Influence of body temperature on indinavir crystallization under loop of Henle conditions

Saima Salahuddin1,2, Dik J. Kok1 and Noor N.-P. Buchholz2*

1Department of Experimental Urology, Erasmus Medical Centre, Dr Molenwaterplein, 3010 GD, Rotterdam, The Netherlands; 2Department of Urology, St Bartholomew’s Hospital, Barts and The London NHS Trust, London EC1A 7BE, UK

Received 1 July 2006; returned 10 August 2006; revised 1 October 2006; accepted 4 October 2006

Objectives: Indinavir is a protease inhibitor used in the therapy of HIV-1+ patients. It causes indinavir stone formation. It has been shown to precipitate in the loop of Henle (LH) at plasma concentrations (conc[P]) of ~8 mg/L. Those experiments were performed at room temperature. Given the influence of temperature on crystallization in general, and solubility of indinavir in particular, we repeated the experiments under physiological (body) temperature conditions.

Methods: Test solutions contained indinavir concentrations of 100–750 mg/L at ionic strengths varying from 0 to 800 mM simulating conditions in the proximal tubule and the LH. Solutions were titrated with base (NaOH) to find the pH value where nucleation is initiated. Experiments were conducted at room temperature (20°C) and repeated under constantly monitored (body) temperature (37°C).

Results: Experiments at 20°C confirmed our previous results. At 37°C, the relationship between pH and indinavir concentration remained inversely proportional. Again, the LH was confirmed as the most likely localization of crystallization. However, at 37°C precipitation occurred at a lower urinary concentration (100 versus 125 mg/L) and within a lower pH range (6.67–7.26 versus 7.23–7.44). This lower urinary concentration corresponds to a lower conc[P] [critical value (CV)] of 6.41 mg/L, as compared with 8.01 mg/L at 20°C.

Conclusions: The CV is even lower at 37°C than previously assumed. Plasma peak concentration above the CV of 6.4 mg/L will induce crystallization in the LH and should be avoided.

Keywords: protease inhibitors, drug kinetics, dosages, in vitro experiments

Introduction

Indinavir is a protease inhibitor used particularly in third world countries in the management of AIDS. Indinavir-urolithiasis is an inherent problem.1 Urinary solubility of indinavir is driven by concentration and pH. We have previously shown that the loop of Henle (LH) is the most likely place for indinavir crystallization, and a critical value (CV) of indinavir plasma concentration (conc[P]) at which intra-tubular crystallization may occur has been reported.2

Those experiments were conducted at room temperature. Since temperature is a driving force in crystallization processes, we repeated those experiments at body temperature reflecting more physiological conditions. The aim of the study was to find a reliable critical indinavir conc[P] which can be considered in balancing between drug efficacy and the risk of stone formation.

Materials and methods

Pure indinavir sulphate powder was provided by the manufacturer [Merck, Sharp & Dohme (MSD), Hoddesdon, Hertfordshire, UK]. TRIZMA® Base (Sigma Chemical Co., St Louis, MO, USA) was used to make artificial urine.

Experiments were conducted at room temperature (20°C) and repeated at body temperature (37°C). A stock solution of 2 g of indinavir in 1 L of TRIZMA® buffer (pH 4) was prepared. Another stock solution of TRIZMA® buffer (10 mM, pH 4) was made to obtain stock solutions of various ionic strengths of 400, 800 and 1600 mM by dissolving appropriate amounts of NaCl. Titration solutions of NaOH were prepared (0.1 and 0.01 mM). All reagents were of analytical purity. All solutions were prepared with Milli-Q® high-quality distilled water.

Appropriate amounts of indinavir stock solution and the buffer solutions of various ionic strengths were mixed, resulting in final
indinavir concentrations of 100, 125, 150, 200, 250, 500 and 750 mg/L, respectively, with various ionic strengths of 0 (buffer solution only), 200, 400 and 800 mM each, respectively. These ionic strengths represent conditions in various parts of the nephron. The mixtures were stirred at pH 4 to maintain indinavir solubility. The mixtures were then titrated with NaOH. In the experiments conducted at 37°C, solutions were kept in a water bath with a monitored temperature of 37 ± 1°C before mixing, and titration and observations were done in a temperature chamber at 37°C.

