

EFFECT OF GRAPEFRUIT JUICE OR CIMETIDINE COADMINISTRATION ON ALBENDAZOLE BIOAVAILABILITY

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Abstract. The assumed metabolic breakdown of albendazole by mucosal CYP3A4 enzymes was studied by coadministering albendazole (10 mg/kg) with grapefruit juice. Concentrations of albendazole sulfoxide (ABZSX), the active metabolite of albendazole, were compared with those after albendazole was administered with water, a fatty meal, or grapefruit juice plus cimetidine (10 mg/kg). In comparison to water, maximum ABZSX concentration (C_{\max}) was enhanced 6.5-fold by a fatty meal (from 0.24 ± 0.09 mg/l to 1.55 ± 0.30 mg/l; mean \pm SD; $P < 0.001$) and 3.2-fold by grapefruit juice (from 0.24 ± 0.09 mg/l to 0.76 ± 0.37 mg/L; $P = 0.031$). When grapefruit juice was combined with cimetidine, C_{\max} was significantly lower than with grapefruit juice alone (0.41 ± 0.29 mg/l and 0.76 ± 0.37 mg/l, respectively; $P = 0.022$). The area under the concentration-time curve from 0 to infinity ($AUC_{0-\infty}$) followed a comparable pattern. Half-life ($T_{1/2}$) was 8.8 ± 4.2 hr and 8.2 ± 4.3 hr after administration with water or a fatty meal ($P = 1.000$). Grapefruit juice shortened $T_{1/2}$ by 46% ($P = 0.026$). We hypothesize that albendazole is metabolized by CYP3A4 enzymes in the intestinal mucosa. This process can be inhibited by grapefruit juice. Cimetidine decreased albendazole bioavailability.

INTRODUCTION

Albendazole is a benzimidazole derivative used for the treatment of intestinal helminthiasis and echinococcosis. Although albendazole is an effective drug for these diseases, the therapeutic response in echinococcosis is difficult to predict.¹ The poor intestinal absorption of albendazole, which can be enhanced by a fatty meal, is a major factor that contributes to this problem.^{2,3} After absorption, the drug is rapidly converted by the liver and probably also by mucosal cells into the active metabolite ABZSX, a mixture of R(+) and S(–) enantiomers. Formation of R(+)ABZSX is catalyzed by microsomal flavin mono-oxidase (FMO) and of S(–)ABZSX by cytochrome P450 enzymes (CYP3A). The extent to which both enzyme systems contribute to this process varies among species and is not well known in humans.^{4,5} This so-called first pass effect is almost 100%. Subsequently, ABZSX is converted by CYP2C enzymes into the inactive metabolite albendazole sulfone.⁶ The activity of CYP enzymes can be inhibited by cimetidine, which has a variable affinity for the CYP subclasses. Cimetidine also affects gastric acid secretion and probably thereby albendazole absorption. Albendazole bioavailability decreased about 50% when it was combined with cimetidine.⁷

Intracellular concentrations of albendazole may also be determined by mucosal CYP enzymes and P-glycoprotein (P-gp), which form a concerted barrier to drug absorption, one by metabolism and the other by efflux into the intestinal lumen.⁸ It is well known that the mucosal metabolism of several drugs (e.g., terfenadine, dihydropyridine calcium channel blockers, HMG-coenzyme A-reductase inhibitors, cyclosporine, cisapride, ethinylestradiol, saquinavir, and midazolam) can be inhibited by grapefruit juice. This has been attributed to inhibition of mucosal CYP3A4 enzymes by grapefruit juice constituents such as the flavonoid naringin and the furanocoumarin 6', 7'-dihydroxybergamottin. This results in less mucosal degradation, enhanced absorption, and enhanced bioavailability of the drug.^{9,10}

However, grapefruit juice also activates P-gp-mediated efflux of drugs (e.g., terfenadine, erythromycin, and lovastatin),

which may partially counteract its effect on inhibition of CYP metabolism.¹¹ It is not yet known whether albendazole is a substrate of CYP enzymes or of P-gp. We studied the effect of grapefruit juice on the assumed CYP3A4-mediated metabolism of albendazole in the intestinal mucosa and compared it with the known enhancement of albendazole bioavailability by a fatty meal. Finally, we studied whether cimetidine, coadministered with grapefruit juice, decreased albendazole bioavailability.

