



Effect of High-Dose Vitamin C on the Steady-State Pharmacokinetics of the Protease Inhibitor Indinavir in Healthy Volunteers

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ABSTRACT

Background: Protease inhibitors (PIs) exhibit a concentration-effect relationship. Maintenance of serum concentrations that suppress viral replication and inhibit selection of resistant mutants is critical. PIs are extensively metabolized by P-450 isoenzymes, primarily CYP3A4. Patients with HIV infection frequently use alternative / complementary agents such as herbs and vitamins. Unfortunately, very little is known about the effect of such substances on the Pharmacokinetics (PK) of PIs. The most commonly used complementary agent reported by patients with HIV infection is Vitamin C, with high doses (≥ 1 gram) being commonly employed. It has been reported that Vitamin C has modulatory effects P-450 enzymes. More specifically increased intake may induce P-450 isoenzymes, including CYP3A. The specific aim of this study was to determine if concomitant use of high-dose Vitamin C (1 gram daily) significantly alters the PK of indinavir (IDV).

Methods: Seven volunteers were studied in a two-period, longitudinal design. All subjects received IDV 800mg every 8 hours x 4 doses. Blood was collected for IDV analysis at pre-dose (0 hr) and 0.5, 1, 2, 3, 4, and 5 hrs after the 4th (steady-state) dose. After a wash out period the sequence was repeated, except that all subjects received a 1 gram daily dose of Vitamin C for a week concluding on the day that blood was collected for IDV concentrations. Vitamin C and IDV were administered at separate times. All subjects were placed on a Vitamin C content-controlled diet during the study. PK parameters were determined by non-compartmental methods.

Results: In healthy volunteers, the addition of Vitamin C supplementation resulted in a significant reduction in IDV C_{max} (-20%, $p = 0.04$) and steady-state AUC_{0-8hr} (-14%, $p < 0.05$). While not statistically significant, the C_{min} was 32% lower with Vitamin C (0.27 vs. 0.18 $\mu\text{g/mL}$, $p = 0.09$). Clearance (0.438 vs. 0.525 L/kg/hr, $p = 0.06$), and $t_{1/2}$ (1.13 vs. 1.02 h, $p = 0.12$) were not found to be significantly different.

Conclusions: Concomitant use of Vitamin C can significantly reduce IDV concentrations. This is the first report of an interaction in humans between Vitamin C and a CYP3A4 substrate. Clinicians should be aware of such interactions and remember to caution patients about concomitant use of complementary agents and PIs.

BACKGROUND:

PIs such as IDV are potent antiretroviral agents used in the treatment of HIV infection. PIs exhibit a concentration-response relationship and maintenance of serum concentrations that suppress viral replication without causing human toxicity is critical.^{1,2} PIs are extensively metabolized by Cytochrome P-450 CYP3A4 isoenzymes.³ Many of the drugs known to interact with PIs do so by inhibiting or inducing the activity / quantity of the CYP3A4. In addition to taking conventional prescription medications, patients with HIV infection frequently use alternative and complementary medicines.^{4,5} Unfortunately, very little is known about the effects of these agents on the pharmacokinetics of antiretroviral agents. A few drug interaction studies with herbal medicines and PIs have been published.⁶ Although herbal medicine use in this population is common, most reports show that vitamin C (Ascorbic acid) is probably the most frequently used alternative or complementary agent in patients with HIV infection.^{4,5} The recommended daily allowance (RDA) for vitamin C supplementation in humans has ranged from 60mg to 90mg, but HIV-positive patients often consume "high-doses" (daily doses of 1,000mg – 3,000mg).^{7,8} Proposed reasons for Vitamin C use by patients with HIV includes enhancement of immune function, reduction of oxidative stress, and anti-HIV viral activity.⁹ Information regarding interactions between vitamin C and protease inhibitors is non-existent. Clinical drug interaction studies in humans involving vitamin C and other CYP3A4 metabolized medications are essentially non-existent. Results from several animal model experiments have shown that vitamin C exerts a modulatory effect on P-450 enzyme systems.¹⁰⁻¹⁴ Many of these studies have reported a positive correlation between vitamin C supplementation or deficiency and an increase or decrease in P-450 activity, respectively. Guinea pig studies suggest that excessive doses of vitamin C can "induce" the P-450 enzyme / activity.¹⁰⁻¹³ It should be mentioned, that most of these animal studies were performed before the scientific community differentiated the different P-450 isoenzyme families. Two recent animal studies have reported a relationship with CYP3A enzymes.¹³⁻¹⁴ The purpose of this study was to determine if the daily administration of high-dose vitamin C (1,000mg daily) significantly altered the pharmacokinetics of IDV.

