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Effects of food and formulation on the relative bioavailability of bismuth biskalcitrate, metronidazole, and tetracycline given for *Helicobacter pylori* eradication

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Aims
To evaluate the effects of food and formulation on the pharmacokinetics of bismuth biskalcitrate, metronidazole and tetracycline when combined in a new 3-in-1 single capsule (BMT) for eradication of *Helicobacter pylori*.

Methods
In a randomized, 3 × 3 cross-over design, 23 healthy males received one dose of BMT in the fed and fasting states and equivalent doses of the three drugs given together but as separate capsules while fasting. Bioequivalence was evaluated according to 90% confidence intervals (CIs) of ratios of geometric least square means for C_{max}, AUC_t, and AUC_∞.

Results
With respect to food, none of the three drugs met bioequivalence guidelines. Bismuth had lower limit CIs ranging from 12% for C_{max} to 25% for AUC_∞. The corresponding values for tetracycline were 59% and 51%. Metronidazole had a lower limit CI of 74% for C_{max}. With respect to formulation, bismuth had lower limits of CIs ranging from 39% for C_{max} to 50% for AUC_t and higher limits of 146% for AUC_t, metronidazole met bioequivalence guidelines, and tetracycline had lower limits of CIs between 72% for AUC_t and 74% for AUC_∞.

Conclusions
Food significantly decreased the relative bioavailability of each drug but formulation was without effect. This decrease may be beneficial when a local gastric action is needed, as confirmed by a near 90% eradication rate when this combined capsule is administered with food to treat gastro-duodenal local infection by *H. pylori*.

Introduction

Eradication of *Helicobacter pylori* requires treatment by several drugs given simultaneously. A well-recognized combination is bismuth, metronidazole, and tetracycline. To comply with this treatment patients must take different numbers of tablets and capsules from different containers several times a day. A new '3-in-1' capsule (BMT) combining bismuth, metronidazole and tetracycline has been developed to increase patient acceptability.

Since this BMT capsule will be given with meals, the food effect on the bioavailability of each component

should be assessed. In addition, if a formulation combines several drugs in a single capsule, a comparison of the bioavailability of each component given in the formulation with that of each drug given in separate capsules is required to rule out chemical interactions.

The objectives of this study were to evaluate the effects of food and formulation on the relative bioavailability of bismuth, metronidazole and tetracycline from this BMT capsule.

Methods

The Institutional Review Board of Algorithme Pharma Inc., Canada and the Canadian regulatory agency approved this study. Participants were enrolled after signing an informed consent form that had been previously revised and approved by both the Institutional Review Board and the Canadian regulatory agency.

Three regimens were administered as a single oral dose according to a three-sequence, three-period, crossover design. Subjects received in a random order three BMT capsules (the recommended single dose) (Helizide™; Axcan Pharma Inc., Mont-Saint-Hilaire, Canada), each containing 140 mg bismuth biskalcitrate +125 mg metronidazole +125 mg tetracycline on one occasion while fasting, on another occasion after a standardized breakfast. The third regimen consisted of one 375-mg metronidazole capsule (Flagyl® 375; Searle, IL, USA) +three 125-mg tetracycline capsules +one 420-mg bismuth biskalcitrate capsule while fasting.

Participants were healthy males (18–50 years) and nonsmokers or ex-smokers for at least 1 year. Exclusion criteria were: a history of hypersensitivity to bismuth, metronidazole, tetracycline; conditions known to interfere with bioavailability; chronic use of bismuth for 6 months before the study; drug or alcohol dependency; significant illness; use of enzyme-modifying drugs within 28 days before the study; a positive urine or serum test for drugs of abuse; positive HBsAg or anti-HCV tests. Subjects were asked not to take any drugs for at least 7 days prior to and during the study.

Subjects arrived at the study site 58 h prior to drug administration and were assigned to a group by randomization. The study drugs were administered on the third day following admission, and after a supervised overnight fast. Each regimen was 4 weeks apart because of the long half-life of bismuth, and the length of the study was 9 weeks.

For the bismuth assays, 2-ml blood samples were collected eight times for two consecutive days prior to drug administration (baseline) and also prior to and at 0.17, 0.25, 0.33, 0.5, 0.67, 0.84, 1, 1.33, 1.67, 2, 2.5, 3, 4, 6, 8, 12, 24, 48, 72 and 96 h post drug administration. For the metronidazole and tetracycline assays, blood samples (2×7 ml) were collected prior to and at 0.33, 0.67, 1, 1.33, 1.67, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 16, 24, 36, 48 and 72 h post drug administration.

Food and beverages containing grapefruit or xanthines were not allowed for 58 h and 96 h, respectively, prior to and during each period. Water was provided *ad libitum* until 2 h predose and then 2 h after dosing. The drugs were given with 240 ml of water.

During fasting, the subjects did not eat breakfast, but lunch, dinner and snack were provided at 11.00 h, 17.00 h and 20.00 h on the 2 days preceding dosing. On the third day they fasted overnight for at least 10 h before dosing, and standard meals and a snack were served 4, 10 and 13 h later.

