Effect of acid secretion blockade by omeprazole on the relative bioavailability of orally administered furazolidone in healthy volunteers

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Aims The administration of omeprazole may interfere with the absorption of orally administered drugs by reducing gastric pH and hence tablet dissolution. The aim of this study was to investigate the effects of a 5 day administration of omeprazole on the pharmacokinetics of furazolidone.

Methods Eighteen healthy (nine male and nine female) volunteers were selected. The study had an open randomized two-period crossover design with a 21 day washout period between the phases. Serum concentrations of furazolidone were measured by reversed-phase h.p.l.c. with ultraviolet detection.

Results Administration of omeprazole caused a significant reduction of $C_{\max}$ [$0.34 \mu\text{g ml}^{-1}$ (range 0.25–0.43) *vs* $0.24 \mu\text{g ml}^{-1}$ (range 0.15–0.34)] with no significant delay in absorption $t_{\max}$ [2.5 h (range 1.85–3.0) *vs* 2.4 h (range 2.06–2.71)].

Conclusions Furazolidone was rapidly absorbed after oral administration. Short-term treatment with omeprazole did alter the relative bioavailability of this drug, probably through an effect on absorption kinetics or first-pass metabolism.

Keywords: furazolidone bioavailability, furazolidone, *Helicobacter pylori*, omeprazole, peptic ulcer

Introduction

More than 50% of the world's population is supposedly infected by *H. pylori* (*Hp*), the most common chronic bacterial infection in humans [1]. This infection is a major pathogenic factor in gastroduodenal disease, including chronic active gastritis, peptic ulcers and gastric neoplasm. Treatment regimens aimed at bacterial eradication are now recommended for patients with symptomatic *Hp* infection [2]. Our present knowledge of the treatments for eradicating *H. pylori* is based on the results of clinical trials involving a combination of two or more drugs, with the efficacy of the drug combinations being determined by trial and error [3]. The development of a simple, safe and effective therapeutic regimen requires knowledge of the pharmacology of the agents to be used and should consider

simplicity, adverse effects and antimicrobial sensitivity [4, 5]. Antibiotic resistance is a growing problem in *Hp* treatment. The resistance rates to drugs commonly used for its eradication in the United States ranges from 20% to more than 50% for metronidazole and from 7% to 14% from clarithromycin [6]. The resistance rate to metronidazole in Brazil ranges from 40% to 50% [7, 8].

Furazolidone has recently been used instead of metronidazole to overcome the high resistance of *Hp* to the latter in therapeutic schedules achieving high eradication rates [9–12]. The bactericidal activity of furazolidone results mainly from the inhibition of enzymes involved in the Krebs' cycle [13]. Severe adverse reactions to this drug, such as serum sickness or acute pulmonary hypersensitivity have been reported [14, 15], but the drug is considered to be safe and well tolerated, with nausea and vomiting being the commonest adverse reactions [13, 16, 17]. Tartrazine, a component of furazolidone tablets in many countries, is suggested as a possible cause of serum sickness rather than furazolidone itself [14].

Although a large amount of qualitative and quantitative data on furazolidone is available for animals, there is only

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limited information on the pharmacokinetics of furazolidone in humans. Although initially reported to be poorly bioavailable, more recent data suggest that the drug is well absorbed and may achieve therapeutic concentration in serum [13, 18, 19].

Since furazolidone is currently being used in association with acid-suppressor agents to eradicate *Hp* [9, 10], and the coadministration of the latter is suggested to interfere with the distribution and possibly the efficacy of antibiotics as well as having a direct effect on *Hp* [20–25], we evaluated the pharmacokinetics of furazolidone after a 5 day treatment with omeprazole.

Methods

Materials

Giarlam[®] (200 mg furazolidone, oral) was purchased from UCI-FARMA (São Bernardo do Campo, SP, Brazil), and Losec (20 mg omeprazole capsules) was a generous gift from Astra Química e Farmacêutica (São Paulo, SP, Brazil). Furazolidone and 3-nitroaniline (Aldrich Chemical Co.) analytical standards were purchased from the Sigma Chemical Co. (St Louis, MO, USA). Other reagents of analytical grade were supplied by Merck Indústrias Químicas (Rio de Janeiro, RJ, Brazil).

Subjects

Eighteen healthy volunteers (nine male and nine female, aged from 19 to 35 years, height from 155 to 180 cm, weight from 55 to 100 kg) were selected after clinical examination and laboratory screening. The latter consisted of blood glucose, urea, creatinine, AST, ALT, gamma GT, alkaline phosphatase, total bilirubin, total protein, albumin, haemoglobin, haematocrit, total and differential white cell counts, routine urinalysis and serology for *H. pylori*. The serological test had a sensitivity of 92% and a specificity of 93.1% (ImmunoComb II *Helicobacter pylori* IgG, Organics Yavne, Israel). Written informed consent was obtained from each volunteer prior to the study, and the protocol was approved by the São Francisco University Ethics Committee in accordance with the Declaration of Helsinki.

