Pharmacokinetics and dissolution of raltegravir

Original article

Comparison of the in vivo pharmacokinetics and in vitro dissolution of raltegravir

in HIV patients receiving the drug by swallowing or by chewing

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Abstract

The pharmacokinetics of raltegravir (RAL) in HIV patients is characterised by high inter/intra-patient variability. We investigated the potential contribution of the drug pharmaceutical formulation on RAL pharmacokinetics.

We firstly compared in vivo the pharmacokinetics of RAL from 67 patients in which the drug was administered by swallowing the intact tablet with those obtained from 13 HIV-infected patients that chewed the RAL tablet due to swallowing difficulties.

Subsequently, we evaluated in vitro the dissolution of RAL tablets under different conditions.

In the in vivo study we found that patients given RAL by chewing the tablets presented pharmacokinetic profiles characterised by significantly higher RAL absorption compared with patients receiving the drug by swallowing. The in vitro studies showed that when the whole tablets were exposed to an acidic medium the release of RAL was very low, whereas when crushed the profiles presented significantly higher concentrations of RAL. Crushed tablets tested in water or in a pH 6.8 buffer exhibited prompt and complete dissolution of RAL.

HIV-infected patients receiving RAL by chewing the tablet showed higher drug absorption and reduced pharmacokinetic variability compared with patients swallowing the intact tablet. This is related to problems in the tablet disintegration and to erratic drug absorption. The amelioration of the RAL pharmaceutical formulation could improve drug pharmacokinetics.
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Introduction

Raltegravir (RAL) is the first approved integrase inhibitor targeting the strand transfer step of HIV integration (14). Studies in HIV treatment-experienced as well as in naïve patients have shown that RAL-containing regimens have potent antiretroviral activity (2). Moreover, the favourable side effect profile in comparison to all other antiretrovirals as well as the minimal impact on lipid homeostasis has made RAL a strong option for the treatment of an array of HIV infected patients (2). Unexpected negative results from recent clinical trials in which RAL-based arms were associated with higher than expected virologic failure compared with protease inhibitor-based therapies have raised, however, some concerns on the clinical efficacy of RAL (7,12,15).

A clear relationship between clinical efficacy and RAL plasma concentrations has not been identified yet, and the pharmacokinetics of the drug appears to have less influence on treatment outcome than other covariates (e.g. baseline HIV RNA, use of other active agents in optimised background therapy, etc) (2). Nevertheless, this concept has been challenged by recent findings. Indeed, a prospective study involving 106 HIV-infected patients experiencing treatment failure under RAL-containing regimens has shown that plasma RAL exposure influences significantly the drug antiviral activity and the eventual selection of resistance mutations (11). This has been confirmed also by the results of the QDMRK trial (7), showing that plasma concentrations of RAL correlate significantly with the likelihood of virologic response in patients given the drug once daily. Despite the lack of establishment of a well-defined therapeutic range for RAL,
these studies indicate that a correlation may exist between the individual RAL plasma concentrations and the clinical outcome of HIV-infected patients, suggesting a potential relevance of therapeutic drug monitoring (TDM) in selected situations.

Limited studies in healthy volunteers and in HIV-infected patients have shown that RAL pharmacokinetics is characterised by high inter-patient variability (3,5,11,13). Recently, we have shown that the pharmacokinetics of RAL in HIV-infected subjects is characterised also by high intra-patient variability (4). Interestingly, in the previously published studies it was shown that gender, race, age, body mass index, food intake, and renal or hepatic insufficiency explain only in part the observed variability in RAL pharmacokinetics (2). More recently it was also shown that the presence of allelic variants in the UGT1A1 gene – that encodes for the enzyme involved in the metabolism of the drug – only minimally affects plasma concentrations of RAL (1,17). Therefore, at the present, the majority of RAL pharmacokinetic variability remains unexplained by genetic and non-genetic factors.

In the present study we investigated whether the observed wide inter- and intra-patient distribution of RAL plasma concentration is eventually related to the drug pharmaceutical formulation.
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Materials & Methods

Patients

All HIV-infected patients given RAL 400 mg twice daily as part of the maintenance highly active antiretroviral therapy (HAART) undergoing TDM as routine clinical practice were included in the present study. HIV patients given concomitant administration of antacids and/or other drugs known to influence significantly the absorption of RAL were excluded from the present study.

