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Effect of Ginkgo Biloba on the Pharmacokinetics of Raltegravir in Healthy Volunteers

Maren Blonk, Angela Colbers, Anne Pointers, Bas Schouwenberg, and David Burger

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Medicinal herbs may cause clinically relevant drug interactions with antiretroviral agents. Ginkgo biloba extract is a popular herbal product among HIV-infected patients because of its positive effects on cognitive function. Raltegravir, an HIV integrase inhibitor, is increasingly being used as part of combined antiretroviral therapy. Clinical data on the potential inhibitory or inductive effect of ginkgo biloba on the pharmacokinetics of raltegravir were lacking, and concomitant use was not recommended. We studied the effect of ginkgo biloba extract on the pharmacokinetics of raltegravir in an open-label, randomized, two-period, crossover phase I trial in 18 healthy volunteers. Subjects were randomly assigned to a regimen of 120 mg of ginkgo biloba twice daily for 15 days plus a single dose of raltegravir (400 mg) on day 15, a washout period, and 400 mg of raltegravir on day 36 or the test and reference treatments in reverse order. Pharmacokinetic sampling of raltegravir was performed up to 12 h after intake on an empty stomach. All subjects (9 male) completed the trial, and no serious adverse events were reported. Geometric mean ratios (90% confidence intervals) of the area under the plasma concentration-time curve from dosing to infinity (AUC$_{0-\infty}$) and the maximum plasma concentration ($C_{max}$) of raltegravir with ginkgo biloba versus raltegravir alone were 1.21 (0.93 to 1.58) and 1.44 (1.03 to 2.02). Ginkgo biloba did not reduce raltegravir exposure. The potential increase in the $C_{max}$ of raltegravir is probably of minor importance, given the large inter-subject variability of raltegravir pharmacokinetics and its reported safety profile.

Approximately 60% of HIV-infected patients use complementary and alternative medicines to treat HIV-related symptoms and the side effects of conventional antiretroviral therapy. Herbal medicines can cause clinically significant interactions with antiretroviral agents, with potential drug failure as a result (17, 26).

Among the most popular herbal products used worldwide is ginkgo biloba extract, which is made from the leaves of the ginkgo biloba tree. It is used for the treatment of peripheral vascular disease and is frequently taken for its claimed beneficial effects on concentration, memory, depressive disorders, and dementia (1). Because cognitive impairment is one of the most feared complications among HIV-infected patients, the popularity of ginkgo biloba within this patient group is easily explained (23). Although ginkgo biloba extract has potential beneficial effects, self-medication with ginkgo biloba may lead to undesirable drug interactions with regular medication. For example, a study in healthy subjects showed that plasma concentrations of midazolam (a CYP3A probe) were significantly reduced after ginkgo biloba intake (22). If this were also true with antiretroviral agents, it could place individual patients at risk for virological failure.

In past years, a few articles have been published about the potential negative effects of ginkgo biloba on the pharmacokinetics of antiretroviral agents. One case report described virological failure in an HIV-infected patient taking an efavirenz-based regimen due to concomitant use of ginkgo biloba. Although the underlying mechanism remained unclear, terpene lactones in ginkgo biloba may lower plasma efavirenz levels by the induction of CYP2B6, CYP3A4, and/or P-glycoprotein (P-gp) (28). Unlike the inductive effects of ginkgo biloba on the pharmacokinetics of midazolam, ginkgo biloba extract did not change the exposure of lopinavir (a protease inhibitor and CYP3A substrate) when used with low-dose ritonavir. The use of low-dose ritonavir, a potent CYP3A inhibitor, may have offset the effect of ginkgo biloba on the pharmacokinetics of lopinavir (22).

In vivo data on ginkgo biloba with other antiretroviral drug classes, such as HIV integrase inhibitors, like raltegravir, are lacking. According to current guidelines, raltegravin in combination with tenofovir/emtricitabine is recommended as one of the preferred regimens for antiretroviral-naïve patients (8, 21). Raltegravir targets the HIV-1 integrase enzyme and prevents the integration of viral DNA into the genome of the host cell. Raltegravir has shown sustained antiretroviral activity, is generally well tolerated, and has little propensity to interact with other drugs. The primary route of metabolism is glucuronidation via UDP-glucuronosyltransferase 1A1 (UGT1A1) in the liver, with minor contributions from UGT1A3 and UGT1A9 (6, 12, 13). In vitro studies suggest that raltegravir is a weak P-gp substrate (20). There is in vitro and animal evidence that ginkgo biloba modulates UGT enzymes. Ginkgo biloba extract and ginkgolides induce the expression of UGT1A1 in human primary hepatocytes, although inhibitory effects on UGT enzymes of ginkgo biloba extract, or one of its components, have been described, as well (16, 18, 19, 29). Other investigations showed that long-term use of ginkgo biloba inhibits P-gp-mediated drug transport, but contrary results have also been reported (1, 9, 10, 30, 31).
Interaction between Ginkgo Biloba and Raltegravir

