

## Effect of Antacids in Didanosine Tablet on Bioavailability of Isoniazid

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**The antacids in two didanosine placebo tablets had no significant effect on the plasma pharmacokinetics of a single oral dose of 300 mg of isoniazid administered to 12 healthy volunteers. These results suggest that isoniazid bioavailability will be unaffected by the antacids in didanosine tablets when the two medications are administered simultaneously to human immunodeficiency virus-seropositive patients.**

The absorption of isoniazid, a widely used antituberculosis agent, is decreased by 20 to 30% when it is administered with aluminum hydroxide gel (4). These findings have led to the recommendation that isoniazid be administered 1 h before ingestion of antacid preparations.

The acid lability of didanosine (2',3'-dideoxyinosine), an antiretroviral agent, at a pH of less than 3 requires that oral formulations of the drug contain buffering agents to minimize acid-induced hydrolysis in the stomach (8). One of the marketed formulations is a chewable tablet containing dihydroxy-aluminum sodium carbonate, magnesium hydroxide, and sodium citrate. These antacids decrease the absorption of other agents, such as ciprofloxacin (14). Because many patients infected with human immunodeficiency virus are receiving didanosine and isoniazid concurrently, the present study was conducted to evaluate the magnitude of the effect of the antacids contained in didanosine tablets on isoniazid bioavailability in healthy volunteers.

This study was approved by the Human Experimental Procedure Committee, Ottawa General Hospital. Written informed consent was obtained from 12 healthy volunteers (11 men and 1 woman) to participate in this randomized, two-period, two-treatment, two-sequence crossover study. There was a 7-day washout period between treatments. Subjects were excluded on the basis of the following: pregnancy, hypersensitivity reactions to any drug, use of any medications, abnormal liver and renal function tests, and abnormal hematology or urinalysis. The mean ( $\pm$  standard deviation [SD]) age and weight of the subjects were  $37 \pm 9$  years and  $74 \pm 11$  kg, respectively.

Subjects were admitted to the Clinical Investigation Unit, Ottawa General Hospital, following an overnight (10-h) fast. In one phase of the study, subjects received a single oral dose of 300 mg of isoniazid (300-mg tablet) (lot 1404209; Pharmascience Inc., Montreal, Canada) with 180 ml of tap water. In the other phase, subjects received two didanosine placebo tablets (15.6 mmol of magnesium and a slightly larger amount of aluminum [proprietary information]; Bristol-Myers Squibb Co., Syracuse, N.Y.) at 2000 h on day 1 and again between 0800 and 0900 h on day 2. Each tablet was chewed for about 30 s and swallowed. The subjects rinsed their mouths with 60 ml

of tap water after swallowing the second tablet. One minute after ingestion of the last antacid tablet on day 2, a single 300-mg tablet of isoniazid was administered with 120 ml of water. On both study days, subjects were allowed to eat 4 h after isoniazid administration.

In both phases, blood samples (5 ml) were collected into Vacutainer tubes containing heparin, immediately before isoniazid administration and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, and 12 h after the dose. They were allowed to equilibrate to room temperature for 10 min and were then centrifuged (10 min, 21°C, 500  $\times$  g) to separate the plasma. Two equal aliquots of plasma sample were transferred to separate polypropylene tubes and immediately frozen in dry ice at  $-70^\circ\text{C}$ . Samples were stored at  $-80^\circ\text{C}$ , together with quality control samples containing isoniazid spiked at three concentrations in plasma, because isoniazid is unstable in plasma at temperatures above  $-20^\circ\text{C}$  when samples are stored longer than 24 h (6).

The regimen for collecting blood samples at the clinical site was based on the results of stability studies conducted in our laboratory. These studies (data not shown) demonstrated that the distribution of isoniazid between whole blood and plasma had reached equilibrium at room temperature at 5 min after blood collection and that isoniazid was stable in blood and plasma in the presence of its metabolites at least for 1 h at room temperature. This allows enough time to isolate plasma. Plasma samples, however, were divided at the clinical center because two freeze-thaw cycles, which are necessary for repeat analysis of previously thawed samples, rendered isoniazid unstable. Isoniazid was considered stable if the 90% confidence limits around the difference between geometric mean concentrations of test and control samples, expressed relative to the controls, fell within the interval from  $-10$  to 11%.