MATERIALS AND METHODS

Study participants. Six healthy male volunteers were included (mean age = 20 years; age range = 19–21 years; mean weight = 69 kg; weight range = 55–92 kg; mean body mass index, or BMI, = 21 kg/m^2 ; BMI range = $17\text{--}25 \text{ kg/m}^2$). Each participant was studied on four subsequent occasions with a washout period of 1 week between each study day. After an overnight fast, a single oral dose of albendazole was administered on an empty stomach in the following order: with water, a fatty meal, grapefruit juice, or grapefruit juice plus cimetidine. We refrained from randomization to prevent a possible long-lasting carry-over effect of grapefruit juice. Food was withheld for 4 hr after the drug was taken, but noncaffeine, nonalcoholic beverages were allowed. A light meal was then offered, and other dietary restrictions were removed. The light meal consisted of a cheese roll, 250-ml of skim milk, and an apple (30 g fat, 49 g carbohydrates, 25 g protein, 575 kcal).

Albendazole (GlaxoSmithKline, UK) from one batch was provided by the hospital pharmacy as a powder in capsules and administered as a single oral dose of 10 mg/kg body weight. The actual albendazole doses were within 3% of calculated doses. The fatty meal consisted of a Big Mac, french fries, and a shake (57 g fat, 150 g carbohydrates, 44 g proteins, 1399 kcal). Frozen grapefruit juice concentrate (Albert Heyn, Zaandam, The Netherlands) was thawed at room temperature and reconstituted with water to a double strength volume of 250 ml for each participant. Cimetidine (10 mg/kg twice

daily) was started 48 hr before albendazole was administered, continued during the study, and stopped thereafter.

Immediately before and 1, 2, 3, 4, 6, 8, 11, 24, and 36 hr after drug administration, venous blood samples were taken from an indwelling catheter placed in the lower arm. Blood was collected into heparinized tubes and centrifuged immediately. Plasma samples were stored at -70°C until assayed.

Written informed consent was obtained from all volunteers. The study was approved by the Institutional Review Board of the Academic Medical Center, Amsterdam.

Albendazole sulfoxide assay. ABZSX concentrations were measured using a modified method.¹² Briefly, plasma samples were slowly defrosted and centrifuged at 4000 rpm for 10 min. Then 400 μl of 3% perchloric acid was slowly added to 400 μl of plasma with the internal standard of hydroxy mebendazole 0.02 g/l. After 30 min the denaturated samples were centrifuged at 14,000 rpm for 10 min. The supernatant was subjected to HPLC using a Kipp Analytica 9209 HPLC autosampler (Jasco FP-920 UV-detector, Hewlett Packard 3390A integrator recorder) and a 100×4.6 3- μm CN spherisorb column. The wavelength was set at 293 nm, the flow rate at 1.0 ml/min, and the injection volume at 100 μl . The mobile phase consisted of 100 mmol acetic acid buffer containing 15% methanol, pH 3.5. Quantitation of ABZSX was calculated by integration using the ratio of the peak height of ABZSX to the internal standard hydroxy mebendazole. HPLC conditions used to measure ABZSX produced well-defined peaks. Retention time of albendazole, albendazole sulfoxide, albendazole sulfone, and the internal standard was 21.0, 7.5, 6.4, and 17.3 min, respectively. A calibration curve was constructed by adding 3.0, 1.0, 0.5, 0.3, 0.1, 0.05, and 0.025 mg/l ABZSX plus 20 μl of the internal standard to plasma. The calibration curve was linear between 0 and 1.5 mg/l ($r = 0.999$). Control specimens were injected with every analytical run: 1.0, 0.5, and 0.1 mg/l ABZSX. The coefficients of variation of these samples were 1.0%, 5.6%, and 10.2%, respectively. The detection limit of the assay was 0.05 mg/l ABZSX. The coefficients of variation of the within- and interday reproducibility were 5 to 10% depending on concentration.

In the same run, ABZ concentration was measured electrochemically in series with ultraviolet detection. A calibration curve was constructed to detect low concentrations of ABZ by adding 0.05, 0.03, and 0.01 mg/l ABZ plus 20 μl of the internal standard to plasma. Concentrations of control specimens were 0.05, 0.03, and 0.01 mg/l ABZ. The detection limit of the assay was 0.050 mg/l ABZ.

