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Body position, olive oil and omeprazole may reduce the absorption of orally administered amoxicillin and metronidazole in rabbits

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Abstract

In 5-20% of patients with peptic ulcer disease, antibacterial agents used to eradicate *Helicobacter pylori* from the gut fail to eradicate the bacteria due to low concentrations of antibacterial agents at the residence of *H. pylori* in the gastric mucosa. The purpose of this study was to assess the influence of omeprazole, olive oil and body position on the pharmacokinetics of amoxicillin and metronidazole. In a randomized controlled animal model study, amoxicillin (50-mg/kg) was administered orally to two groups of rabbits (n=8 in each group) in which a group had previously received 2-mg/kg omeprazole and 10-ml/kg of olive oil orally, and restrained on their left lateral position while the other group serving as control received 10-ml/kg of water. Another two groups of rabbits (n=8 in each group) were treated in a similar manner except that each rabbit received metronidazole (30-mg/kg) instead of amoxicillin. The plasma levels of the medicines were monitored over 12 hr and pharmacokinetic parameters were then determined using non-compartment model. The C_{max} (μg/ml), t_{max} (hr), k (μg/hr), $t_{½}$ (hr), and $AUC_{0-\infty}$ (μg.hr/ml) of amoxicillin were 6.67±4.82, 3.99±2.3, 1.37±0.42, 0.51±1.64 and 44.13±18.92, respectively while the values for metronidazole were 4.41±8.97, 4.01±2.64, 0.73±0.52, 0.94±1.32 and 28.65±24.62. Unlike metronidazole, significant reduction in the mean C_{max} (P=0.004) and $AUC_{0-\infty}$ (P=0.018) were observed in the pharmacokinetic profiles of amoxicillin following administration of olive oil and omeprazole while lying on the left lateral position. It is concluded that the administration of olive oil and omeprazole, while lying in the left lateral position, reduces the absorption rate of both amoxicillin and metronidazole in rabbits. The implication is that patients lying on their left lateral position that are being treated with omeprazole may not eat meals containing high amount of olive oil as it can adversely alter the pharmacokinetics of antibiotics and increase bacterial resistance.

Keywords: Amoxicillin, metronidazole, absorption, olive oil, pharmacokinetics, rabbits.

Introduction

In vitro, *Helicobacter pylori* is susceptible to most antibiotics but when administered to humans, no single-antibiotic therapy has been able to achieve a high eradication rate [1-2]. Concomitant administration of antimicrobial(s) with an antisecretory agent improves the eradication of *H. pylori* as compared to individual drug administration [3]. Some of the medicines that are still being used in clinical practice to eradicate *H. pylori* include amoxicillin, metronidazole and omeprazole (a proton pump inhibitor). However, the recommended therapies do not often result in eradication in as much as 20% of the patients in some cases [1].

The failure to effectively eradicate *H. pylori* has often been attributed to acquired resistance, poor compliance, insufficient antibiotic penetration into the infection site, and/or a low level of drug stability at this location. Both *in vitro* and *in vivo* studies have shown that the transport of medicines across the gastric mucosa is a major limiting factor in eradicating the organism [4-5]. Unlike the small intestine which has a much larger surface area (because of the presence of numerous folds and projections including villi and microvilli), the stomach has a relatively smaller surface area for absorption of medicines. The pH of the stomach is acidic and this provides an unfavourable condition for the stability, and hence the bioavailability, of many medicines in the gastric mucosa. Studies on transport of medicines across the gastric mucosa have shown that the proton pump inhibitor, omeprazole, increases antibiotic concentration in the gastric mucosa [6-7] possibly by the combined inhibitory effect on acid secretion in the stomach and its ability to delay gastric emptying [8-9]. Boulby and his colleagues [10-11] have demonstrated that by predisposing volunteers with olive oil, test meals can be delayed in the stomach when the subjects are lying on their left lateral position. Nevertheless, the combined effect of omeprazole and olive oil in subjects lying on their left lateral position has not been investigated.