Omeprazole administration

The study had an open randomised two-period crossover design, with a 21 day washout period between the phases. In the OME(–) phase (control, no omeprazole pretreatment), the volunteers were hospitalized at 21.00 h after having had a normal evening meal. Following an overnight fast, they received a single tablet of furazolidone (200 mg, Giarlam[™]) with 200 ml of water at 07.00 h.

During the OME(+) phase (omeprazole pretreatment), the volunteers came to the clinical pharmacology and gastroenterology unit daily for 5 days (Monday to Friday), where they received one capsule of omeprazole (20 mg, Losec[™]) at 09.00 h and 20.00 h. On Friday evening, they were admitted to the clinical pharmacology and gastroenterology unit, and received another capsule of omeprazole at 09.00 h. After an overnight fast, furazolidone was administered as described above for the OME(–) group.

During both study periods blood samples for serum furazolidone (FUR) quantification were taken from a convenient forearm vein before and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 18 and 24 h following furazolidone administration. At each time point, one 5 ml blood sample was taken via a 'butterfly' cannula into a tube. The samples were centrifuged at 2000 *g* for 10 min, and the sera then separated and stored at –20°C until assayed for their FUR concentrations.

Methodology

The h.p.l.c. system consisted of a Shimadzu LC-6AD pump, an SPD-10 A VP u.v. detector operated at 362 nm and a Shimadzu SIL-10AD VP automatic injector. A precolumn (4.0 × 3.0 mm) and an analytical column (Phenomenex, Lichrosorb C₁₈, 10 µm, 250 mm × 4.6 mm, i. d.) were used and operated at room temperature. The mobile phase was a mixture of methanol, water and acetic acid (40:59.5:0.5 v/v). The analytes and internal standard were eluted isocratically at a flow rate of 1.2 ml min^{–1}.

3-nitroaniline (30 µl) (internal standard 80 µg ml^{–1} mobile phase) were added to 1 ml of serum and the mixture was transferred to a Supelclean[™] LC-18 cartridge (SUPELCO, USA). Furazolidone was extracted and eluted into 1 ml methanol which was then evaporated under nitrogen at 40°C. The residue was dissolved in 250 µl of mobile phase and a 50 µl aliquot of each sample was then injected into the h.p.l.c. system.

The limit of quantification was 0.05 µg ml^{–1} and the lower limit of detection was 0.01 µg ml^{–1}. Under these conditions, the calibration curves for furazolidone in serum (0.1–1.5 µg ml^{–1}) was linear and passed through the origin ($r=0.9997$). Coefficients of variation were 3.9% at the lowest concentration and 8.9% for concentrations above 1.3 µg ml^{–1}.

Pharmacokinetic and statistical analysis

The maximum serum concentration ($C_{\max}$) and the time taken to reach this concentration ($t_{\max}$) were obtained directly from the individual concentration vs time curves. A first-order terminal elimination rate constant (k_e) for furazolidone was derived by log-linear regression of

selected data points from the concentration *vs* time curves describing a terminal log-linear decaying phase. The half-life ($t_{1/2}$) was estimated from this rate constant ($t_{1/2} = \ln(2)/k_e$). The respective area under the time-concentration curves from 0 to 24 h for furazolidone ($AUC(0,24\text{ h})$) was calculated by the linear trapezoidal method. Extrapolation of $AUC(0,24\text{ h})_{\text{MET}}$ to infinity ($AUC(0,\infty)$) was done by addition of the value C_{24}/k_e (where C_{24} = plasma furazolidone concentration 24 h after furazolidone administration). All the variables were analysed by parametric (one way ANOVA) and nonparametric tests (Mann–Whitney test, Wilcoxon test). Student's *t*-test was also used to compare the $AUC(0,24\text{ h})$ and C_{max} data. Individual $AUC(0,24\text{ h})$, C_{max} , $t_{1/2}$, k_e ratios and t_{max} differences between the OME(+) (test) and OME(–) (reference) phases were calculated. The 95% confidence intervals (CI) for individual ratios were calculated and their inclusion into the bioequivalence interval (80–125%) was evaluated. In addition, the 95% CI for individual t_{max} differences were also calculated and the inclusion of the zero value in this range analysed. A *P* value of 0.05 was taken as the lower limit for significance in all statistical tests.

Results

No side-effects were reported by any of the volunteers after the administration of either omeprazole or furazolidone. The clinical and biochemical tests were not affected by drug administration and remained within the reference values.

Ten of the volunteers were *Helicobacter* positive and eight were negative. However, since the C_{max} , $t_{1/2}$, and $AUC(0,24\text{ h})$ values for these two group did not differ, as assessed by comparison of the means using Student's *t*-test (data not shown), they were considered as one group for analysis.

Furazolidone was well absorbed after oral administration (Figure 1, Table 1). Omeprazole treatment did not alter t_{max} but significantly decreased C_{max} (Figure 1, Table 1). Treatment with omeprazole significantly decreased the relative bioavailability of furazolidone, because the 95% CI of the ratios (expressed as a percentage) of $AUC(0,24\text{ h})$, $AUC(0,\infty)$ and C_{max} and the individual $AUC(0,\infty)$ did not fall within the range 80–125% (Table 1).