Pharmacokinetic analysis. C_{max} and T_{max} (time to reach C_{max}) were read directly from the observations. Terminal phase elimination rate constants (k_{el}) for ABZSX were determined from the postpeak linear decay portion using three to five terminal samples. The corresponding elimination half-lives ($T_{1/2}$) were calculated as $0.693/k_{\text{el}}$. $\text{AUC}_{0-\infty}$ was calculated by the linear trapezoidal method, and 8.3% of the total AUC was extrapolated.

Statistical analysis. The study was not randomized. To account for the influence of sequence on the results, analysis of variance (ANOVA) was used first to test the statistics of all four groups at once. After significance was found, Wilcoxon's signed rank test was used to compare the pharmacokinetic data between treatment groups (SPSS for Windows).

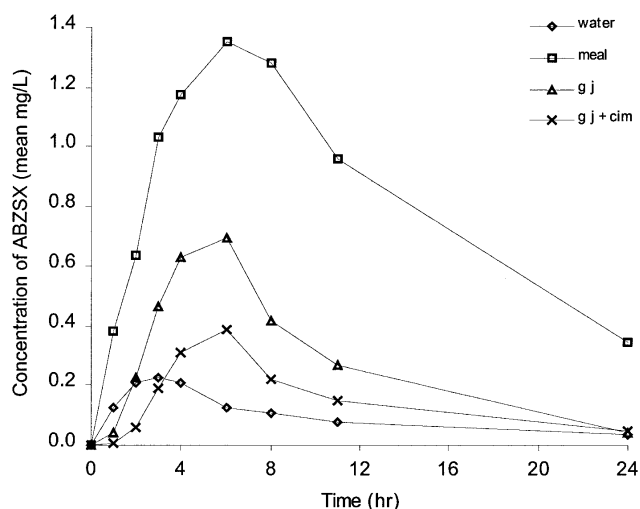


FIGURE 1. Mean ABZSX concentration-time curves in six participants after a single oral dose of albendazole (10 mg/kg) combined with water, a fatty meal (meal), grapefruit juice (g j), or grapefruit juice plus cimetidine (g j + cim).

RESULTS

The participants did not encounter adverse events from the single oral dose of albendazole. ABZSX concentrations could be readily detected in the high-performance liquid chromatography assay. The concentrations of the parent compound albendazole and the inactive metabolite albendazole sulfone were too low for quantitation. All volunteers responded uniformly when albendazole was combined with a fatty meal or with grapefruit juice. Mean C_{max} was enhanced 6.5-fold by a fatty meal and 3.2-fold by grapefruit juice ($P < 0.001$ and 0.031, respectively; Figure 1). C_{max} decreased when grapefruit juice was coadministered with cimetidine in all participants but one. In the latter regimen, mean C_{max} was 46% lower than after grapefruit juice alone ($P = 0.022$). In the sequence of regimens studied, C_{max} (mean \pm SD) was 0.24 ± 0.09 mg/l (water), 1.55 ± 0.30 mg/l (fatty meal), 0.76 ± 0.37 mg/l (grapefruit juice), and 0.41 ± 0.29 mg/l (grapefruit juice plus cimetidine), respectively. The interindividual variability of C_{max} was great and varied from 19% to 71% (Figure 2).

T_{max} shifted from 2.5 (1–4) hr to 5.3 (3–8) hr when albendazole was administered with a fatty meal, to 5.3 (4–6) hr with grapefruit juice and to 5.2 (4–6) hr when grapefruit juice was coadministered with cimetidine (mean and range; P s = 0.0026, 0.04, and 0.818, respectively).

$\text{AUC}_{0-\infty}$ was enhanced 9.4-fold by a fatty meal and 3.1-fold by grapefruit juice ($P = 0.002$ and $P = 0.031$, respectively). In the sequence of regimens studied, $\text{AUC}_{0-\infty}$ was 2.08 ± 0.61 mg/h.l (water), 19.64 ± 6.81 mg/h.l (fatty meal), 6.52 ± 5.09 mg/h.l (grapefruit juice), and 3.52 ± 1.85 mg/h.l (grapefruit juice plus cimetidine), respectively. The interindividual variability of $\text{AUC}_{0-\infty}$ was great and varied from 29% to 78% (Figure 3). When grapefruit juice was coadministered with cimetidine, $\text{AUC}_{0-\infty}$ decreased 46% compared with grapefruit juice alone ($P = 0.118$).