Pharmacokinetic sampling and safety assessments. Blood samples for assessment of the pharmacokinetic parameters of raltegravir were collected during a 12-hour period at 0 (predose), 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, and 12 h after intake of a single dose of 400 mg of raltegravir on days 15 and 36. Blood samples were collected in heparinized tubes and centrifuged for 10 min at 2,930 × g at 20°C. Plasma was transferred to polypropylene tubes and stored at −40°C until further bioanalysis. Blood samples for serum biochemistry and hematology were taken on days 15 and 36, as well as before and during ginkgo biloba treatment (days 1 and 8 of treatment). Subjects were asked about the presence of adverse events at each visit day. Screening for drugs of abuse in urine was performed on days 15 and 36; blood glucose testing and urinalysis were carried out on day 36. Pregnancy was checked by performing a human chorionic gonadotropin (hCG) blood test on all female subjects on days 15 and 36.

Compliance. All intake of medication at the clinical trial unit was supervised and recorded by the study personnel. Intake of ginkgo biloba tablets at home was monitored by the use of microelectronic monitoring system (MEMS) caps (Aardex Ltd., Zug, Switzerland), which record the opening of the medication bottle. In addition, ginkgo biloba tablets were counted on each visit day during ginkgo biloba treatment to assess adherence. Subjects were asked to write down the exact times of intake in a booklet.

Bioanalysis of raltegravir in plasma. The concentrations of raltegravir in plasma were analyzed by use of a validated reversed-phase high-pressure liquid chromatography (HPLC) method with fluorescence detection. Sample preparation consisted of a liquid-liquid extraction by adding 500 μl of acetate buffer (pH 4.0; 0.2 M), 5 ml of hexane/dichloromethane (1:1 [vol/vol]), and 50 μl of internal standard (lormetazepam in methanol-water [1:1 [vol/vol]]) to 500 μl of plasma. The samples were mixed on a vortex mixer for 5 min, followed by centrifugation at 11,500 × g for 5 min. After freezing at −40°C for 5 min, the organic supernatant was decanted and evaporated at 37°C under a stream of nitrogen gas. The residue was reconstituted in 200 μl of eluent (acetonitrile-phosphate buffer, pH 4.8; 20 mM; 35:65 [vol/vol]). Forty microliters of the reconstituted solution was injected onto a SymmetryShield RP 18 column (3.5 μm; 100 by 4.6 mm). The flow rate was set at 1.5 ml/min. Raltegravir was detected by the use of a fluorescence detector (λexcitation 240 nm; λemission 412 nm). The lower limit of quantification was 0.014 mg/liter. The linear calibration ranges in plasma were from 0.014 to 10 mg/liter. The validation results displayed accuracies of the quality control samples of 100%, 102%, and 107% at plasma concentrations of 0.060, 0.400, and 4.000 mg/liter. At the same concentrations, the precision values (within-day coefficients of variation [CV]) were 3.7%, 1.8%, and 0%, respectively. The raltegravir assay was performed at the laboratory of the Pharmacy of Radboud University Nijmegen Medical Centre (Nijmegen, The Netherlands) and was externally validated through the International Interlaboratory Quality Control Program for Measurement of Antiretroviral Drugs in Plasma (5).

Pharmacokinetic analysis. Pharmacokinetic parameters for raltegravir were calculated by noncompartmental methods using the WinNonlin software package (version 5.2; Pharsight, Mountain View, CA) and the linear log trapezoidal rule. Based on the individual plasma concentration-time data, the following pharmacokinetic parameters of raltegravir were determined: the area under the plasma concentration-time curve from dosing to infinity (AUC0–∞) (in mg · h/liter), the area under the plasma concentration-time curve from 0 to 12 h after intake (AUC0–12) (in mg · h/liter), the maximum plasma concentration of the drug (Cmax) (in mg/liter), the time to reach Cmax (Tmax) (in h), the apparent volume of distribution (V/F) (in liters), the apparent oral clearance (CL/F) (in liters/h), and the apparent elimination half-life (T1/2) (in h).