Clinical samples from both regimens for each subject were analyzed on the same day to minimize contributions of the analysis to variability. Determination of isoniazid in plasma was based on the high-performance liquid chromatographic (HPLC) procedure of Hutchings et al. (5), except that the nitrile HPLC column was maintained at  $32^\circ\text{C}$  and was eluted with an acetonitrile:aqueous mobile phase (6.25:93.75, vol/vol; pH 2.26) saturated with silica gel. The aqueous portion contained 5 mmol of phosphoric acid and 0.625 mmol of heptane sulfonic acid. Quantitation was based on peak-height ratios of isoniazid to the internal standard (iproniazid) over the concentration (C) range of 0.1 to 10  $\mu\text{g ml}^{-1}$  in plasma by using weighted ( $1/C^2$ ) linear regression analysis ( $R^2 > 0.99$ ).

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TABLE 1. Mean pharmacokinetic parameters of isoniazid administered alone and with antacid<sup>a</sup>

Parameter	Value with treatment <sup>b</sup>		ANOVA for crossover design					
	INH alone	INH plus antacid	%Δ <sup>c</sup>	95% confidence limits	P	Intrasubject %CV	Intersubject pooled %CV	%Δ <sub>80%</sub> <sup>d</sup>
C <sub>max</sub> (μg ml <sup>-1</sup> )	6.01 ± 2.40	6.05 ± 1.74	4.1	-27.0 and 48.6	0.805	39.1	nm	64.2
T <sub>max</sub> (h)	0.57 ± 0.34	0.58 ± 0.47	3.2	-61.7 and 68.2	0.914	70.2	17.0	89.1
AUC <sub>0-tz</sub> (μg · h ml <sup>-1</sup> )	16.9 ± 10.9	16.0 ± 9.2	-0.5	-15.0 and 16.4	0.948	17.2	61.2	-19.6
CL <sub>O</sub> (liters h <sup>-1</sup> )	28.0 ± 22.9	26.0 ± 18.1	-0.4	-14.2 and 15.7	0.958	16.4	63.3	-18.8
CL <sub>O</sub> (liters h <sup>-1</sup> kg <sup>-1</sup> )	0.37 ± 0.27	0.34 ± 0.21	-0.4	-14.2 and 15.7	0.958	16.4	62.5	-18.8
MTRT (h)	3.38 ± 1.45	3.33 ± 1.62	-4.2	-12.2 and 4.4	0.288	9.5	45.9	-11.3
t <sub>1/2z</sub> (h)	2.47 ± 1.19	2.42 ± 1.20	-1.8	-9.0 and 5.4	0.591	8.0	47.3	-10.1
V <sub>Z</sub> /F (liters)	69.6 ± 24.7	67.0 ± 19.5	-2.8	-16.2 and 12.8	0.679	16.3	18.6	-18.7
V <sub>Z</sub> /F (liters kg <sup>-1</sup> )	0.94 ± 0.27	0.90 ± 0.21	-2.8	-16.2 and 12.8	0.679	16.3	17.3	-18.7

<sup>a</sup> Abbreviations: INH, isoniazid; C<sub>max</sub>, observed peak plasma drug concentration; T<sub>max</sub>, observed time of peak concentration; t<sub>1/2z</sub>, apparent terminal half-life; AUC<sub>0-tz</sub>, area under the plasma concentration-time curve from time zero to time (t<sub>z</sub>) of last measurable concentration; CL<sub>O</sub>, apparent oral plasma clearance; MTRT, mean total residence time; V<sub>Z</sub>/F, apparent terminal volume of distribution; CV, coefficient of variation; nm, not measurable.

<sup>b</sup> A 300-mg single dose of INH and two didanosine placebo tablets (every 12 h for two doses) were used. Values are the arithmetic means ± SD (n = 12).

<sup>c</sup> % change of the least-squares geometric treatment mean (C<sub>max</sub>, AUC, CL<sub>O</sub>, V<sub>Z</sub>/F, and MTRT) or the least-squares arithmetic treatment mean (T<sub>max</sub> and t<sub>1/2z</sub>) of the INH-plus-antacid treatment relative to that of the INH-alone treatment. A negative value refers to a decrease.

<sup>d</sup> % change of the least-squares means (see footnote c) that can be detected with a power of 80% at the 5% level of significance (α = 0.05/2).

