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In-vitro crystallization of indinavir in the presence of ritonavir and as a function of pH

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Abstract

The aim of this study was to investigate the in-vitro crystallization of indinavir as a function of pH alteration and in the presence of another protease inhibitor, ritonavir. Crystallization processes were studied for indinavir sulfate, indinavir free base and a commercial indinavir capsule dosage form, respectively. Crystallization induction times were determined with varying initial concentration of supersaturated solution, and in the presence or absence of seed material. In-vitro induction times were found to be significantly shorter for the indinavir capsule dosage form compared with that of indinavir sulfate and indinavir free base. Induction times were inversely proportional to the final concentration in pH 7 buffer for all materials, and were significantly shortened in the presence of seeds. The crystal morphology of indinavir varied under different crystallization conditions. This study demonstrated the potential for precipitation of indinavir upon pH elevation, while also suggesting that the presence of impurities or seeding material significantly shortens the induction time for indinavir crystal formation. This induction time period falls well within the gastric emptying time following the intake of a high-caloric meal, and within small intestinal transit time. The results of this study are in agreement with the clinical observation that a high-calorie protein meal significantly reduces the oral bioavailability of indinavir in man, accompanying a pH elevation in the stomach.

Introduction

Indinavir is an HIV protease inhibitor widely used in antiretroviral therapy. It is a lipophilic weak base that exhibits a pH-dependent solubility profile. Indinavir is metabolized largely by the liver and excreted in the faeces. However, 20% of the ingested dose is excreted in the urine within 24 h, including about 11% in the form of unchanged drug and the remaining as six metabolites (Balani et al 1995, 1996), with most of the unchanged drug appearing in the first 4 h (Balani et al 1995). Shortly after drug administration, the concentration of indinavir in the urine often exceeds its intrinsic solubility.

Despite the manufacturer's suggestion to take this drug with plenty of water to ensure adequate hydration, the major side effect associated with indinavir therapy is the formation of renal calculi, which has been reported with a frequency of 4–12% (according to the package insert). Indinavir was reported to cause not only nephrolithiasis but also crystal-induced acute renal failure. Recently, a case study was reported on the sudden unexpected death of a 60-year-old male in Sweden (Rajs et al 2000) who had been on indinavir therapy for 4 years until his death. Death was attributed to sudden cardiac dysfunction as a consequence of severe nephropathy, which in turn was likely due to kidney damage as the result of indinavir treatment.

Crystallization of indinavir has been observed in clinical settings. Researchers in France have used stereomicroscopy and Fourier transform infrared spectrophotometry (FTIR) to analyse urinary stones from patients who were enrolled in a large therapeutic program for indinavir sulfate, Crixivan (Daudon et al 1997). The organic compound in the core and in the surrounding layers of the urinary stones was identified as indinavir monohydrate free base. Light microscopy of urinary sediment from patients with HIV infection who were treated with indinavir sulfate (Gagnon et al

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1998) revealed that indinavir crystals appear as colourless, polarizable, pointed needles. They may occur singly but most often are grouped in various formations, such as rosettes, fans and starbursts. The needles are frequently arranged in rectangular plates and sheaves; these distinctive groupings vary greatly in size, and large forms are common. Similar indinavir crystal groupings also occurred in the presence of leucocytes and erythrocytes (Gagnon et al 2000).

A previous clinical study in HIV-infected patients illustrated the potential impact of meal-induced gastric pH change on the systemic bioavailability of indinavir (Carver et al 1999). The objective of this preliminary study was to demonstrate in-vitro crystallization of indinavir as a function of pH change, induction time and the presence of other HIV protease inhibitors. Induction times were also estimated and compared with small intestinal transit time.

Materials and Methods

Induction time and in-vitro crystallization upon pH change

Three materials were used: indinavir (free base), indinavir sulfate and filtered Crixivan capsule formulation. Indinavir free base was extracted from the capsule formulation and characterized (data not shown). Indinavir sulfate was a gift from Merck & Co. Crixivan capsules were obtained from the University of Michigan hospital pharmacy.