METHODS AND ANALYSIS:

Subjects:

• Seven healthy volunteers (6 male, 1 female) between the ages of 18-55 years.

• **Inclusion:** All subjects had to give written informed consent before participating in the study. All subjects underwent an initial screening visit with a complete physical examination and baseline laboratory testing. All participants had to be within 30% of their ideal body weight. The subjects had to have normal serum chemistries, hematology, lipids, hepatic function tests, BUN, serum creatinine, glucose, and urinalysis including a drug screen for illicit substances.

• **Exclusion:** Patients were excluded if they had a history of diabetes, renal disease, nephrolithiasis, chronic gastrointestinal diseases, blood dyscrasias or if taking substances or medications known to significantly induce or inhibit CYP3A4 or P-Glycoprotein activity.

Study Design:

• Prospective open-label, longitudinal, two-period time series study with a washout period.

• Protocol: Plasma samples were collected from the subjects for determining IDV pharmacokinetic parameters at pre-dose (0 hr) and 0.5, 1, 2, 3, 4, and 5 hrs after four (steady-state) doses of IDV 800mg eight hours apart. After a 7-day "wash-out" period, subjects were started on 1,000 mg of vitamin C for 7 days. The subjects were also restarted on IDV 800mg every eight hours beginning on the 6th day of vitamin C administration. After four doses of IDV, plasma was collected again to determine the IDV pharmacokinetics. Vitamin C and IDV were administered at least 3 hours apart.

• All subjects were placed on a Vitamin C content-controlled diet (using the USDA Nutrient Database Standard Reference¹⁵) to calculate vitamin C content in food and drink) throughout the study.

Indinavir Plasma Concentration Pharmacokinetic and Statistical Analysis:

• Plasma IDV samples were analyzed at the Antiviral Pharmacology Laboratory, University of Colorado Health Sciences Center, using a validated HPLC assay.

• The lower limit of quantification for IDV was 0.020 $\mu\text{g/mL}$.

• IDV pharmacokinetic parameters were determined by non-compartmental analysis using Win Nonlin® version 2.0 (Pharsight Corp., Mountain View, CA).

• The AUC for the dosage interval (AUC_{0-8hr}) was extrapolated with the elimination rate constant calculated by log-linear regression of the terminal phase of the concentration-time curve.

• Means were compared using t-tests and ANOVA as appropriate.

• Statistical analyses were performed with Excel® (Microsoft, Redmond, WA) and JMP® Version 5 (SAS Institute, Cary, NC).

Mean Indinavir Plasma Concentration-Time Profile

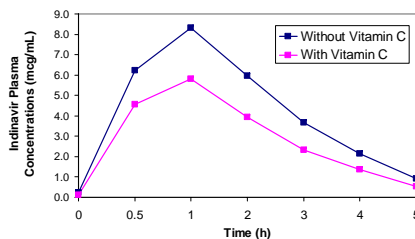


Table 1. Steady-State Indinavir Pharmacokinetic Parameters*

Parameter	Without Vitamin C	With Vitamin C	P value
C_{max} ($\mu\text{g/mL}$)	10.3 \pm 1.5	8.2 \pm 2.9	$p = 0.04$
T_{max} (h)	0.9 \pm 0.2	1.0 \pm 0.0	$p = 0.20$
AUC_{0-8hr} ($\mu\text{g} \cdot \text{h} / \text{mL}$)	26.4 \pm 7.2	22.7 \pm 8.1	$p = 0.009$
C_{min} ($\mu\text{g/mL}$) #	0.27 \pm 0.17	0.18 \pm 0.80	$p = 0.09$
CL/F (L/kg/h)	0.44 \pm 0.05	0.53 \pm 0.11	$p = 0.06$
$t_{1/2}$ (h)	1.13 \pm 0.2	1.02 \pm 0.1	$p = 0.12$