Discussion

Furazolidone was well absorbed after oral administration, being detectable in serum 30 min after administration and reaching a maximum serum concentration ($0.34\text{ }\mu\text{g ml}^{-1}$) within the first 3 h (Figure 1, Table 1), with a $t_{1/2}$ of 4.87 h. A 5 day treatment with omeprazole adversely affected the relative bioavailability of furazolidone as

Figure 1 Serum furazolidone *vs* time curves (mean $\pm$ s.e.mean) obtained in serum of healthy volunteers after the oral administration of 200 mg of furazolidone in the presence (●), and absence of omeprazole (○).

indicated by a decrease in the peak serum concentration (C_{max}) of furazolidone and by the lack of inclusion of the 95% CI of the ratios (expressed as a percentage) of $AUC(0,24\text{ h})$, $AUC(0,\infty)$ and C_{max} in the range 80–125%.

Anti-secretory drugs can potentially alter the pharmacokinetics of antibiotics by reducing the gastric juice viscosity and/or volume [29], interfering with the chemical stability of drugs [30], slowing gastric emptying [31], and possibly reducing the dissolution of solid formulations. In the present work no differences were observed in the t_{max} of furazolidone between both phases, thus indicating that the effect of omeprazole may be via a reduction in the amount of furazolidone available at the site of absorption and not by a delay in its delivery.

Since omeprazole is not supposed to interfere with intestinal absorption, the pharmacokinetic changes observed are likely to have resulted from an interaction between the two drugs before they reached the intestine, probably by a reduction in the dissolution or an increase in the degradation of furazolidone. Other possible pharmacokinetic effects include induction of the first pass metabolism of furazolidone by omeprazole. Indeed, as with other benzimidazole derivatives, omeprazole has been shown to inhibit or induce the oxidative metabolism of various drugs [32–35]. The trend towards a decrease in half-life supports this possibility.

H. pylori is present on the gastric epithelial surface and may be associated beneath and within the adherent mucous gel. Thus agents used to eliminate this micro-organism must achieve a bactericidal concentration at these sites [36]. This can occur by direct or indirect mechanisms, namely by dissolution and distribution inside

Table 1 Furazolidone pharmacokinetic parameters in serum after a single oral (200 mg) dose of furazolidone in 18 healthy volunteers during [OME(+)] and without [OME(-)] pretreatment with omeprazole.

Pharmacokinetic parameters (geometric mean)	OME (-)	OME (+)	OME (+)/OME (-) ratios (expressed as %)	P value
AUC(0,24 h) ($\mu\text{g ml}^{-1} \text{ h}$)	0.89	0.73	82	0.150
(CI 95%)	(0.67, 1.10)	(0.43, 1.03)	(64.20, 93.64)	
AUC(0, ∞) ($\mu\text{g ml}^{-1} \text{ h}$)	0.90	0.74	82	0.133
(CI 95%)	(0.68, 1.11)	(0.44, 1.04)	(64.71, 93.69)	
C_{max} ($\mu\text{g ml}^{-1}$)	0.34	0.24	70.6	0.023*
(CI 95%)	(0.25, 0.43)	(0.15, 0.34)	(41.2, 110.9)	
K_e (h^{-1})	0.21	0.36	171	0.164
(CI 95%)	(0.15, 0.27)	(0.22, 0.50)	(40.7, 185.2)	
$t_{1/2}$ (h)	4.87	3.90	80	0.077
(CI 95%)	(2.63, 7.11)	(2.10, 5.71)	(48.68, 131.6)	
t_{max} (h)	2.5	2.4	98	0.946
(CI 95%)	(1.85, 3.04)	(2.06, 2.71)	(77.23, 129.4)	
Clearance ($\mu\text{g ml}^{-1}$)	0.28	0.32	114	0.66
(CI 95%)	(0.18, 0.39)	(0.19, 0.45)	(94.74, 115.4)	

the gastric cavity immediately after ingestion, or by secretion into the gastric juice after intestinal absorption.

If a direct mechanism of antibiotic action plays a major role in eradication therapy, interference with chemical stability of antibiotics or even with their distribution within the stomach, due to a decrease in gastric juice volume, may lessen drug efficacy. On the other hand, if an indirect mechanism of antibiotic action is responsible for *H. pylori* eradication, any interference with the intestinal absorption or metabolism of the drug may also affect the outcome of the treatment. These mechanisms are not mutually exclusive and may be acting simultaneously as has been suggested for the combination of metronidazole and clarithromycin [20, 22, 23].

Furazolidone is considered to be highly effective against *H. pylori*, and low resistance rates or the absence of resistance to this agent have been reported [37]. However, dual therapy using this agent and omeprazole has achieved only modest eradication rates (50%) [38], which could be partially, explained by our findings. The use of triple therapies containing furazolidone, a proton pump inhibitor, and clarithromycin has resulted in high eradication rates [12, 39]. This apparent contradictory observation could be explained by a pharmacokinetic interaction between omeprazole and clarithromycin. Indeed, acid blockade has been suggested to increase the amount of clarithromycin transferred on gastric juice [24, 40].

Our results emphasize the need for improved knowledge of the pharmacology of and interactions between drugs used in *H. pylori* eradication therapies.

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