Stock solutions of 10 mg mL^{-1} were made from each source with 0.01 M HCl. Final pH values of all stock solutions were 2.0. Stock solutions from the Crixivan capsule formulation were filtered through qualitative wet-strengthened filter paper (no. 114 Whatman) by gravity filtration. A small amount of these stock solutions was added to pH 7.0 McIlvaine buffer (5.9 mM citric acid, 54.8 mM dibasic sodium phosphate and 1.16 M sodium chloride) to reach the desired final concentration. Induction times were estimated with the observation of a first visible crystal by eye. Shapes of crystals were then evaluated under an inverted microscope.

In-vitro crystallization of indinavir in the presence of ritonavir

Capsules of another protease inhibitor, ritonavir, were obtained from the University of Michigan hospital pharmacy. The content of the capsules was dissolved in 0.1 M HCl and centrifuged at 3000 rev min^{-1} for 10 min. The supernatant was then filtered through qualitative wet-strengthened filter paper (no. 114 Whatman) by gravity filtration. Final stock solutions of ritonavir had a pH of 2 and concentration of 10 mg mL^{-1}. Indinavir free base was used in this part of the crystallization study and a stock solution of indinavir was made in 0.01 M HCl at a concentration of 10 mg mL^{-1}. A small amount of ritonavir and indinavir in acidic medium was added to pH 7 McIlvaine buffer to prepare supersaturated solutions of 0.5 mg mL^{-1} of each drug. Instantaneous precipitation was observed for ritonavir but not for indinavir. Ritonavir suspension (20 μL), serving as a seed for crystallization, was then added to the supersaturated indinavir solution.

Results

Crystallization of indinavir with a change in pH

Supersaturated solutions were prepared by raising the pH of the solution, and induction times, defined as the time elapsed before the first sign of crystal was observed, were recorded in Table 1. In the absence of crystal seeds or other impurities, the supersaturated solutions could be maintained for a much longer period than small intestinal transit time (3–4 h). In vitro induction times were significantly shorter for the filtered capsule formulation,

Table 1 Approximate induction times for indinavir, indinavir sulfate and indinavir dosage form upon pH change[a].

Chemical identity	Final concn at pH 7.0[b] (mg mL^{-1})	Seed[c] added?	Approximate induction time (h)	Crystal shape[d]
Indinavir (free base)	0.064	No	72	Needle
	0.128	No	48	Needle
Indinavir sulfate	0.3	No	24	Needle
	0.3	Yes	12	Needle
	0.4	No	12	Needle
	0.4	Yes	0.5	Plates
Crixivan (filtered dosage form)	0.3	No	1.5	Plates
	0.3	Yes	0.33	Plates
	0.4	No	1	Plates
	0.4	Yes	0.17	Plates

[a]Concentrated stock solutions were 10 mg mL^{-1} at pH 2 in HCl. [b]Small amount of stock solution was added to McIlvaine buffer to reach desired final concentration. [c]Indinavir cystals served as seeding material. [d]Observed under inverted microscope (morphology pictures are presented in Figure 1).

which included formulation excipients in addition to the active ingredient, indinavir sulfate. Induction times were also significantly shortened when seed was added to the supersaturated solution. Induction time is inversely proportional to the final concentration in pH 7 buffer for all materials. Supersaturated solutions from the filtered capsule formulation formed visible and dense crystals within the projected average small intestinal transit time under all circumstances studied, suggesting the possibility for in-vivo precipitation. In addition, it was observed that the shape of crystals formed within short induction time (cubic) was different from that formed over longer induction time (needle) (Figure 1A, B).