The purpose of this study therefore, is to assess the influence of omeprazole and olive oil in animals kept on their left lateral position on the pharmacokinetics of amoxicillin and metronidazole. We have chosen an animal model for this study with the hope that the outcomes can be applied in a clinical trial aimed at reducing the therapeutic failure rate in the treatment of gastric ulcers.

Materials and Methods

Study Design

In a randomized controlled study, rabbits were divided into four groups with two groups receiving, omeprazole and single dose of either amoxicillin or metronidazole orally as well as olive oil and kept on their left lateral position. Animals in the remaining two groups, serving as controls, received single dose of either amoxicillin or metronidazole orally.

Animals

Locally bred healthy white rabbits (n = 32; average weight, 1.36 ± 0.15 kg; weight range, 1.12 – 1.72 kg) were purchased in Benin City. The animals were placed in individual cages, nourished *ad libitum* with drinkable water and standard rabbit feed (Bendel Feed and Flower Mills Ltd, Ewu, Edo State, Nigeria) and allowed to acclimatize to the laboratory environment for four weeks. During this period, the animals were treated with terramycin (200 mg/kg, May & Baker Ltd., England) in the first week to exclude bacteria infections and blood samples (smear) were collected on microscope slides from each animal through the marginal ear vein each week and examined under a microscope for presence of parasites and bacteria. Animals with blood infections were excluded from the experiment and replaced [12].

The animal cages were cleaned daily and disinfected with saponified cresol (Izal®, SmithKline Beecham, Brenford, England). Superficial infection by parasites was controlled through regular disinfection of the cages with lindane insecticide, Gammalin 20® (Chemical & Allied Products Ltd., Nigeria) as previously described [12].

Procedure

This study was conducted after appropriate ethical approval. Amoxicillin syrup powder (Amoxil®, Smithkline Beecham, Lagos) was reconstituted with boiled and cooled drinking water to a concentration of 50-mg/ml and used within 48 hr. Metronidazole (Emgyl®, Emzor Pharmaceutical Company, Lagos) suspension (40-mg/ml) was purchased from local pharmacy in Benin City. The quality of the medicines was ascertained using standard procedures. All rabbits used were allowed to fast overnight before administration of oil and/or medicines orally. Blood samples were collected from each animal just before administration of oil and/or medicines (using a standard stainless steel feeding needle). All the animals were allowed access to drinking water and feed 2 hr after initiation of administration of oil or medicines.

Group 1 animals: Each rabbit (n=8) received 10-ml/kg olive oil (Goya en España, Spain) to form a layer around the antrum any pylorus on top of the aqueous meal in the stomach [11] and restrained to its left lateral position followed by standard suspension of amoxicillin equivalent to 50-mg/kg of body weight and omeprazole (in alkaline suspension of sodium bicarbonate [13]) equivalent to 2-mg/kg. The lateral position of the animals was achieved by physically restraining each animal in a custom built adjustable cage in which it was kept for 2 hr.

Group 2 animals: Each rabbit (n=8) received 10-ml/kg olive oil (Goya en España, Spain) and restrained to its left lateral position followed by standard suspension of metronidazole equivalent to 30-mg/kg of body weight and omeprazole (in alkaline suspension [13]) equivalent to 2-mg/kg. The lateral position of the animals was maintained for 2 hr.

Group 3 animals (amoxicillin control): Each rabbit (n=8) received oral syrup of amoxicillin equivalent to 50-mg/kg of body weight and 10-ml/kg of water.

Group 4 animals (metronidazole control): Each rabbit (n=8) received oral metronidazole suspension equivalent to 30-mg/kg of body weight and 10-ml/kg of water.