Study design

We firstly compared in vivo the pharmacokinetics of RAL from patients receiving the drug by swallowing the whole tablet with those obtained from HIV-infected patients that chewed the tablet due to swallowing difficulties (no specific contraindications for the administration of the drug by chewing are given in the ISENTRESS® full prescribing information sheet).

Subsequently we evaluated in vitro the dissolution of RAL tablets under different conditions, namely in water, in acidic solution and in pH 6.8 buffer. Dissolution tests were performed comparing RAL whole tablets (that mimics swallowing administration) with tablets crushed by grinding in mortar and pestle (that mimics administration of the tablet by chewing).

In vivo pharmacokinetic evaluations

The present study is based on a retrospective analysis of routine pharmacokinetic evaluations carried out as day-by-day clinical practice for the optimisation of drug
dosing in HIV-infected patients. For most antiretrovirals, TDM is usually performed by assessing the single plasma trough concentration. However, this approach is not feasible for RAL that indeed requires routine assessment of predicted AUC (5). The assessment of RAL plasma concentrations was therefore carried out as routine TDM based on the collection of blood samples within the first 4 hours after the morning RAL dose.

On the morning of the pharmacokinetic studies, blood samples were collected for the measurement of RAL plasma trough concentrations. Then patients took morning RAL dose as per their practice in the presence of the nursing staff. Subsequently, additional blood samples were collected at 1, 2, 3 and 4 hours after the morning drug dose. A maximum deviation from the scheduled sampling times of ± 5 minutes was considered as acceptable. A light standardised breakfast (coffee and semi-skimmed milk with biscuits or rusks, often served with orange juice) was served to all patients 90-120 min after drug intake. Patients had free access to water.

Palatability of RAL chewed tablet was assessed on the day of the pharmacokinetic evaluation by the patients using a 3-point scale (0=poor, 1=fair, 2=good) when responding to the question: How palatable do you feel the study medication is? The safety of RAL chewed tablet was assessed by the recording of adverse events.

The study was performed in accordance with the Declaration of Helsinki and in compliance with guidelines of good clinical practice.

**Assessment of RAL plasma concentrations**

RAL concentrations in plasma samples were determined by a HPLC method coupled with tandem mass spectrometry (LC-MS/MS) based on the assay originally developed by Fayet et al (9). The method was validated in agreement with the Consensus...
Guidelines on Bioanalytical Method Validation (10). Our laboratory participates in an external quality control programme for the continuous monitoring of the method’s performance (the Dutch KKGT). The method was linear over the RAL concentration ranges of 10 to 10000 ng/mL. Between and within-day imprecision and inaccuracy were less than 15%.

Drug release studies
Dissolution tests were conducted according to USP 30 Apparatus 2 guidelines (paddle method, Model AT7, Sotax, Basel, CH) with 900mL dissolution medium maintained at 37 ± 0.5°C and agitated at 50 rpm (n = 6). The media studied included purified water, pH 1.0 (0.1N HCl), and pH 6.8 (50mM phosphate buffer). At each sampling time, 1.5 mL of dissolution medium were withdrawn, filtered (0.22 μm syringe filter, Millipore, USA) and stored in polyethylene test tubes at 4°C until the analysis. Crushed tablets were prepared by grinding in mortar and pestle for 5 min and the obtained powders were transferred into dissolution vessel by wax paper.

Samples were analysed for drug content using a high performance liquid chromatography (HPLC) system with a photodiode array detector (Model Alliance, Waters, Milford, MA, USA) set at a wavelength of 260 nm. For the analysis the 50 μL of the samples were diluted with 450 μL of purified water in HPLC glass vials, vortexed for 30 s and injected. Concentrations were confirmed by HPLC MS/MS after appropriate dilution of the sample.
Statistical analyses

Normal distribution of the continuous variables was confirmed by the Kolmogorov-Smirnov test (eventually retested after logarithmic transformation). Not-normally distributed variables were expressed as median (interquartile range, IQR) and normally-distributed variables as means ± standard deviation (SD).