Crystallization of ritonavir and indinavir in the presence of ritonavir

Very fine crystals of ritonavir were formed instantaneously upon supersaturation. The crystals remained very fine under the microscope for a period of 3 h. The picture of crystal morphology of ritonavir, presented in Figure 2A, B, was taken 3 h after addition of ritonavir stock solution into pH 7 buffer and consequent spontaneous precipitation. Spontaneous precipitation of indinavir also occurred upon addition of ritonavir crystals as seed material. The crystal shape of indinavir in the presence of ritonavir remained needle like. However, most of the crystals were arranged in aggregate forms such as rosettes or sheaves. Surface area was significantly increased with this morphology.

Discussion

The weak base indinavir, with pK_as at 3.7 and 5.9, is highly water soluble at basal gastric pH. However, given the pH-solubility profile of indinavir, it was expected that this drug would precipitate out at high gastric pH, limiting drug availability for absorption in the gastrointestinal tract (Li et al 2000). Previously in our laboratory, a clinical study with HIV-infected patients was conducted to evaluate the effect of in-vivo gastric pH change on the pharmacokinetic profile of indinavir (Carver et al 1999). Results from this clinical study demonstrated that elevated gastric pH, caused by a protein meal in this case, is related to a reduction of the systemic bioavailability of indinavir. Specifically, a 680-kcal low-viscosity liquid protein meal, co-administered with a single oral dose of 600 mg of Crixivan, produced a 68% reduction in AUC and 74% reduction in C_{max} compared with the fasted state. This reduction was by far the most significant when compared with other treatments such as fat, carbohydrate and high-viscosity meals, all of which caused various extents of reduction in the oral bioavailability of indinavir in all subjects. In addition, intake of high-calorie protein meals also produced significant and prolonged elevation of gastric pH from a baseline of 2 to a pH value of 6 immediately after food intake (Figure 3). The gastric pH was maintained between pH 4 and 6 for at least 4 h after meal intake. Such prolonged gastric pH elevation was not observed with any other meal treatments. A hypothesis for the mechanism of this protein-meal-induced negative food effect is based on in-vivo precipitation of indinavir upon pH change in the stomach, which leads to a decreased