Determination of blood levels of antibiotics

Uncoagulated blood (0.5 ml) was collected from each rabbit at 0, 0.25, 0.5, 1, 1.5, 2, 4, 8, 12 hr from the marginal ear vein, centrifuged at 2,500 *g* immediately and stored at -20 ^{o}C until analysed (within 24 hr). Amoxicillin and metronidazole concentrations in the blood were determined in triplicates (within 24 hr of sample collection) by the agar diffusion method using *Bacillus subtilis* ATCC 6633 as test organism for amoxicillin [14-15] and *Clostridium perfringens* as test organisms for metronidazole [16].

For standard curves, amoxicillin and metronidazole were diluted in pooled rabbit plasma and the medicine concentrations in plasma were derived from the standard curves. The limit of detection of the bioassay was 0.4 mg/L for both compounds. The linearity of the standard curve was assessed by a regression coefficient to be >0.97 (r^2) for both medicines, and the percent coefficient of variation of the assay was 8.9% (amoxicillin) and 9.2% (metronidazole), as determined for six concentrations within the range of 0.4 to 20 mg/L. The between-day and within-day coefficients of variation for replicates were equal to 4.9% and 9.2% (amoxicillin) and 7.1% and 8.9% (metronidazole), respectively.

Data analysis

For each rabbit, pharmacokinetic parameters, maximum plasma concentration (C_{max}), time corresponding to C_{max} (t_{max}), elimination rate constant (k), elimination half-life ($t_{½}$) and area under the plasma concentration versus time curve from zero to infinity ($AUC_{0-\infty}$) were determined by non-compactment model using the least-squares method [17] for elimination rate constant (k) calculation with Microsoft Excel (Microsoft Inc., USA). The descriptive statistical data (mean and standard deviation) are reported. Group data comparison was carried out with Instat (GraphPad Inc, USA) using ANOVA. At 95% confidence interval, 2-taile *P* values less than 0.05 were considered to be significant.

Results

The plasma concentration – time profiles for amoxicillin and metronidazole are given in Figure 1 while the calculated pharmacokinetic parameters are given in Table 1. Variable plasma concentration profiles of amoxicillin and metronidazole were observed in the rabbits that were given olive oil and omeprazole prior to the administration of the antibiotics. These variations mainly occurred at the absorption phase of amoxicillin and metronidazole. In 3 of the 8 rabbits that received amoxicillin (37.5%) and 4 of those that received metronidazole (50%), the profiles were not significantly different from those of the control animals

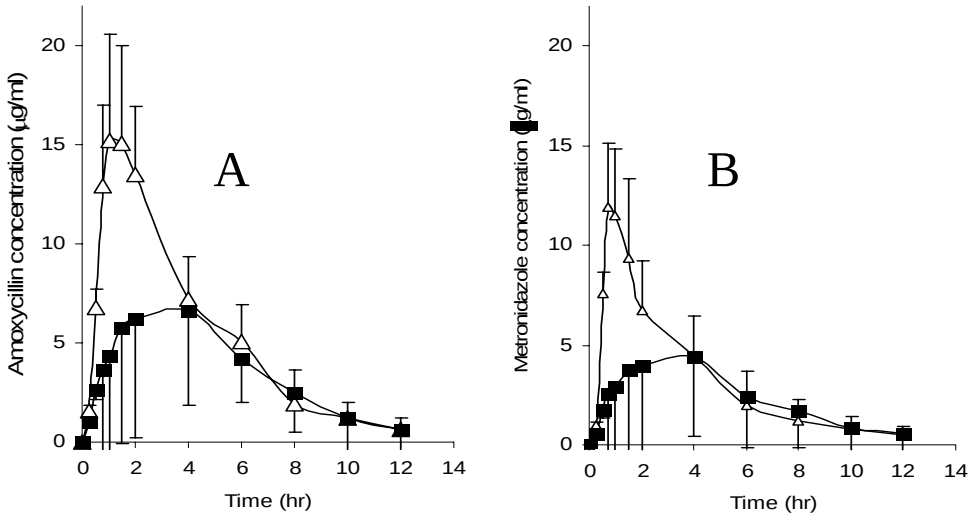

Figure 1: Plasma concentration versus time profiles of amoxicillin (A) and metronidazole (B) in rabbits in the presence (■) or absence (Δ) of orally administered olive oil and omeprazole. The line curves represent the mean plasma concentrations of amoxicillin and metronidazole with their standard deviations reflected.