Titration steps were performed every minute under continuous observation of optical density up to the pH at which crystallization was observed.

Experiments were repeated at least three times each, and the average pH value at which crystallization occurred for a given concentration and ionic strength was calculated.

For each precipitation point the corresponding pH and ionic strength values have been established by a nephron model. Assuming a 61% plasma-protein binding of indinavir, 100% filtration of plasma indinavir, and a 2-fold secretion component in the proximal tubule, the corresponding plasma concentration will be calculated by the following equation:

\[ \text{conc[P]} = \frac{\text{indinavir conc[nephron]} \times \text{indinavir secretion concentration capacity} \times \text{fraction of filterable plasma indinavir}}{\text{conc[U]}} \]

where indinavir conc[P] corresponds to the indinavir concentration in the plasma; indinavir conc[nephron] corresponds to the indinavir concentration in the corresponding nephron; indinavir secretion corresponds to indinavir secretion in the proximal tubule (estimated at 2); concentrating capacity corresponds to the concentration capacity in the corresponding part of the nephron, and fraction of filterable plasma indinavir corresponds to filterable indinavir (≈39%).

### Discussion

Indinavir, usually boosted with ritonavir (400/100 mg twice a day), is currently the cheapest form of protein inhibitor therapy and plays a role in third world countries where access to affordable yet efficient therapy is paramount. Indinavir competitively attaches to viral protease thus preventing maturation of HIV virions. Indinavir-uro lithiasis is an inherent problem and occurs in up to 13% of cases. This led to its disuse in developed countries after development of better but more expensive alternatives.

We have previously shown that the LH is the most likely place for indinavir crystallization and a CV of indinavir conc[P], at which intra-tubular crystallization may occur, has been reported. Although boosted regimens have reduced the overall indinavir dosage whilst maintaining efficacy through the use of ritonavir as a pharmacokinetic indinavir inhibitor, its conc[P] can still rise well beyond CV.

Urinary solubility of indinavir is driven by concentration and pH. For a previously used dose of 800 mg and an average 1500 mL per 24 h urine output, the urinary indinavir concentrations have been estimated at 0.2–0.3 mg/mL within 3 h after drug administration. This would be already at the limit of its solubility, even at low urinary pH. In the LH, pH increases transiently but consistently and the tubular fluid is concentrated. The extent of the pH increase and the concentrating factor are directly related to the length of the loop because of the multiplier mechanism. In the longest loops pH increases to 7.4, and the fluid is concentrated 20-fold. Such conditions favour indinavir crystal formation. The number of affected nephrons depends on the filtered load

### Table 1. Crystallization thresholds (pH)

<table>
<thead>
<tr>
<th>conc[U]</th>
<th>20°C</th>
<th>37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>7.18 (±0.03)</td>
<td>7.61 (±0.03)</td>
</tr>
<tr>
<td>250</td>
<td>7.30 (±0.04)</td>
<td>7.63 (±0.05)</td>
</tr>
<tr>
<td>300</td>
<td>7.32 (±0.05)</td>
<td>7.65 (±0.06)</td>
</tr>
<tr>
<td>400</td>
<td>7.34 (±0.06)</td>
<td>7.67 (±0.07)</td>
</tr>
<tr>
<td>500</td>
<td>7.36 (±0.07)</td>
<td>7.69 (±0.08)</td>
</tr>
<tr>
<td>100</td>
<td>7.41 (±0.08)</td>
<td>7.70 (±0.09)</td>
</tr>
<tr>
<td>125</td>
<td>7.43 (±0.09)</td>
<td>7.72 (±0.10)</td>
</tr>
<tr>
<td>150</td>
<td>7.45 (±0.10)</td>
<td>7.74 (±0.11)</td>
</tr>
<tr>
<td>200</td>
<td>7.47 (±0.11)</td>
<td>7.76 (±0.12)</td>
</tr>
<tr>
<td>250</td>
<td>7.49 (±0.12)</td>
<td>7.78 (±0.13)</td>
</tr>
<tr>
<td>300</td>
<td>7.51 (±0.13)</td>
<td>7.80 (±0.14)</td>
</tr>
<tr>
<td>400</td>
<td>7.53 (±0.14)</td>
<td>7.82 (±0.15)</td>
</tr>
<tr>
<td>500</td>
<td>7.55 (±0.15)</td>
<td>7.84 (±0.16)</td>
</tr>
<tr>
<td>750</td>
<td>7.57 (±0.16)</td>
<td>7.86 (±0.17)</td>
</tr>
</tbody>
</table>