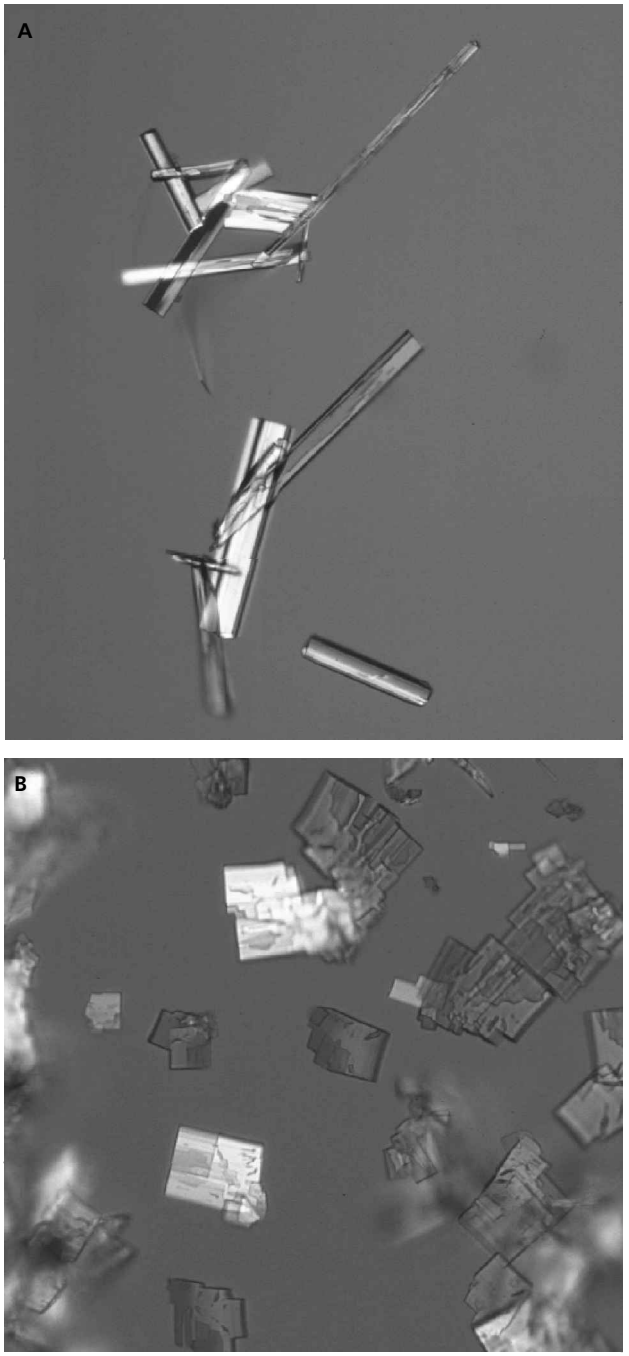

Figure 1 Representative crystal morphology of indinavir upon pH change. A. Needle-shaped indinavir crystals formed over long induction time (original magnification: × 40). B. Cube-shaped indinavir crystals formed over short induction time period (original magnification: × 40). For conditions resulting in above different crystal morphologies, refer to Table 1.

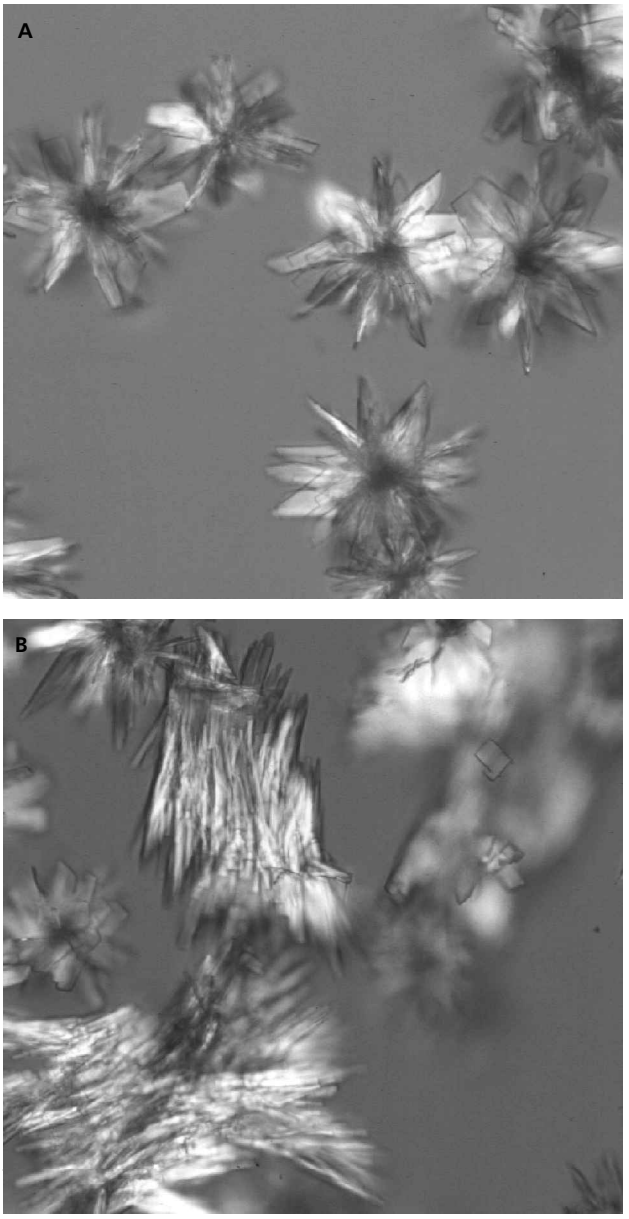

Figure 2 Representative crystal morphology of indinavir in the presence of ritonavir. A. Indinavir crystal groupings in the form of rosettes (original magnification: × 40). B. Indinavir crystal groupings in plates and sheaves, with rosette arrangements in the background (original magnification: × 40).