Between-patient variability in the main RAL pharmacokinetic parameters were expressed as the coefficient of variation (CV%). RAL AUC$_{0-4}$ was estimated using the trapezoidal rule. RAL AUC$_{0-12}$ was estimated using the following algorithm (5):

$$AUC_{0-12} = 2.082C_0 + 0.821C_1 + 1.238C_2 - 0.210C_3 + 4.280C_4 + 783.1$$

Comparisons of the main RAL pharmacokinetic parameters between patients given the drug by swallowing or by chewing were performed using unpaired T-test or the corresponding non parametric test as deemed appropriate according to the data distribution.
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Results

In vivo RAL pharmacokinetics

A total of 106 RAL pharmacokinetic profiles from 0 to 4 hours after the morning drug dose were collected from 80 HIV-1-infected patients. Ninety-three out of the 106 pharmacokinetics evaluations were assessed in 67 patients given the drug by swallowing the intact tablet. The remaining 13 pharmacokinetics evaluations were obtained from 13 HIV-infected patients that chewed RAL tablet due to swallowing difficulties. Baseline demographic characteristics of the patients included in the present study were given in Table 1.

As shown in Figure 1, patients taking RAL by chewing presented regular pharmacokinetic profiles, characterised by single sharp drug peak and significantly higher RAL absorption – assessed by RAL C<sub>max</sub> and AUC<sub>0-4</sub> (Table 1) – compared with patients taking the drug by swallowing the intact tablet (RAL C<sub>max</sub>: 5404 ± 3032 vs. 3128 ± 2588 ng/mL, p=0.004; RAL AUC<sub>0-4</sub>: 11634 ± 7288 vs. 7007 ± 5803 ng*h/mL, p=0.011). The higher RAL absorption determined also a non significant trend for higher daily drug exposure in patients taking the drug by chewing vs. by swallowing (RAL AUC<sub>0-12</sub>: 15024 ± 9693 vs.11803 ± 9544 ng*h/mL, p=0.084).

Large inter-patient variability in the main RAL pharmacokinetic parameters was observed in both groups of patients (Table 2). However, with the exception of RAL trough concentrations, patients taking RAL by chewing had a 30-60% reduction in the coefficient of variation associated with the main RAL pharmacokinetic parameters (Table 2).
Most of the patients taking RAL by chewing found the palatability fair (n=12), while in the remaining palatability was rated as poor (n=1). No adverse events were reported during the study.

In vitro studies

The in vitro studies showed that the intact tablets presented relatively slow release profiles probably due to lacking disintegration compared with crushed tablets in all the media considered (Figure 2 A-C). In particular, for intact tablets tested in an acidic medium the RAL concentrations were very low, reaching less the 10% of the dose after 2 hours (Figure 2B), owing to well-known poor solubility of RAL at low pH (Isentress Product Monograph, available at http://www.merck.ca, last access April 11, 2012). Crushed tablets tested in water and in a pH 6.8 buffer exhibited prompt and complete dissolution of RAL with the total amount of the drug dissolved in 15 min (Figure 2A, 2C). When crushed tablets were tested in acid, the powder tended to float on the fluids at the beginning of the test. After few seconds however, the powder was totally wetted and a white dispersion formed in the dissolution vessel. The dissolution profiles obtained from crushed tablets in the acidic medium presented relatively higher concentrations of RAL compared to those from intact tablets, with an evident supersaturation peak possibly explained by RAL potassium salt that almost immediately recrystallised as free acid.
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**Discussion**

The present study documents for the first time that HIV-infected patients taking RAL by chewing show higher drug absorption and less inter-patient pharmacokinetics variability compared with patients taking the drug by swallowing. These results may explain the previously observed large inter- and intrapatient variability in the daily concentrations of RAL (3-5,11,13), whose pharmacokinetics is not influenced significantly by genetic and non-genetic characteristics of the patients (1,2,17). Our results points towards a significant role of the drug pharmaceutical formulation *per se* in determining the variable and irregular pharmacokinetics of RAL. This conclusion is sustained not only by the pharmacokinetic profile of RAL release *in vivo* but also by *in vitro* analyses.

The *in vitro* dissolution of RAL tablets was tested under different conditions (pH 1, pH 6.8 buffer and water) and by comparing the dissolution of RAL intact tablets (that mimics drug administration by swallowing) with tablets crushed by grinding in mortar and pestle (that fairly mimics drug administration by chewing the tablet). Using this approach we documented that *in vitro* the dissolution of RAL from whole tablets is very low in acid conditions, while it fairly improved in a neutral environment (water and pH 6.8). Conversely, crushed tablets tested in water and pH 6.8 buffer exhibited prompt and complete dissolution of RAL, with the total amount of the drug dissolved in 15 min.

Also when tested in acid conditions, crushed tablets showed higher concentrations of RAL compared to the whole tablets.