$T_{1/2}$ was 8.8 ± 4.2 hr when albendazole was administered with water and 8.2 ± 4.3 hr when administered with a fatty meal ($P = 1.000$). Grapefruit juice shortened $T_{1/2}$ by 46%, from 8.8 ± 4.2 hr to 4.1 ± 1.6 hr ($P = 0.026$). Cimetidine

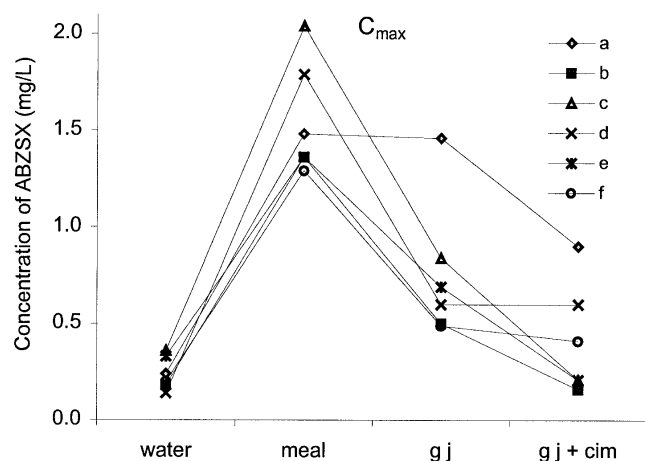


FIGURE 2. C_{\max} of ABZSX in six participants after a single dose of albendazole (10 mg/kg) combined with water, a fatty meal (meal), grapefruit juice (g j), or grapefruit juice plus cimetidine (g j + cim).

coadministration partially corrected $T_{1/2}$ from 4.1 ± 1.6 hr to 6.1 ± 1.9 hr ($P = 0.065$).

DISCUSSION

The first two thirds of the small intestine is a major site of CYP3A4-mediated metabolism of orally administered drugs. The content of mucosal CYP enzyme activity in the jejunum varies greatly among individuals. The interindividual variability contributes to the variability in blood levels of orally administered drugs that are substrates of CYP3A4.¹³⁻¹⁶ Mucosal CYP3A4 activity can be inhibited by grapefruit juice and occurs probably instantaneously. In a human volunteer, mucosal CYP3A4 activity estimated in mucosal biopsies taken 4 hr after grapefruit juice was administered decreased 47%.¹⁰ Another study in humans demonstrated a 62% selective downregulation of CYP3A4 activity in biopsies taken from the small intestinal mucosa.¹⁷

In this study, we focused on the question of whether albendazole is subject to mucosal CYP enzyme metabolism. Compared with albendazole administered with water, grapefruit juice enhanced C_{\max} 3.2-fold and $AUC_{0-\infty}$ 3.1-fold. This observation suggests that albendazole is metabolized by mucosal CYP3A4 enzymes. The observed enhancement of albendazole bioavailability may be an overall effect of both mucosal CYP3A4 inhibition and P-gp activation by grapefruit juice. Activated P-gp may partially counteract the inhibitory effect on CYP3A4 by transmembrane efflux. This mechanism is, however, tentative. It has not been demonstrated yet that albendazole is a substrate of P-gp.

An unexpected finding is the 46% shortening of $T_{1/2}$, which cannot be understood from the known mechanism of action of grapefruit juice. Grapefruit juice is assumed to selectively inhibit the activity of CYP3A4 enzymes in the intestinal mucosa and not to affect hepatic CYP3A4 activity.¹⁷ In vivo studies support this experimental observation. Grapefruit juice enhanced drug bioavailability after oral administration but not after intravenous administration of midazolam or cyclosporine.^{18,19} The question arises whether estimation of elimination half-life in our participants is affected by two obvious factors. First, sampling points are limited because of a