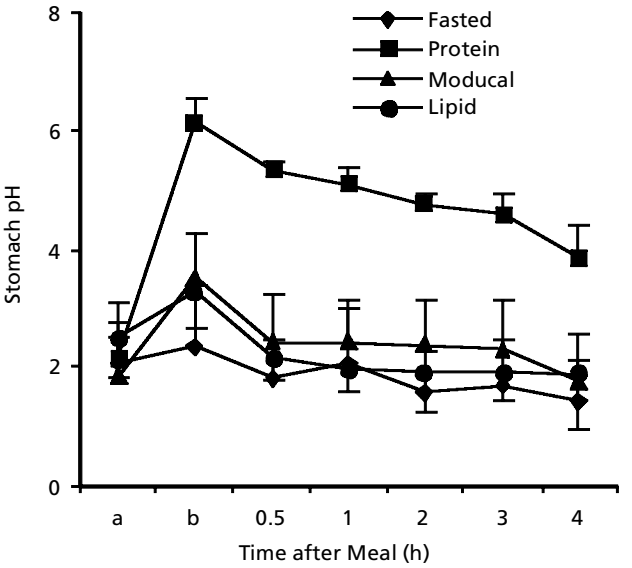

Figure 3 Stomach pH changes after meal administration in a controlled clinical study. a, pre-meal, fasted; b, time of meal ingested; n = 7 (reproduced with authors' permission from Carver et al (1999)).

total amount of drug in solution available for absorption. The absorption rate for poorly soluble, high-dose drugs such as HIV protease inhibitors depends largely on dissolution rate, which in turn depends on solid surface area (Li et al 2000). While it is difficult to study crystallization in a clinical setting by sampling the gastric contents, an in-vitro crystallization study serves as a reasonable representation under appropriate experimental conditions.

Results from this study demonstrated the potential for precipitation/crystallization of indinavir upon pH change of the drug solution. Results confirmed that precipitation does occur when pH of the bulk solution is elevated and buffered. The data suggest that the presence of impurities or seeding material, whether they are excipients from formulation or crystals of indinavir or ritonavir, significantly shortens the induction time of indinavir crystal formation. This time falls well within gastric emptying time following the intake of a high-caloric meal and also within the small intestinal transit time. This is most relevant to an in-vivo situation where impurities, excipients and other food components are all present in the gastrointestinal tract.

The clinical ramification of this study is reflected in the reduced systemic bioavailability of indinavir subsequent to intake of a protein meal, in conjunction with a change of in-vivo gastric pH monitored during the clinical study (Carver et al 1999). The mechanism of the negative food effect from the protein meal is likely due to the reduced amount of indinavir in solution as a consequence of precipitation/crystallization in the stomach with co-administration of the protein meal. In addition, indinavir is mainly metabolized by CYP3A and exhibits non-linear pharmacokinetics, suggesting a saturable clearance process. The pH change of the medium in which indinavir initially dissolves, namely gastric fluid, not only has an impact on the total amount of drug in solution available for absorption, but also determines the luminal concentration of the drug in the small intestine as a function of gastric emptying. The rate of drug absorption, therefore, is dependent upon the rate of drug delivery across the epithelium as well as the rates of intestinal first-pass clearance processes.

Conclusions

The results of this study demonstrated the potential for precipitation of indinavir upon pH elevation, while

also suggesting that the presence of impurities or seeding material significantly shortens the induction time for indinavir crystal formation. This induction period falls well within the gastric emptying time following the intake of a high-caloric meal, and within small intestinal transit time. The results of this study are in agreement with the clinical observation that a high-calorie protein meal significantly reduces the oral bioavailability of indinavir in human subjects, accompanying a pH elevation in the stomach.

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