Introduction

After the discovery of the human Immunodeficiency virus (HIV), a retrovirus, as the causative agent of the acquired immunodeficiency syndrome (AIDS), scientists have been searching exhaustively for drugs that attack this virus and inhibit replication. At this time, three of these so-called antiretroviral drugs have been licensed in the Netherlands: zidovudine (AZT, Retrovir®), didanosine (ddl, Videx®) and zalcitabine (ddC, Hivid®). Due to the rapid introduction of the antiretroviral agents, the clinical pharmacokinetics were only sparsely studied at the time these drugs became available. The purpose of our studies was to investigate the clinical pharmacokinetics of these antiretroviral agents in HIV-infected subjects in more detail. Special attention was paid to interactions between antiretroviral drugs and other drugs.

Bioanalysis

Bioanalytical methods have been developed for four antiretroviral agents: immunological assays for zidovudine and zalcitabine; high-pressure liquid chromatographic (HPLC) assays for AMT (a metabolite of zidovudine), didanosine and stavudine (an investigational antiretroviral agent). A comparison of two immunological methods, a radioimmunoassay and a fluorimunoassay, for the determination of zidovudine showed that both methods were similar in respect to sensitivity, selectivity, accuracy and reproducibility. Both methods were a clear improvement on existing HPLC methods in terms of sensitivity, time required for analysis and sample volume. The radioimmunoassay is less expensive than the fluorimunoassay and was therefore our method of choice for pharmacokinetic studies with zidovudine. In vitro experiments had suggested that AMT was responsible for zidovudine-induced bone-marrow toxicity. Although we could demonstrate by HPLC analysis that AMT was indeed present in the plasma of HIV-infected patients, its concentrations were much lower than expected. It is therefore not likely that AMT contributes to bone-marrow suppression by zidovudine.

Until recently, no bioanalytical method was available for the determination of zalcitabine. Zalcitabine doses are quite low (0.75 mg three times a day) and plasma concentrations are usually not above 20 ng/ml. Previously published HPLC methods have higher detection limits. Combined chromatographic-mass-spectrometric assays are more sensitive, but require instrumentation not readily available in most hospitals. We have developed a radioimmunoassay for the determination of zalcitabine, using immunocarbons from Sigma, with the required detection
limit of 1 ng/ml. Sample pretreatment of plasma, urine and cerebrospinal fluid (CSF) was carried out by solid-phase extraction with silica columns.

Clinical pharmacokinetics
Clinical pharmacokinetic studies have been performed with zidovudine and didanosine, the two most widely used agents in the Netherlands at the time of our studies. We have conducted a population pharmacokinetic study of zidovudine in 68 patients, resulting in 95 full pharmacokinetic curves. Clearance of zidovudine appeared to be lower in women than in men, was decreased in patients with advanced disease compared with asymptomatic persons, and increased proportionally with the patient's body weight. In another study we analysed plasma and CSF samples of 39 patients with AIDS who underwent lumbar puncture to establish a diagnosis of a neurological disorder (total number of samples: 50). It appeared that the concentration-time course was different in plasma compared with CSF. Elimination of zidovudine from CSF was much slower than from plasma, and there was no clear relation between zidovudine doses and CSF concentration. This last observation may be the explanation of the comparable efficacy of currently used low doses of zidovudine in the prevention and treatment of AIDS-dementia complex.

The pharmacokinetics of zidovudine were studied in a patient with HIV-associated nephropathy and different stages of renal dysfunction. Zidovudine clearance was only decreased when the renal function was severely impaired (creatinine clearance <10 ml/min). At the same time this patient could no longer tolerate the drug because of anaemia.

At the time didanosine was licensed, there was only experience with this drug in a sachet formulation. The marketed formulation was a chewable-dispersable tablet. We have therefore conducted a bioequivalence study in 15 HIV-infected patients. The tablets showed indeed 20-25% more bioavailability than the sachets, as represented in the manufacturer's dose recommendations. To our amazement, plasma concentrations in patients with a low body weight (less than 60 kg) were relatively lower than in patients with a higher body weight, despite a weight-adjusted dose of didanosine.

The frequent withdrawal of blood samples for pharmacokinetic studies is not a sound basis for the investigation of pharmacokinetic-pharmacodynamic studies. A strategy using so-called limited sampling models is less inconvenient for the patient, less laborious and less expensive. We have demonstrated in two separate investigations that the determination of zidovudine or didanosine in only 1 or 2 plasma samples is sufficient to estimate the area under the concentration–time curve (AUC).

Drug interactions
In most cases, information about drug–drug interactions is minimal at the time the drugs are licensed. For antiretroviral agents this lack of knowledge is a matter of even more concern because patients usually take a number of drugs concomitantly. We have therefore reviewed the literature on drug interactions with antiretroviral agents. In addition, we have carried out a number of interaction studies.

We investigated a possible interaction between rifampicin and zidovudine or didanosine. Rifampicin, widely known as an inducer of metabolizing enzymes in the liver, decreased plasma levels of zidovudine by 50%. This interaction is probably also based on enzyme induction because plasma levels of the primary metabolite of zidovudine, zidovudine glucuronide, were higher. Rifampicin did not consistently influence didanosine levels, which is in agreement with the fact that didanosine is not metabolized in the liver. Two studies of the presumed interaction between paracetamol and zidovudine were performed. Although literature suggested otherwise, no pharmacokinetic interaction could be demonstrated.

Because combination antiretroviral therapy is becoming more popular, antiretroviral drugs may also interact with each other. We studied the combination of zidovudine and didanosine in 5 patients with HIV infection. Although plasma concentrations of zidovudine were lower in 4 out of the 5 patients when both drugs were combined, these differences were small, statistically non-significant and probably clinically not relevant. Didanosine concentrations were not altered.

We have investigated the effect of zidovudine on phenytoin levels in HIV-infected patients and compared these data with data from HIV-negative persons with epilepsy. No drug interaction between zidovudine and phenytoin could be detected. It was observed that phenytoin levels were quite different from reference data. Possible explanations were a higher incidence of liver dysfunction, drug interactions (folic acid, clarithromycin) and a lower serum albumin level in HIV-infected patients.

Conclusions
The research described in this thesis has given us more insight into the various aspects of the clinical pharmacokinetic behaviour of antiretroviral agents in...
HIV-infected individuals. Especially our knowledge of the pharmacokinetics of zidovudine has been enhanced, but data for didanosine and zalcitabine are still scarce. In all situations, the pharmacokinetics in subgroups of patients may be altered, also as a result of drug interactions. The next years special attention should be paid to pharmacokinetic-pharmacodynamic relationships. The above described research has created a basis for this kind of investigation.

Results have also been published in the following papers