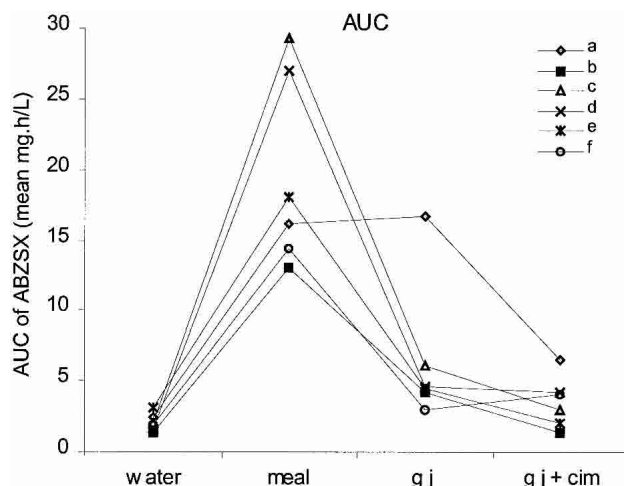


FIGURE 3. $AUC_{0-\infty}$ of ABZSX in six participants after a single dose of albendazole (10 mg/kg) combined with water, a fatty meal (meal), grapefruit juice (g j), or grapefruit juice plus cimetidine (g j + cim).

significant delay of T_{\max} after a fatty meal and grapefruit juice. Second, ABZSX formed during absorption of the drug may not be the only and primary source of plasma ABZSX. Another, secondary source of plasma ABZSX could be albendazole, which is absorbed without being metabolized. In the latter case, decay of ABZSX is affected by a possible difference of the elimination rate constant of ABZ and ABZSX.

However, we assume that estimation of $T_{1/2}$ in our subjects is not affected by the two factors mentioned previously. First, in comparison with water, elimination half-life was not affected when albendazole was combined with a fatty meal ($P = 1.00$). Second, the parent compound albendazole could not be detected quantitatively in plasma and does, therefore, not contribute to plasma ABZSX concentration. Among our participants, ABZSX formed during absorption of albendazole is the only source of plasma ABZSX concentration and the only determinant of elimination half-life.

The second question of our study was whether cimetidine coadministered with grapefruit juice would decrease albendazole bioavailability. In the current study, this regimen resulted in significantly lower C_{\max} than after grapefruit juice alone ($P = 0.022$). The cimetidine-mediated decrease of C_{\max} is concordant with what we observed in a previous pharmacokinetic study. Cimetidine coadministered with albendazole decreased C_{\max} by 52% with a remarkable reduction of the interindividual variability.⁷ We hypothesized that cimetidine exerts its effect via inhibition of gastric acid secretion and that albendazole absorption is pH dependent. A tentative alternative explanation is that cimetidine inhibits the CYP-mediated metabolism of albendazole into S(-) ABZSX. Then, the FMO-mediated pathway that converts albendazole into R(+) ABZSX may become the limiting factor and result in lower ABZSX concentrations.

Finally, we compared the effect of grapefruit juice with the known 4- to 8-fold enhancement of albendazole bioavailability by a fatty meal.² In our study, a fatty meal enhanced C_{\max} 6.5-fold and $AUC_{0-\infty}$ 9.4-fold, significantly more than the effect of grapefruit juice on C_{\max} and $AUC_{0-\infty}$ ($P_s = 0.028$ and 0.046 , respectively). Several mechanisms may be involved in

the enhancement of albendazole bioavailability: (1) the administration of fatty food or grapefruit juice itself, which is supported by the data of the current study; (2) stimulation of gastric acid secretion, which is supported by the observation that albendazole absorption is probably pH dependent.⁷ Furthermore, the mere increase of residence time in the acidic environment of the stomach we observed in our present study may also have contributed to the enhanced absorption of albendazole; (3) stimulation of bile salt secretion, for which no data are available but which deserves further attention.

In comparison with other studies, ABZSX concentrations in our participants were lower despite the higher dose we administered of the same commercially available drug.²⁰ However, the interindividual variability between individuals is known to be great when albendazole is administered on an empty stomach.^{2,20–22} We hypothesize that this is related to differences of gastric pH in the individuals studied.

To avoid a possible long-lasting carry-over effect of grapefruit juice, the study was not randomized. To account for the influence of sequence on the results, a one-way ANOVA was used first. The statistics of all four groups were significantly different for C_{\max} and $AUC_{0-\infty}$ ($P = 0.000$). Therefore, Wilcoxon's ranked sum test was used as a posthoc test to compare the results between treatment groups.

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