrale commisie mensgebonden onderzoek
CD(4/8)	Cluster of differentation (4/8), subtype of white blood cells
CDC	Centers for disease control and prevention
CHAPAS	Children with HIV in Africa – Pharmacokinetics and Adherence of Simple antiretroviral regimens
CI	Confidence interval
CL	Clearance
C_{last}	Last observed plasma concentration
C_{max}	Maximum plasma concentration
CMO-an	Commisie mensgebonden onderzoek Arnhem-Nijmegen
cov	Covariate
CV	Variation coefficient
CYP	Cytochrome
D	Absorption phase
d4T	Stavudine
Darunavir/r	Darunavir boosted with ritonavir

DIONE	Darunavir/ritonavir once daily in treatment-naïve adolescents
EFV	Efavirenz
EMA	European medicines agency
ETA	Estimates of variability
EudraCT	European clinical trials database
F	Fraction of an extravascular dose of drug that is absorbed
FDA	US Food and drug administration
FDC	Fixed-dose combination
FNA	Formulary of the Dutch pharmacists
FP7	Seventh framework program from the European Union
FTC	Emtricitabine
GEE	Generalized estimating equations
GMP	Good manufacturing practice
GMR	Geometric mean ratio
HIV	Human immmunodeficiency virus
HIV-NAT	The HIV Netherlands Australia Thailand research collaboration
HPLC	High performance liquid chromatography
HSV	Herpes simplex virus
IDV/r	Indinavir boosted with ritonavir
INSERM	Institut national de la santé et de la recherche médicale
IQR	Interquartile range
ISRCTN	International standard (randomised controlled) trial number
k	Exponent
Ka	Absorption rate constant
KNMP	Royal Dutch pharmacists association
KONCERT	A Kaletra ONCE daily Randomised Trial of the pharmacokinetics, safety and efficacy of twice-daily versus once-daily lopinavir/ritonavir tablets dosed by weight as part of combination antiretroviral therapy in HIV-1 infected children (PENTA 18)
LLOQ	Lower limit of quantification
LOCF	Last observation carried forward
Lopinavir/r	Lopinavir boosted with ritonavir
LPV	Lopinavir
LPV/r	Lopinavir boosted with ritonavir
MEC	Medical ethical committee
NCT number	National clinical trial number (clinicaltrials.gov)

NKFK	Dutch knowledge centre for paediatric pharmacotherapy
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NONMEM	Nonlinear mixed effects modelling
NPDE	Normalised prediction distribution error
NRTI	Nucleoside reverse transcriptase inhibitor
NVK	Dutch paediatric association
NVP	Nevirapine
NVZA	Dutch association of hospital pharmacists
NWO	Dutch organisation for scientific research
OFV	Objective function value
P	Parameter estimate
PCA	Principal component analysis
PD	Pharmacodynamics
PENTA	Paediatric European network for treatment of AIDS
PHPT	Program for HIV prevention and treatment
PI	Protease inhibitor
PIP	Paediatric investigation plan
PK	Pharmacokinetic(s)
pKa	Acid dissociation constant
PUMA	Paediatric use marketing authorisation
Q	Intercompartmental clearance
q.d.	Once daily (quaque die)
RNA	Ribonucleic acid
RONDO	Pharmacokinetics of a once-daily regimen of lopinavir/ ritonavir in HIV-infected children
RSE	Relative standard error
RTV	Ritonavir
SAE	Serious adverse event
SAS	Statistical analysis system
SD	Standard deviation
SF	Sugar free
SGC	Soft gel capsule
SPSS	Statistical package for the social sciences
STEP	Safety and toxicity of pharmaceutical excipients for paediatrics
T_0	Time of start of dosing interval
$t_{1/2}$	Elimination half-life
TAM	Thymidine associated mutation
TDF	Tenofovir disoproxil fumarate

TDM   Therapeutic drug monitoring
$T_{\max}$ Time of maximum plasma concentration
US FDA   US Food and drug administration
UV   Ultraviolet
V   Volume of distribution
VALID   Development of a new paediatric formulation of valaciclovir
               for the prophylaxis and treatment of VZV and HSV
               infections in children
VAS   Visual analogue scale
VZV   Varicella zoster virus
WHO   World health organization
ZDV   Zidovudine
ZonMW   The Netherlands organisation for health research and
               development

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Dankwoord

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Curriculum vitae

Diane Bastiaans behaalde in 1994 haar VWO-diploma aan het Augustinianum in Eindhoven. Ze studeerde farmacie aan de Universiteit Utrecht en studeerde in 1998 af bij de vakgroep Biofarmacie op het onderwerp 'Specific active immunotherapy using liposomal peptide antigens.'

Na het behalen van haar apothekersbul in 2001 werkte ze als projectapotheker in het toenmalige Diaconessenhuis in Eindhoven. Ze werd opgeleid tot ziekenhuisapotheker door Vincent Brenninkmeijer in het Maxima Medisch Centrum, Eindhoven/Veldhoven. In de laatste jaren van haar opleiding specialiseerde zij zich in de richting van de kindergeneeskunde en sloot zij zich aan bij de Special Interest Group Kindergeneeskunde van de Nederlandse Vereniging van Ziekenhuisapothekers (NVZA) en het Nederlands Kenniscentrum Farmacotherapie bij Kinderen (NKFK).

Vanaf 2007 werkte ze als ziekenhuisapotheker in het Laurentius ziekenhuis in Roermond met als belangrijkste aandachtsgebied medicatieveiligheid. In 2009 ging ze werken als ziekenhuisapotheker in het Radboud Universitair Medisch Centrum en combineerde dit met de uitvoer van haar promotie onderzoek. Aanvankelijk lag de focus van haar onderzoek vooral op de farmacokinetiek van antiretrovirale middelen bij kinderen. Met de toekenning van een subsidie door ZonMW aan het 'VALID-project' richtte haar onderzoek zich steeds meer op verschillende aspecten rondom kinderformuleringen.

Diane is getrouwd met Ben Schreur en samen hebben zij twee dochters.