Sample size and statistical analysis. For the identification of a clinically relevant drug interaction, the bioequivalence approach was used, as described previously (25). The main pharmacokinetic parameter to be evaluated in this respect is the exposure to raltegravir, expressed as the AUC. Sample size calculation was performed using the method for two-period designs of Diletii et al. (7). The required sample size was calculated (power of 80%) assuming no difference in the AUC of raltegravir with or
without ginkgo biloba and an estimated intrasubject coefficient of variation of the log-transformed AUC values for raltegravir of 20%. The required number of participants was 16. Taking dropouts into account, a total of 18 subjects were included. Geometric mean ratios (GMRs) with 90% confidence intervals (CI) were calculated for AUC\(_{0-12}\), AUC\(_{0-12}\) h, \(C_{max}\), and \(T_{1/2}\) after log transformation of within-subject ratios of raltegravir combined with ginkgo biloba versus raltegravir alone. GMRs with 90% CI falling entirely within the range of 0.80 to 1.25 were considered to indicate no significant interaction. Statistical and descriptive analyses were carried out using SPSS for Windows, version 16.0.1 (SPSS, Chicago, IL), and Microsoft Office Excel 2007.

### RESULTS

#### Baseline characteristics.
A total of 18 subjects (9 male and 9 female, all Caucasian) were enrolled in the study and received treatment. The mean (range) age, body weight, and body mass index were 38 (22 to 55) years, 72 (52 to 93) kg, and 23 (19 to 28) kg/m\(^2\), respectively. The subjects were in good general health, according to medical histories, physical examinations, vital signs, and laboratory data. All included subjects completed the trial and were available for statistical evaluation.

#### Compliance.
The compliance with the ginkgo biloba treatment of all 18 subjects was good, as indicated by their statements about the intake of the drug doses as noted in the booklets, the number of ginkgo biloba tablets counted on each visit day, and the MEMS caps (data not shown). Only two subjects admitted to having missed one ginkgo biloba dose.

#### Pharmacokinetics.
The pharmacokinetic parameters and the plasma concentration versus time curves of raltegravir in the presence and absence of steady-state ginkgo biloba are shown in Table 1 and Fig. 1. Ginkgo biloba increased the maximum plasma concentration \((C_{max})\) and the area under the plasma concentra-

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Raltegravir alone</th>
<th>Geometric mean</th>
<th>95% CI</th>
<th>Raltegravir + ginkgo biloba</th>
<th>Geometric mean</th>
<th>95% CI</th>
<th>GMR</th>
<th>90% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>(AUC_{0-12}) (mg · h/liter)</td>
<td>6.35(^b)</td>
<td>4.39–9.18</td>
<td>7.44</td>
<td>5.10–10.9</td>
<td>1.21(^b)</td>
<td>0.93–1.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(AUC_{C_{max}}) (mg · h/liter)</td>
<td>5.93</td>
<td>4.21–8.34</td>
<td>7.33</td>
<td>5.01–10.7</td>
<td>1.24</td>
<td>0.97–1.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C_{max}) (mg/liter)</td>
<td>2.08</td>
<td>1.39–3.12</td>
<td>3.01</td>
<td>2.00–4.52</td>
<td>1.44</td>
<td>1.03–2.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(T_{max}) (h)</td>
<td>2.00</td>
<td>1.13–3.00</td>
<td>2.00</td>
<td>1.50–2.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(CL/F) (liters/h)</td>
<td>63.0(^b)</td>
<td>43.6–91.1</td>
<td>53.8</td>
<td>36.8–78.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V/F) (liters)</td>
<td>288(^b)</td>
<td>193–429</td>
<td>220</td>
<td>148–325</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(T_{1/2}) (h)</td>
<td>3.17(^b)</td>
<td>2.61–3.86</td>
<td>2.83</td>
<td>2.42–3.32</td>
<td>0.93(^b)</td>
<td>0.73–1.17</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) For \(T_{max}\), the median and interquartile range are reported.

\(^b\) Two subjects were excluded because the \(T_{1/2}\) could not be determined.