Our *in vivo* and *in vitro* findings, suggest that the tablets administered to patients by swallowing do not fully disintegrate in the stomach leading to a negligible drug release from the tablets for the entire duration of the gastric residence. In order to release the
active ingredients tablets need to reach the duodenum where RAL presumably can find an environment (neutral pH) that can ease its dissolution. The gastric residence times of non-disintegrating dosage form with dimensions greater than 10 mm are extremely variable, mainly linked to the presence or absence of food and to the type of food ingested (6). In the case an empty stomach (fasting state) the transit time of pharmaceutical dosage forms up to 12-14 mm in size is relatively rapid and depends on the recurrence of strong contractions of the gastric muscular tissues every about 2 hours (also known as housekeeper waves) which transfer the stomach content to the duodenum. In fed state, the gastric residence time of relatively big solid dosage forms is linked the duration of the digestive phase, which in turn depends on the amount and type of food ingested. In contrast, solutions or suspensions of solids and small units (<4-7 mm) can freely pass the pylorus that is partially open, even during the digestion process, being thus less influenced by the variability in gastric emptying times. Thus the lack of disintegration of the RAL tablets and the relatively low solubility of the active ingredient in acidic environment that are highly affected by the gastric residence times are consistent with the lower absorption of RAL observed in patients who swallowed the intact tablets compared to that of patients who chewed the tablets. Besides the higher reproducibility in the stomach transit, dispersion obtained by chewing the tablet can also promote the dissolution of the active ingredient in acidic medium by exploiting the supersaturation of RAL potassium salt \textit{in vivo} and consequently enhance the RAL absorption.

The present study was not designed and powered to assess whether the observed improvement in RAL pharmacokinetics eventually impact on patient outcome. The
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potential contribution of RAL pharmacokinetics on HIV virologic response is still a
matter of debate. Indeed, phase II/III trials have shown that RAL plasma concentrations
and drug exposure correlated with efficacy outcome, whereas no relevant clinical
associations were found with RAL C_{trough} (11,13,16). This latter finding was not
unexpected, given the failure of RAL C_{trough} to reliably predict daily drug exposure,
expressed as RAL AUC_{0-12} (5). In the present study, we have shown that patients taking
RAL by swallowing the whole tablets experienced a wide distribution in the main drug
pharmacokinetic parameters. However, this variability is greatly reduced in patients that
chewed RAL tablets. Accordingly, it can be reasonably speculated that the
administration of RAL by chewing the tablet – by providing higher RAL absorption
with reduced variability – may improve the way to use this drug in the clinical practice.
The possibility that the pharmacokinetics of RAL could be further improved by
dissolving the pulverized tablets in water before taking the drug is an intriguing
hypothesis that needs to be investigated.

The use of RAL once-daily has been considered an attractive option to promote
patients’ adherence to the therapeutic regimen. However, a recent phase III trial
involving antiretroviral-naive patients treated with RAL 800 mg once daily or 400 mg
twice daily both in combination with tenofovir/emtricitabine has shown that RAL once-
daily was associated with significantly lower virological response compared with the
twice-daily administration (8). Nevertheless, by considering that: I) the inhibition of
RAL to the integrase enzyme is irreversible (2); II) administration of RAL by chewing
the tablet produces significantly higher drug absorption, and III) more drug available for
the binding to the integrase may result in a higher extent of target enzyme inhibition, it
cannot be excluded that the administration of RAL at 800 mg/once daily by chewing the tablet may improve the virologic response compared with RAL at 800 mg/once daily by swallowing the whole tablet, and allow a better patient compliance compared with RAL at 400 mg twice daily. In our opinion this is an intriguing hypothesis that needs further investigation.

In conclusion, we have shown that HIV-infected patients taking RAL by chewing the tablets have higher drug absorption and lower drug inter-subject pharmacokinetic variability compared with patients taking the drug by swallowing the whole tablets. We also show that this may depend on issues of appropriate tablets disintegration in the gastric environment leading to erratic drug release. The improvement of the drug pharmaceutical formulation could potentially increase the response of HIV-infected patients to RAL-based regimens.
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Funding
No specific funding has been received for this study. The study was carried out as part of our routine work.