Within- and between-batch coefficients of variation (CVs) for concentration data were less than 10% for 42 quality control samples analyzed in duplicate over 14 analytical runs. The CV was 11% for the slope (n = 14) and is a reflection of between-batch precision for peak-response data. The pooled within-batch CV for the peak-height response of the internal standard was 7% over 14 runs (n ~ 50 samples per run). A random selection of clinical samples (n = 28) chosen for repeat analysis had an average relative deviation of 7% (SD, 11%) from the original concentration.

The plasma isoniazid concentration-versus-time (t) data were analyzed by nonlinear, weighted (1/C), least-squares regression (PCNONLIN 4.2; SCI Software, Lexington, Ky.) to estimate the terminal disposition rate constant (λ<sub>z</sub>). The Gauss-Newton fitting algorithm was used for the regression analysis. The model fit was evaluated by Akaike's information criterion, plots of weighted residuals versus time or versus weighted predicted concentration, and the sum of the squared residuals. The highest observed concentration and the corresponding sampling time were defined as C<sub>max</sub> and T<sub>max</sub>, respectively. The terminal disposition half-life (t<sub>1/2z</sub>) was calculated from the quotient of (ln 2)/λ<sub>z</sub>. The area under the plasma concentration-time curve from time zero to the time of the last measurable sample (AUC<sub>0-tz</sub>) and area under the first moment of the plasma concentration-time curve (AUMC<sub>0-tz</sub>) were estimated by the linear trapezoidal method. Extrapolations from the time of the last measurable sample (t<sub>z</sub>) to infinity were estimated by adding C(t<sub>z</sub>)/λ<sub>z</sub> to AUC<sub>0-tz</sub> and (t<sub>z</sub> + λ<sub>z</sub><sup>-1</sup>) · C(t<sub>z</sub>)/λ<sub>z</sub> to AUMC<sub>0-tz</sub>, where C(t<sub>z</sub>) is the model-predicted plasma concentration at t<sub>z</sub>. The segment of total AUC and AUMC that was extrapolated to infinity averaged 6.7% (SD, 2.5%) and 24.6% (SD, 7.3%), respectively.

The apparent oral plasma clearance (CL<sub>O</sub>) was calculated by dividing the dose by the AUC from 0 h to infinity (AUC<sub>0-∞</sub>). The apparent terminal volume of distribution (V<sub>Z</sub>/F) was estimated from the quotient of CL<sub>O</sub>/λ<sub>z</sub>. F is the bioavailability. The mean total residence time (MTRT) was calculated from the AUMC<sub>0-∞</sub>/AUC<sub>0-∞</sub> ratio.

Differences in mean pharmacokinetic parameters of isoniazid between treatments were evaluated by analysis of variance (ANOVA) appropriate for a crossover study (10). Concentration-dependent parameters, C<sub>max</sub> and AUC and AUMC, and parameters derived from them (CL<sub>O</sub>, V<sub>Z</sub>/F, and MTRT) were

logarithmically transformed before ANOVA, and inference is based on geometric means. Time-dependent parameters, t<sub>1/2z</sub> and T<sub>max</sub>, were analyzed on the raw scale, and inference is based on arithmetic means. Effects of treatment, treatment-by-acetylator-status interaction, and period were tested by the mean-square residual (MSR), and effects of sequence were tested by the subject-within-sequence mean-square term. The difference between treatment means was considered significant for a P value of <0.05. Results are expressed as arithmetic mean ± SD in the text and tables. The percent change (Δ) of the least-squares means for the isoniazid-plus-antacid treatment, relative to those for the isoniazid-alone treatment, that can be detected with a power of 80% at the 5% level of significance was estimated by the following formulae for point hypothesis testing. For log-transformed data

$$\Delta 80\% = [e^{(t_{\beta, n_1 + n_2 - 2} + t_{\alpha/2, n_1 + n_2 - 2}) \cdot SE} - 1] \times 100\% \text{ for } \% \text{ increase}$$

$$\Delta 80\% = [e^{-(t_{\beta, n_1 + n_2 - 2} + t_{\alpha/2, n_1 + n_2 - 2}) \cdot SE} - 1] \times 100\% \text{ for } \% \text{ decrease}$$