* Dose = 800mg q 8 hours, # pre-dose trough

RESULTS:

• The mean steady-state IDV C_{max} concentration was significantly reduced (20 %) after 7 days of Vitamin C administration (10.3 \pm 1.5 vs. 8.2 \pm 2.9 $\mu\text{g/mL}$, $p = 0.04$).

• The mean steady-state AUC_{0-8hr} of IDV significantly decreased (14%) after 7 days of Vitamin C administration (26.4 \pm 7.2 vs. 22.7 \pm 8.1 ($\mu\text{g} \cdot \text{h} / \text{mL}$), $p = 0.009$).

• While not statistically significant, the IDV C_{min} was 32% lower with Vitamin C (0.27 \pm 0.17 vs. 0.18 \pm 0.80, $\mu\text{g/mL}$, $p = 0.09$).

• IDV oral clearance (0.44 \pm 0.05 vs. 0.53 \pm 0.11 L/kg/h, $p = 0.06$), and $t_{1/2}$ (1.13 \pm 0.2 vs. 1.02 \pm 0.1 hrs, $p = 0.12$) were not found to be significantly different.

DISCUSSION:

This is the first study that has evaluated the effect of vitamin C in "high" doses on the pharmacokinetic exposure of an HIV protease inhibitor. The reduction in IDV C_{max} and AUC_{0-8hr} after a week of vitamin C was striking. The difference in mean trough concentrations was not statistically different, although the troughs were lower after vitamin C administration. A larger sample size may have detected a difference in this parameter as well. The effect of the interaction was quite profound in 5 of the 7 subjects. One of the other volunteers not showing a difference in IDV concentrations was a female, which raises the question of a gender effect. Slight changes in CYP3A activity have been reported to fluctuate with menstrual cycle phases.¹⁶ However, hormonal fluctuations within the menstrual cycle have not caused significant changes in CYP3A metabolism in previous studies.¹⁶ There has been much debate about which protease inhibitor pharmacokinetic-pharmacodynamic parameters correlate best with antiviral effect. Most reports suggest that C_{max} and AUC are the parameters that have most closely been associated with antiviral efficacy and prevention of resistance.^{1,2,17} However, at least one recent study has reported a correlation between maximizing C_{max} concentrations and improved CD4+ immune response.¹⁸ In light of this, our findings would suggest that high doses of vitamin C could lead to a reduction of protease inhibitor effectiveness. It is possible that the degree of interaction between IDV and Vitamin C could be more profound with even larger doses of vitamin C (e.g., 3 gram) or longer courses of vitamin C. Maximum induction of P-450 enzymes may not be seen until 10 days or more.¹⁰ Vitamin C appears to have some relationship with P-450 enzymes, including CYP3A. Therefore, this is a very likely mechanism for the observed interaction. P-glycoprotein is a substance that appears to have a related role to CYP3A4 in the disposition of certain drugs, including protease inhibitors.¹⁹ The relationship between vitamin C and P-glycoprotein has not been well studied. A recent *in vitro* study reported that Vitamin C might inhibit P-glycoprotein activity.²⁰ This would mean that Vitamin C could actually cause an increase in IDV bioavailability by that mechanism. At present time it is difficult to separate the contributions of each of these mechanisms.²¹ Future studies are needed to identify the exact mechanism of this interaction.

SUMMARY:

- The use of high doses of vitamin C appears to be associated with a reduction in plasma IDV concentrations.
- Clinicians must consider patient use and interaction of alternative and complementary medicines with antiretroviral therapy.

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