Intracellular Accumulation of Tipranavir/Ritonavir

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INTRODUCTION
Measurement of plasma concentrations of PIs is a reliable tool but only the fraction reaching the intracellular compartment is expected to exert action. Aim of our study was to evaluate intracellular penetration of TPV/RTV in multiexperienced patients administered with 2 NRTIs + TPV/RTV 500/200 mg BID. Plasma and PBMCs TPV and RTV concentrations were measured at the end of dose interval (C\text{t\text{rough}}) in 14 pts; five pts. underwent also to complete 12-h PK sampling (1.5, 3.5, 7 and 12 hours after dose intake). Plasma TPV and RTV concentrations were measured by validate HPLC methods while intracellular ones in PBMCs by RP-HPLC coupled with an ESI-MS detector. Values were given as mean ± SD. TPV C\text{t\text{rough}} in PBMCs and plasma were 7176 ± 6465 and 43080 ± 30319 ng/ml, (ratio 0.15 ± 0.036). RTV C\text{t\text{rough}} in PBMCs and plasma were 1319 ± 1101 and 345 ± 386 ng/ml, (ratio 4.86 ± 1.68). Correlations between plasma TPV C\text{t\text{rough}} and PBMCs TPV C\text{t\text{rough}} (R=0.969, p=0.0001), plasma RTV C\text{t\text{rough}} (R=0.776, p=0.001), PBMCs RTV C\text{t\text{rough}} (R=0.837, p=0.001) were observed. This is the first report on intracellular penetration of TPV/RTV: tipranavir showed to have a poor intracellular accumulation (near 15%) while RTV showed a concentration within the cells up to four-fold higher than in the plasma. Further studies are warranted to investigate such differential accumulation and the clinical impact of these findings. Correlation between intracellular and plasmatic concentrations of TPV support the use of the latter as a tool for TDM in the clinical setting.

METHODS
We evaluated multiexperienced patients administered two NRTIs + TPV/RTV 500/200 mg BID for at least three months, and with self-reported optimal adherence. After having obtained informed consent, plasma and peripheral blood mononuclear cells (PBMCs) paired samples were obtained at the end of dose interval (C\text{t\text{rough}}) in 14 patients. Five out of the 14 also underwent complete 12h PK sampling (1.5, 3.5, 7 and 12 hours after last dose intake). Plasma concentrations of TPV were measured by a previously published method (12). RTV plasma concentrations were quantified by a validated HPLC-UV method. PBMCs were isolated from whole blood by density gradient centrifugation on Ficoll (700 g, 25 min, 4°C) and washed with cold phosphate-buffered saline (PBS), and then stored at -80°C. Precise cell number and median cell volume were calculated using a Coulter electronic cell counter. Intracellular levels of TPV and RTV were measured by a validated assay based on a liquid/liquid extraction coupled with an HPLC-MS method, [modified from Ford et al., Antimicrob Agents Chemother. 2004 July; 48(7):2388-93]. Briefly, internal standard (quinoa-
line) was added to thawed PBMC aliquots (samples, standards and quality controls) previously stored in methanol:water (70:30), and the mixtures were sonicated, vortexed and centrifuged at 12000 rpm (10 minutes, 4°C). Supernatants were collected in glass shots and dried with a vacuum-spin. Dry samples were reconstituted in acetonitrile:water (40:60) and 40 µl injected in a reverse-phase C-18 Atlantis column (150 x 2.1 mm) (Waters). RTV and TPV masses, m/z 721.4 and m/z 603.3 respectively, were monitored with a ZQ mass system, in Electron Spray Ionization +. Median individual cell volume, calculated by Coulter cell counter (Beckman Coulter Z2), was used to obtain an accurate quantification of intracellular drug levels. Results were expressed as mean ± SD.

**RESULTS**

TPV C<sub>trough</sub> in PBMCs and plasma were 7176 ± 6465 and 43080 ± 30319 ng/ml, (ratio 0.15 ± 0.036). TPV C<sub>max</sub>, AUC and half-life in PBMCs and plasma were 10732 ± 5794 and 71289 ± 27062 ng/ml (ratio 0.14 ± 0.04); 70043 ± 39185 and 560639 ± 261411 ng*h/ml (ratio 0.12 ± 0.05) and 5.38 ± 1.66 and 6.28 ±1.29 hours, respectively (Table 1 and Figure 1). RTV C<sub>trough</sub> in PBMCs and plasma were 1319 ± 1101 and 345 ± 386 ng/ml, (ratio 4.86 ± 1.68). RTV C<sub>max</sub>, AUC and half-life in PBMCs and plasma were 1617±644 and 1182 ±673 ng/ml, (ratio 1.48 ± 0.5); 14514 ± 5493 and 7448 ± 3625 ng*h/ml (ratio 2.03 ± 0.54) and 7.81 ± 1.67 and 3.36 ± 1.1 hours, respectively (Table 2 and Figure 2). Overall, correlations between plasma TPV C<sub>trough</sub> and PBMCs TPV C<sub>trough</sub> (R=0.969, p<0.0001), plasma RTV C<sub>trough</sub> (R=0.776, p=0.001), PBMCs RTV C<sub>trough</sub> (R=0.837, p=0.837, p<0.0001) were observed (Table 3).