and for raw data

$$\Delta 80\% = [(t_{\beta, n_1 + n_2 - 2} + t_{\alpha/2, n_1 + n_2 - 2}) \cdot SE/X] \times 100\% \text{ for } \% \text{ increase or decrease}$$

where t<sub>β</sub> and t<sub>α/2</sub> are the appropriate values from the t distribution with n<sub>1</sub> + n<sub>2</sub> - 2 df, n<sub>1</sub> = n<sub>2</sub> = 6 (number of subjects per sequence), t<sub>β, 10</sub> = 0.87905 for β = 0.2, t<sub>α/2, 10</sub> = 2.2282 for α = 0.05, SE is the standard error calculated as [(n<sub>1</sub> + n<sub>2</sub>) · MSR/2n<sub>1</sub>n<sub>2</sub>]<sup>1/2</sup>, and X is the grand mean of the two least-squares treatment means.

For logarithmically transformed data, the approximate intrasubject and intersubject CVs (CV<sub>w</sub> and CV<sub>b</sub>, respectively) for each pharmacokinetic parameter were calculated as CV<sub>w</sub> ~ (MSR)<sup>1/2</sup> and CV<sub>b</sub> ~ [(MS - MSR)/2]<sup>1/2</sup>, respectively, where MS is the subject mean square from the ANOVA. The respective equations were divided by X to obtain the exact CV values for T<sub>max</sub> and t<sub>1/2z</sub> data.

Mean pharmacokinetic data for isoniazid after each treatment are summarized in Table 1, and the mean plasma concentration-versus-time data are graphically illustrated in

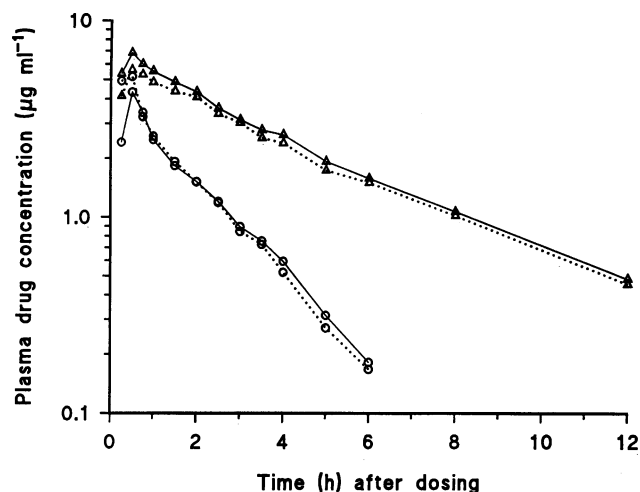


FIG. 1. Mean isoniazid plasma concentration-time profiles for six fast acetylators (circles) and six slow acetylators (triangles) after administration of a single oral dose of 300 mg of isoniazid either alone (—) or 1 min after the last (second) dose of two didanosine placebo tablets containing antacids (· · ·). Mean concentration data that were less than the lower limit of quantitation of the assay ( $0.1 \mu\text{g ml}^{-1}$ ) are not shown.

Fig. 1. Subjects were classified as fast acetylators on the basis of an isoniazid  $t_{1/2z}$  of less than 130 min (7). Isoniazid administered alone was rapidly absorbed with  $T_{\text{max}}$  values averaging 0.57 h (11 of 12 values of  $<1.0$  h) and, depending on acetylator status, was eliminated quickly in six fast acetylators ( $t_{1/2z}$ ) =  $1.38 \pm 0.26$  h) and more slowly in six slow acetylators ( $t_{1/2z}$ ) =  $3.55 \pm 0.50$  h). Four of 10 Caucasians, an African American, and an East Indian were fast acetylators.