Transparency declaration
DC has received educational grants from Merck Sharp & Dome (MSD) and from Janssen-Cilag.
CG has received educational grants from Abbott, Boehringer Ingelheim, Bristol Myers Squibb, Merck Sharp & Dome (MSD) and Janssen-Cilag.
MG and GR have receive educational grants from Abbott, Boehringer Ingelheim, Bristol Myers Squibb, Gilead, Merck Sharp & Dome (MSD), VIIV and Janssen-Cilag.
EC has received educational grants from Zambon Italia and Nicox.
For the remaining authors none were declared.
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References


Figure 1
Raltegravir time-concentration profiles in 80 HIV-patients given the drug by swallowing the whole tablet (n=67) or by chewing the tablet before swallowing (n=13).
Figure 2
In vitro dissolution profiles of whole tablets versus crushed tablets of raltegravir in water (panel A), at pH 1 (panel B) and at pH 6.8 (panel C).
Table 1. Demographic characteristics of HIV-1 patients given raltegravir by swallowing the intact tablet with or by chewing the tablet due to swallowing difficulties.

<table>
<thead>
<tr>
<th></th>
<th>Raltegravir chewed (n=13)</th>
<th>Raltegravir swallowed (n=67)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males, %</td>
<td>62%</td>
<td>68%</td>
</tr>
<tr>
<td>Caucasians, %</td>
<td>85%</td>
<td>88%</td>
</tr>
<tr>
<td>Age, years</td>
<td>49 ± 13</td>
<td>45 ± 9</td>
</tr>
<tr>
<td>Weight, Kg</td>
<td>69 ± 11</td>
<td>68 ± 15</td>
</tr>
<tr>
<td>AST, IU/L</td>
<td>39 ± 23</td>
<td>30 ± 9</td>
</tr>
<tr>
<td>ALT, IU/L</td>
<td>36 ± 30</td>
<td>38 ± 27</td>
</tr>
<tr>
<td>Serum Creatinine, mg/dL</td>
<td>1.0 ± 0.3</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>CD4 cell count, cells/μL</td>
<td>599 ± 447</td>
<td>580 ± 424</td>
</tr>
<tr>
<td>Concomitant HAART, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- protease inhibitor-based</td>
<td>46%</td>
<td>64%</td>
</tr>
<tr>
<td>- tenofovir-based</td>
<td>38%</td>
<td>22%</td>
</tr>
<tr>
<td>- other</td>
<td>16%</td>
<td>14%</td>
</tr>
</tbody>
</table>

AST: aspartate aminotransferase, ALT: alanine transaminase, HAART: highly active antiretroviral therapy
Table 2. Main raltegravir pharmacokinetic parameters measured in HIV-1 patients in which the drug was administered by swallowing the intact tablet with those obtained from HIV-infected patients that chewed the RAL tablet due to swallowing difficulties.

<table>
<thead>
<tr>
<th>Main pharmacokinetic parameters</th>
<th>Raltegravir Chewed (n=13)</th>
<th></th>
<th>Raltegravir Swallowed (n=93)</th>
<th></th>
<th>p-value^</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrough, ng/mL</td>
<td>350 ± 414</td>
<td>118</td>
<td>544 ± 655</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>C1h, ng/mL</td>
<td>5397 ± 3043</td>
<td>56</td>
<td>2110 ± 2132</td>
<td>101</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C2h, ng/mL</td>
<td>3480 ± 2415</td>
<td>69</td>
<td>2256 ± 2342</td>
<td>104</td>
<td>0.044</td>
</tr>
<tr>
<td>C3h, ng/mL</td>
<td>1975 ± 1490</td>
<td>75</td>
<td>1872 ± 1858</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>C4h, ng/mL</td>
<td>1212 ± 1004</td>
<td>82</td>
<td>1360 ± 1537</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>Cmax, ng/mL</td>
<td>5404 ± 3032</td>
<td>56</td>
<td>3128 ± 2588</td>
<td>83</td>
<td>0.004</td>
</tr>
<tr>
<td>Tmax, min*</td>
<td>60 (60 – 120)</td>
<td>25</td>
<td>180 (0 – 240)</td>
<td>63</td>
<td>0.028</td>
</tr>
<tr>
<td>AUC0-4, ng*h/mL</td>
<td>11634 ± 7288</td>
<td>62</td>
<td>7007 ± 5803</td>
<td>83</td>
<td>0.011</td>
</tr>
<tr>
<td>AUC0-12, ng*h/mL</td>
<td>15024 ± 9639</td>
<td>64</td>
<td>11803 ± 9544</td>
<td>83</td>
<td>0.084</td>
</tr>
</tbody>
</table>

*median (interquartile range)
CV: coefficient of variation
^refers to the comparison of the raltegravir pk parameters between the two groups