**DISCUSSION**

This is the first report on the intracellular penetration of TPV/RTV in the clinical setting. A new method for the measurement of intracellular concentration of TPV and RTV was validated relying on precise individual PBMCs count and median cell volume calculation with a Coulter cell counter. TPV unexpectedly showed a poor intracellular accumulation (near 15%), lower than other PIs.

### Table 1. Plasma and intracellular PK parameters of TPV (results are expressed as mean ±SD, ng/ml)

<table>
<thead>
<tr>
<th></th>
<th>C&lt;sub&gt;trough&lt;/sub&gt; (ng/ml) (n=14)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml) (n=5)</th>
<th>AUC (ng*h/ml) (n=5)</th>
<th>Half-life (hrs) (n=5)</th>
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<tbody>
<tr>
<td>PBMCs</td>
<td>7176 ± 6465</td>
<td>10732 ± 5794</td>
<td>70043 ± 39185</td>
<td>5.38 ± 1.66</td>
</tr>
<tr>
<td>Plasma</td>
<td>43080 ± 30319</td>
<td>71289 ± 27062</td>
<td>560639 ± 261411</td>
<td>6.28 ± 1.29</td>
</tr>
<tr>
<td>Ratio PBMC/plasma</td>
<td>0.15 ± 0.036</td>
<td>0.14 ± 0.04</td>
<td>0.12 ± 0.05</td>
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</table>

### Table 2. Plasma and intracellular PK parameters of RTV (results are expressed as mean ±SD, ng/ml)

<table>
<thead>
<tr>
<th></th>
<th>C&lt;sub&gt;trough&lt;/sub&gt; (ng/ml) (n=14)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml) (n=5)</th>
<th>AUC (ng*h/ml) (n=5)</th>
<th>Half-life (hrs) (n=5)</th>
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<tr>
<td>PBMCs</td>
<td>1319 ± 1101</td>
<td>1617 ± 644</td>
<td>14514 ± 5493</td>
<td>7.81 ± 1.67</td>
</tr>
<tr>
<td>Plasma</td>
<td>345 ± 386</td>
<td>1182 ± 673</td>
<td>7448 ± 3625</td>
<td>3.36 ± 1.10</td>
</tr>
<tr>
<td>Ratio PBMC/plasma</td>
<td>4.86±1.68</td>
<td>1.48 ± 0.5</td>
<td>2.03±0.54</td>
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### Table 3. Correlation between TPV C<sub>trough</sub> and RTV C<sub>trough</sub> (n = 14, Spearman's test)

<table>
<thead>
<tr>
<th>Plasma TPV C&lt;sub&gt;trough&lt;/sub&gt;</th>
<th>PBMCs TPV C&lt;sub&gt;trough&lt;/sub&gt;</th>
<th>R=0.969, p&lt;0.0001</th>
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<tr>
<td>Plasma TPV C&lt;sub&gt;trough&lt;/sub&gt;</td>
<td>Plasma RTV C&lt;sub&gt;trough&lt;/sub&gt;</td>
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<td>PBMCs RTV C&lt;sub&gt;trough&lt;/sub&gt;</td>
<td>R=0.837, p&lt;0.0001</td>
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is noteworthy that TPV is protein binding: >99.9% and this characteristic can influence its disposition in body fluids and compartments.

On the other hand, RTV showed a cell concentration up to fourfold higher than in the plasma. Reasons for this peculiarity need to be further investigated, but a possible interplay at the transmembrane influx/efflux pumps system could be anticipated.

From a clinical viewpoint, these findings may have implications in terms of toxicity. TPV was described to be an inducer of CYP3A4. In fact, any kind of concomitant PIs, known to be substrates of the latter, showed a marked reduction of plasma concentration in the presence of TPV. In addition, the plasma concentration of RTV, when used as a booster of TPV at 200 mg bid dosing, was described to be equivalent or inferior to the those obtained when used as a booster of other PIs at the dose of 100 mg bid (e.g., with LPV or DRV). Due to the pattern of dose- and concentration-related toxicity of RTV, a potentially increased risk of the latter when used as a booster of TPV did not appear to be relevant. However, TPV/RTV in both trials and clinical setting showed a not negligible risk of hepatotoxicity, possibly related to TPV plasma exposure (13).

Our data could shed some light on this issue. Intracellular accumulation of TPV-associated RTV was significantly higher than the available data of other boosted PIs, where RTV plasma and intracellular concentrations were shown to be substantially equivalent. Moreover, a correlation between both plasma and intracellular TPV and RTV levels was found. Therefore, in spite of low plasma levels, RTV accumulation within the cells could lead to some degree of toxicity, especially in subjects with the highest cellular levels. For instance, the above cited high TPV plasma exposure associated with the risk of hepatotoxicity could reflect such a condition, identifying subjects with the highest plasma and, especially, intracellular
RTV exposure. RTV had shown a clear indirect relationship between exposure and risk of hepatotoxicity, being associated with the latter when used at a full dose (600 mg bid) as an antiviral agent and not when used at baby doses as a booster of other PIs (14,15).

In any case, the correlation between intracellular and plasma concentrations of TPV and RTV support the use of RTV as a tool for TDM in the clinical setting.

Further studies are warranted to elucidate the reasons for such differential accumulation and to investigate the clinical impact of these findings in terms of efficacy and toxicity.

REFERENCES