![FIG 1](http://aac.asm.org/) Geometric mean plasma concentrations following a single dose of 400 mg of raltegravir in the presence and absence of steady-state ginkgo biloba (semilog scale on the inset).
The apparent elimination half-life of raltegravir did not appear to be influenced by ginkgo biloba. The median time to reach $C_{\text{max}}$ of raltegravir was 2.0 h irrespective of ginkgo biloba treatment. For raltegravir coadministered with ginkgo biloba relative to raltegravir alone, the GMRs (90% confidence intervals) were 1.21 (0.93 to 1.58) for AUC$_{0-\infty}$, 1.44 (1.03 to 2.02) for $C_{\text{max}}$, and 0.93 (0.73 to 1.17) for $T_{1/2}$ (Table 1). Two subjects were excluded from the calculation of the GMRs of AUC$_{0-\infty}$ and $T_{1/2}$ because the $T_{1/2}$ and AUC$_{0-\infty}$ could not be determined. Figure 2 shows the individual-subject changes in the $C_{\text{max}}$ and AUC$_{0-\infty}$ of raltegravir alone and coadministered with ginkgo biloba. Although a small majority of the subjects showed an increase in the $C_{\text{max}}$ upon coadministration, considerable variation in the amount and direction of the effect was seen, as well as variation between individuals. This also applies to the observed individual changes in the AUC$_{0-\infty}$. The

**FIG 2** Individual changes in the maximum plasma concentration (top) and the area under the concentration-time curve (bottom) of raltegravir alone versus raltegravir coadministered with ginkgo biloba. (Two subjects were excluded because the $T_{1/2}$ and AUC$_{0-\infty}$ could not be determined.)
coefficients of variation of the AUC values of raltegravir alone and raltegravir with ginkgo biloba were 66% and 51%, respectively.

**Adverse events and safety assessments.** The study medication was generally well tolerated, and no serious adverse events were reported. There were no discontinuations due to adverse events, and all subjects completed the trial. Sixteen subjects reported a total of 32 adverse events. Five (16%) adverse events were considered possibly drug related (all were classified as grade 1). Diarrhea was reported by two subjects during ginkgo biloba treatment, and one subject reported a transient headache, which was possibly related to raltegravir and/or ginkgo biloba. One subject developed a grade I triglyceride elevation after administration of raltegravir, which returned to normal within 7 days. One subject had a mild elevated gamma-glutamyltransferase (γ-GT), which remained elevated during the study.

**DISCUSSION**

Herb-drug interactions are an important consideration in HIV-infected patients because herbs are frequently used, often not reported, and a potential cause of drug failure. A popular herbal product among HIV-infected individuals is ginkgo biloba extract, despite a lack of evidence for effectiveness or safety within this special patient group. Because human and preclinical data were inconclusive with regard to ginkgo biloba’s potential to modulate UGT and P-gp activity, a drug interaction study with raltegravir in healthy volunteers was carried out.