conc[U], urinary concentration of indinavir in mg/L; IS, ionic strength (in mM); IS 0.0, buffer solution (no ionic strength); *conditions at the end of the proximal tubule; **conditions at the descending loop of Henle.
of indinavir. Notably, pH also increases in the collecting ducts beyond the solubility capacity of indinavir, but is dependent upon the acid–base regulatory control through humoral mechanisms and is, therefore, conditional. However, this will often create an environment of low solubility and crystallization.

This study not only confirms the site of indinavir crystallization in the LH, but also shows that at 37°C crystallization may occur at a lower conc[P] than reported before and may affect more nephrons.

A relationship between high conc[P] and urinary symptoms, and, in turn, symptom improvement with decrease in dosage, has been observed in 80% of patients. The peak concentrations following the oral intake of different indinavir dosages are disproportionate and appear to be critical in raising urinary concentration and, therefore, the risk of stone formation.

This peak concentration in healthy subjects at the recommended doses of 400–800 mg has been reported between 8 and 10 mg/L, but higher values have been found in HIV-1-infected patients, especially those on ritonavir-boosted regimens.

In this study, assuming normal glomerular and tubular function and 39% of unbound indinavir in the glomerular filtrate (conc[U]), as well as a 2-fold secretion of indinavir in the proximal tubule, we did not consider any patho-physiological conditions which could affect renal function and could therefore increase indinavir conc[P] and conc[U]. For this reason, we call the above calculated conc[P] of 6.4 mg/L a ‘CV’ at which crystallization will ensue in the most efficient part of renal tubules.

However, in clinical practice liver insufficiency, inter-individual pharmacokinetic differences, variations in plasma-protein binding of indinavir, tubular cell injury pre-disposing to crystal-adherence and -agglomeration, and pre-existing risk factors for non-indinavir urolithiasis can all further promote the crystallization process. Therefore, a substantial number of patients would have an even higher conc[P] than calculated under standard conditions reaching the CV much faster than expected.

Indinavir conc[P] should achieve a 95% inhibition of virion reproduction (CI95).

There is no relationship between maximum indinavir plasma concentration (conc[P]max) and a decrease in viral load. In contrast, a clear relationship has been established between the trough plasma concentration (conc[P]min) and a decrease in viral RNA.

Considering all the above, conc[P]max will commonly exceed CV resulting in clinical symptoms and drug withdrawal. Drug regimens should aim at the conc[P]min not exceeding CV whilst keeping conc[P]min above CI95.

In conclusion, indinavir plasma peak concentration (conc[P]max) exceeding the CV of 6.4 mg/L is the determining factor in the induction of crystallization in the LH and should be avoided.

Acknowledgements

We would like to thank Dr Graham Griffith and Dr Richard Hilditch, Department of Clinical Pathology, United Lincolnshire Hospitals, Lincoln, UK for their support and assistance. We declare that this study was not financially supported in any way.

Transparency declarations

None to declare.

References

5. Wasmuth JC, la Porte CJ, Schneider K et al. Comparison of two reduced-dose regimens of indinavir (600 mg vs 400 mg twice daily) and ritonavir (100 mg twice daily) in healthy volunteers (COREDIR). Antivir Ther 2004; 9: 213–20.
Indinavir crystallization