that were not given olive oil and omeprazole. Significant reduction in the mean C_{max} (*P*=0.004) and $AUC_{0-\infty}$ (*P*=0.018) but increase in mean t_{max} (*P*=0.003) were observed in the pharmacokinetic profiles of

amoxicillin following administration of olive oil and omeprazole, and lying on the left lateral position (P=0.004). However, only the mean t_{max} significantly increased in the metronidazole data in the presence of olive oil and omeprazole and lying in the left lateral position (P=0.018). The mean elimination rates of amoxicillin and metronidazole were not significantly affected by the administration of olive oil and omeprazole, and lying on left lateral position.

Table 1: Pharmacokinetics of orally administered amoxicillin and metronidazole in rabbits (n=8) restrained to their left lateral positions after receiving olive oil and omeprazole orally. The control animals (n=8) were not restrained on their left lateral position.

Parameter	Control (mean±sd)	Olive oil and omeprazole administered (mean±sd)*	p value
Amoxicillin			
C_{max} (μg/ml)	15.25±5.31	6.67±4.82	0.004
t_{max} (hr)	1.0±0.56	3.99±2.3	<0.003
k (μg/hr)	0.75±2.91	1.37±0.42	NS
$t_{½}$ (hr)	0.93±0.24	0.51±1.64	0.018
$AUC_{0-\infty}$ (μg.hr/ml)	67.18±15.5	44.13±18.92	NS
Metronidazole			
C_{max} (μg/ml)	9.34±3.24	4.41±8.97	NS
t_{max} (hr)	1.5±0.34	4.01±2.64	0.018
k (μg/hr)	0.57±3.65	0.73±0.52	NS
$t_{½}$ (hr)	1.21±0.19	0.94±1.32	NS
$AUC_{0-\infty}$ (μg.hr/ml)	40.13±12.63	28.65±24.62	NS

**Rabbits were restrained to their left lateral position for 2 hr after administration of olive oil, amoxicillin and metronidazole (8 rabbits each were used for amoxicillin and metronidazole).*
C_{max}, maximum plasma concentration; T_{max}, time corresponding to C_{max}; k, elimination rate constant.
$AUC_{0-\infty}$, area under the plasma concentration versus time curve from zero to infinity; NS, not significant.

Discussion

Amoxicillin and metronidazole are two compounds with different physicochemical properties that are frequently combined with omeprazole in the eradication of *H. pylori* [18]. Being a zwitterion with pK_a values of 2.68 (carboxylic acid), 7.49 (amine) and 9.63 (phenolic hydroxyl), amoxicillin is often ionized across the whole range of pH in the gastrointestinal tract (GIT). With a pK_a of 2.52, metronidazole will occur mainly in the unionized form in the GIT at pH above 4.5 [19]. Usually, it is the unionized form of a compound that is readily absorbed.