Steady-state ginkgo biloba increased the mean exposure to raltegravir (AUC<sub>0-∞</sub>) by 21% and the C<sub>max</sub> by 44%. However, the large 90% confidence interval for the GMRS partly overlaps the predefined range of 0.80 to 1.25 in the bioequivalence approach and reflects the variability in the individual changes in the AUC values and C<sub>max</sub> of raltegravir. Based on our findings, raltegravir exposure after a single dose was not clinically significantly reduced by concomitant use of ginkgo biloba extract at steady state, making it less likely that ginkgo biloba is a UGT inducer, as suggested by in vitro research. The observed mean increase in the C<sub>max</sub> by concomitant use of ginkgo biloba is more likely to be caused by a change in oral bioavailability than by inhibition of the metabolism of raltegravir, because the apparent elimination half-life of raltegravir remained unaffected. The slight increase in the raltegravir AUC<sub>0-∞</sub> when combined with ginkgo biloba is largely due to the observed increase in the C<sub>max</sub> (Fig. 1). The difference in the C<sub>max</sub> of raltegravir alone versus raltegravir with ginkgo biloba within subjects could be (partly) due to the normal intrasubject variability in raltegravir pharmacokinetics instead of an effect caused by ginkgo biloba. It is known that raltegravir pharmacokinetics exhibits considerable intra- and intersubject variability instead of an effect caused by ginkgo biloba. It is known that raltegravir pharmacokinetics exhibits considerable intra- and intersubject variability (2, 4). A possible explanation for the increase in the C<sub>max</sub> and bioavailability of raltegravir when combined with ginkgo biloba could be the inhibition of P-gp by ginkgo biloba. In vitro characterization of raltegravir transport by drug transporters indicates that raltegravir is a weak P-gp substrate. P-gp is an active ATP-dependent efflux pump and is encoded by the ABCB1 gene. Efflux mechanisms, such as P-gp, are responsible for transporting a broad range of compounds out of the intestinal epithelial cells back into the intestinal lumen and play an important role in oral drug absorption. The effect of chronic use of ginkgo biloba extract on the pharmacokinetics of the P-gp substrate talinolol was studied in healthy volunteers. The observed increase in the C<sub>max</sub> by 33% and in the AUC by 21% without any significant alterations in the T<sub>1/2</sub> and T<sub>max</sub> of talinolol supports our hypothesis. The variation in the change of the oral bioavailability of raltegravir and subsequent C<sub>max</sub> values could be a reflection of individual variation in the inhibitory potential of P-gp by ginkgo biloba. The expression and transport activities of P-gp may differ between individuals due to individual variations in the highly polymorphic ABCB1 gene. Therefore, the extent of the inhibition of P-gp may vary accordingly. Although inhibition of P-gp-mediated efflux of raltegravir by ginkgo biloba is an interesting hypothesis, one must be cautious in translating findings obtained from in vitro experiments directly to the clinical setting. Raltegravir transport by P-gp is not yet confirmed in human studies. There are currently no clinical data indicating that the pharmacokinetic profile of raltegravir may be affected by selective P-gp inducers or inhibitors.

It is well known that the pharmacokinetics of raltegravir displays large intersubject variability, which was observed in our study, as well (CV in AUC<sub>0-∞</sub>, 66%) (2, 4). Contributing factors, in general, are differences in absorption due to food intake or pH effects, genetic polymorphisms associated with altered UGT1A1 activity, and potential drug interactions. In this study, raltegravir was administered in a fasted state to minimize intersubject variability. Nevertheless, differences in gastric pH and therefore absorption of raltegravir probably led to variability in pharmacokinetic parameters between subjects, and maybe within subjects, as well. Individuals with decreased UGT1A1 expression caused by UGT1A1*28 polymorphism (approximately 7 to 19% of the Caucasian population is homozygous for UGT1A1*28) are known to have moderately elevated plasma levels of raltegravir. However, this increase in the plasma level is not considered to be of clinical importance. Pharmacogenetic testing was not performed in our study, and UGT1A1*28 polymorphism might have contributed to the intersubject variability, as well. In two subjects, the elimination half-life of raltegravir could not be determined because of secondary peaks in the plasma concentration-time curve. These secondary peaks are frequently observed in pharmacokinetic studies of raltegravir and can be attributed to either delayed absorption or enterohepatic circulation, which is not uncommon for UGT substrates (4, 6). The pharmacokinetic parameters observed in this study were compared with data from Wenning et al., as these healthy subjects (all with the UGT1A1*1/*1 genotype) were exposed to similar study conditions, i.e., a single intake of a 400-mg raltegravir tablet on an empty stomach. No major differences between our data and these historical controls could be observed.

The combined use of chronic ginkgo biloba and a single dose of raltegravir was well tolerated. No serious events were reported during the trial. The reported adverse events related to the study medication were mild and transient. In clinical practice, raltegravir is well tolerated, with no dose-related toxicities identified so far. Given that there have been no acute safety findings associated with peak raltegravir concentrations, the somewhat higher C<sub>max</sub> values for raltegravir when coadministered with ginkgo biloba compared with intake of raltegravir alone are not expected to lead to any meaningful clinically significant safety concerns.

Our study was undertaken in healthy volunteers, limiting our interpretation in HIV-infected individuals with concomitant medication and comorbidity. Because of the inconsistencies and controversies in the literature regarding the exact action of ginkgo biloba...
biloba extract on metabolizing enzymes or drug transporters and the variation in effects seen in this study, it is not possible to draw any definite conclusions. However, the study does provide data to support the idea that raltegravir can be used safely for the management of HIV infection when taken in combination with ginkgo biloba. No decrease in the AUC of raltegravir was observed, and the increases in the maximum plasma concentrations are not considered to be of clinical importance, due to the normal intersubject variability of raltegravir pharmacokinetics and the good safety profile of raltegravir.

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