The ANOVA indicated no effects of treatment, treatment-by-acetylator-status interaction, or period for all pharmacokinetic parameters. A sequence effect, probably attributable to the unequal assignment of slow and fast acetylators (four versus two) in each sequence, was observed for  $V_z/F$ , uncorrected for body weight. The pharmacokinetic parameters determined when the didanosine placebo tablets were administered with isoniazid were not significantly different ( $P > 0.29$ ) from those determined for isoniazid alone. Table 1 shows less than a 5% difference between treatment means. Except for  $T_{\text{max}}$  and  $C_{\text{max}}$  values, an increase of more than 25% and a decrease of more than 20% between means, relative to the reference isoniazid alone, can be detected at an  $\alpha$  level of 0.05 and a  $\beta$  level of 0.2 and is a reflection of  $CV_w$  values of  $<20\%$ .  $C_{\text{max}}$  was considered highly variable within a subject ( $CV_w > 30\%$ ). Of 12 subjects, 9 had AUC ratios of test to reference between 88 and 114%. Of the remaining three subjects, one subject (slow acetylator, Caucasian) showed a large decrease in  $AUC_{0-12}$  (ratio, 59%) and  $C_{\text{max}}$  (ratio, 32%) values and two subjects (fast acetylators, Caucasian and East Indian) had high AUC ratios (145 and 147%) and  $C_{\text{max}}$  ratios (177 and 292%) when isoniazid was taken with the buffered tablets. There was a slight decrease in the  $CV_b$  values of clearance and volume of distribution when these parameters were corrected for body weight.

In this study, the observed pharmacokinetic parameters ( $T_{\text{max}} \sim 0.6$  h,  $C_{\text{max}} \sim 6 \mu\text{g ml}^{-1}$ ) and the calculated pharmacokinetic parameters (AUC,  $CL_Q$ ,  $t_{1/2z}$ , and  $V_z/F$ ) of isoniazid are comparable to those cited in the literature for a 300-mg single oral dose of the drug (9, 11). Malabsorption of

isoniazid has been reported in patients with AIDS coinfecting with *Mycobacterium tuberculosis* (1, 13). Therefore, the  $C_{\text{max}}$  and AUC values reported here may be higher than those expected in patients with advanced human immunodeficiency virus infection. The proportion of Caucasians that were slow acetylators (60%) is in agreement with the incidence of slow isoniazid inactivators (58.8%) among 102 Canadian students (2).

The  $CV_w$  values for AUC and  $C_{\text{max}}$  are about twice those reported for a comparative bioavailability study of three tablet formulations of isoniazid (17 versus 11% and 39 versus 18%, respectively) (16). The higher variabilities in absorption within a subject resulted mainly from one subject with AUC and  $C_{\text{max}}$  ratios of  $<60\%$  and two subjects with ratios of  $>140\%$ . The large increase in AUC and  $C_{\text{max}}$  values for the two subjects during antacid treatment was mostly a consequence of a single high isoniazid concentration at 30 min postadministration.

Previous studies have shown that the simultaneous administration of an aluminum hydroxide gel (Amphojel) decreased the peak plasma drug levels and delayed or decreased the absorption of isoniazid by 20 to 30% in 11 patients with tuberculosis (4). The effect of magaldrate (Riopan), an aluminum-magnesium compound, on plasma isoniazid concentrations was less pronounced. The authors proposed that the reduced absorption seen with aluminum hydroxide is caused by delayed gastric emptying and not by decreased acidity (4). This appears to be supported by the absence of any significant effect of the  $H_2$  blockers, cimetidine and ranitidine, on isoniazid absorption (12).

The results of the present study, however, indicate that the bioavailability of isoniazid was not influenced by the antacids or other excipients in the didanosine tablets. The most likely reason why the magnitude of the antacid interaction was greater with the aluminum hydroxide gel (4) than with the didanosine formulation was because of the large dose of aluminum hydroxide ingested (about twice that contained in two didanosine tablets). There was no evidence to suggest that the didanosine placebo tablets delayed gastric emptying, except in one subject in whom  $T_{\text{max}}$  was increased from 0.25 to 2 h. This subject also showed a large decrease in isoniazid absorption in the presence of the didanosine buffer, but his  $C_{\text{max}}$  value ( $3.7 \mu\text{g ml}^{-1}$ ) was well above the MIC for *M. tuberculosis* (0.025 to  $0.05 \mu\text{g ml}^{-1}$  [3]). The marked increase in AUC and  $C_{\text{max}}$  values for isoniazid in the presence of antacids, as observed in two subjects, has also been noted in one alcoholic patient when an aluminum hydroxide gel or magaldrate was given (4).

We conclude that the antacids in two didanosine tablets, the recommended dose to ensure adequate buffer capacity (15), do not affect the plasma pharmacokinetics of a single oral dose of 300 mg of isoniazid in healthy volunteers.

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