In this study, the pharmacokinetic profile of amoxicillin in the control rabbits is similar to that reported in humans following oral administration and the rate of absorption of the compound in the rabbits was fast and equivalent to that observed in humans [19]. When it is administered orally, omeprazole is rapidly absorbed from the gut and in an acidic environment it is activated to a sulphenamide derivative that binds irreversibly at the secretory surface of parietal cells to the H+/K+ ATPase to inhibit the transport of hydrogen ions into the stomach thereby inhibiting acid secretion [20]. However, omeprazole has been reported not to alter the pharmacokinetics of amoxicillin in humans [21], possibly because of its inability to alter the ionic state of amoxicillin (a zwitterion). The observed alteration in the pharmacokinetics of amoxicillin in the presence of omeprazole and olive oil while lying on the left lateral position may not be related to the effect of omeprazole. This is because a preliminary report [22] has shown that the pharmacokinetic profile of amoxicillin in 5 rabbits fed on olive oil while lying on their left lateral positions was not altered by the administration of omeprazole (P<0.05). The significant reduction in the peak plasma concentration (C_{max}) of amoxicillin and significant increase in the time (t_{max}), the C_{max} occurred, may be related to the delay in gastric emptying produced by the effect of olive oil and lying on the left lateral position [10-11]. Omeprazole, olive oil and body position did not affect the elimination rate constant and hence the half-life of amoxicillin in the rabbits suggesting that only the absorption phase of amoxicillin was affected.

It is difficult at this stage to explain the reasons for the observed variability in the pharmacokinetic profiles of amoxicillin following the administration of omeprazole, olive oil and lying on the left lateral position as it was observed that the pharmacokinetics of amoxicillin in some of the animals were not significantly

affected by the treatment. The oral bioavailability of amoxicillin in humans is 93% [23] and previous work has demonstrated high absorption of the compound in the rabbits' gut [15]. Following the administration of omeprazole, olive oil and lying on the left lateral position, the overall significant decrease in the AUC of amoxicillin is suggestive of reduced and incomplete absorption of amoxicillin in the rabbits. This is likely due to slow release of the compound from the stomach into the small intestine where the compound is mainly absorbed into the blood stream as a result of the olive oil and delay in gastric emptying, as well as possibly degradation in the stomach (resulting from delayed gastric emptying).

The mean peak plasma concentration of metronidazole observed in the control animals is similar to that reported in a previous study on the pharmacokinetics of suppositories in rabbits [24]. As with amoxicillin, the rate of absorption in the rabbits was fast and equivalent to that observed in humans after oral administration. Following administration of the compound (omeprazole), olive oil and lying on the left lateral position, the pharmacokinetic profiles of the rabbits obtained in the rabbits indicates that the absorption profile of metronidazole was not affected in 50% of the rabbits by the treatment (omeprazole, oil and body position) adopted in this study. This is an indication that the treatment may not be useful in clinical situation. However, omeprazole is known to alter the lipophilicity of metronidazole [19] and has been shown to raise the gastric pH to above pH 5 [25-26]. This would normally favour the formation of unionized form of metronidazole and hence increase absorption in the GIT which does not appear to have happened in this case. In addition, the finding that lying on the left lateral position and olive oil delays gastric emptying [10-11] would have supported increase in the gastric absorption of metronidazole which was not significantly reflected in the pharmacokinetic data obtained. It does appear that absorption of metronidazole in the stomach and other parts of the GIT was not enhanced by the combined effect of omeprazole, olive oil and lying on the left lateral position. Our results adds credence to the earlier report that omeprazole decreases intragastric concentrations of metronidazole [19] as the expected increase in lipophilicity of metronidazole by omeprazole, and hence gastric absorption of the compound, was not reflected in the pharmacokinetic data.

In our present study, significant differences in the pharmacokinetic data determined was only observed in the rate of absorption as indicated by the increase in the mean t_{max}. With the significant variability in the plasma levels of metronidazole in 50% of the rabbits, the effect of the omeprazole, oil and body position on the pharmacokinetics of metronidazole in rabbits can be described as variable.

Conclusion

The result presented in this study has shown that the administration of olive oil and omeprazole while lying in the left lateral position reduces the absorption rate of both amoxicillin and metronidazole in rabbits. The implication is that the pharmacokinetics of antibiotics can be adversely altered in patients taking olive oil while lying on their left lateral position and receiving omeprazole treatment. This can increase the resistance of *H. pylori* to the antibiotics being used to eradicate the bacteria.

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