In the with food regimen, the subjects received breakfast, lunch, dinner and a snack at 6.30 h, 11.00 h, 17.00 h and 20.00 h on the 2 days preceding dosing. On the third day they fasted overnight for at least 10 h until a standardized high-fat breakfast was served 30 min before drug administration. Standard meals and a snack were served 4, 10 and 13 h later. All laboratory personnel were blinded to formulations and feeding conditions.

Samples were assayed for metronizadole by high-pressure liquid chromatography (HPLC) with ultraviolet detection in the laboratories of Algorithme Pharma (575 boul Armand Frappier, Laval Qc, Canada H7V 4B4; http://www.algopharm.com, contact@algopharm.com). Quality control samples were analysed in duplicate at low (150 ng ml^{-1}), medium (6000 ng ml^{-1}), and high (15000 ng ml^{-1}) concentrations with each analytical batch. Between-run coefficients of variation (precision) ranged from 2.2% to 3.9%. Percent nominal values (accuracy) ranged from 98.4% to 102.0%.

Samples were assayed for tetracycline by HPLC with mass spectrometric (MS) detection in the laboratories of Algorithme Pharma. Quality control samples were analysed in duplicate at low (30 ng ml^{-1}), medium (800 ng ml^{-1}), and high (1700 ng ml^{-1}) concentrations with each analytical batch. Between-run coefficients of variation (precision) ranged from 5.1% to 6.9%. Percent nominal values (accuracy) ranged from 96.8% to 99.0%.

The assays for bismuth used Inductively Coupled Plasma with MS detection and were performed in the laboratories of Cirion Biopharma Research Inc. (230 rue Bernard-Belleau, suite 169, Laval Qc, Canada H7V 4A9; http://www.cirion.ca, info@cirion.ca). Quality control samples were analysed in triplicate at 500, 2500, 17 500 and 28 000 pcg/ml. Between-run coefficients of variation (precision) ranged from 5.7% to 8.3%. Percent nominal values (accuracy) ranged from 96.7% to 105.0%.

The following pharmacokinetic parameters were determined: C_{max}, t_{max}, AUC_t, terminal elimination constant (β), elimination half-life ($t_{1/2}$), and AUC_∞. AUCs and C_{max} were log transformed. Geometric least square (GLS) means, 90% confidence intervals (CIs), mean, standard deviation, and coefficient of variation were calculated and comparisons were made by analysis of variance (ANOVA) or Mann–Whitney tests. The significance was assessed at 5% and corrected when appropriate by Bonferroni tests. Bioequivalence was established by C_{max}, AUC_t, and AUC_∞ if 90% CIs for GLS mean ratios fell within the 80% to 125% acceptance range.

Results

Twenty-three healthy male Caucasians were studied. Their mean plasma concentration *vs.* time profiles for bismuth, metronidazole, and tetracycline under the different conditions are shown in Figures 1–3.

Analysis of blood samples (data not shown) confirmed complete washout of bismuth between periods. There were no significant differences in the pharmacokinetic parameters for bismuth between fasting and fed conditions except for t_{max}, but bioequivalence criteria were not met. The lower limits of the CIs ranged from 12% for C_{max} to 25% for AUC_∞. Comparisons for bismuth between the BMT capsules and the individual drugs given separately in the fasting state did not show any difference for any parameter, but bioequivalence criteria were not met. The lower limits of the CIs ranged from 39% for C_{max} to 50% for AUC_t and higher limit of 146% for AUC_t.

Comparisons between fasting and fed conditions showed significant differences for metronidazole with respect to C_{max}, t_{max}, AUC_t, and AUC_∞. The fasting condition gave values consistently larger than the fed condition except for t_{max}. The lower limit of the CI was 74% for C_{max}, which did not meet the requirements for bioequivalence. Comparisons between the BMT capsule and the drugs given separately while fasting showed no differences in the pharmacokinetic parameters for metronidazole. The 90% CIs on the ratio of means for C_{max}, AUC_t, and AUC_∞, were all within the accepted ranges for bioequivalence.

C_{max}, t_{max}, AUC_t, and AUC_∞ for tetracycline were significantly different in the fasted compared with fed state. The bioequivalence criteria were not met since the lower limits of the CIs ranged from 51% for AUC_t to 59% for C_{max}. Comparisons between the BMT capsule and the individual drugs given separately while fasting showed no difference in the pharmacokinetics of tetracycline, but bioequivalence requirements were not met since the lower limits of the CIs ranges between 72% for AUC_t and 74% for AUC_∞.

Nine participants reported 22 adverse events, 16 of which were deemed related to the medication. One

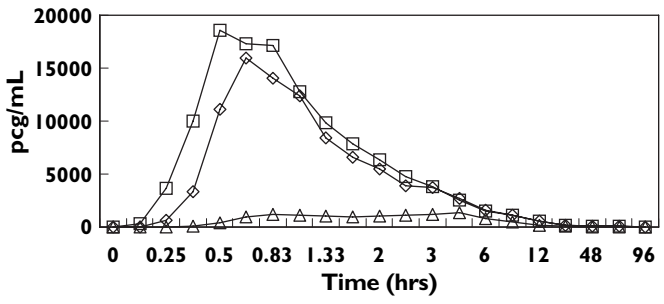

Figure 1

Mean plasma bismuth concentrations *vs.* time following the administration of the combined BMT capsule in the fasting state (◇), after food (△), and when the drugs were given as separate capsules in the fasting state (□)

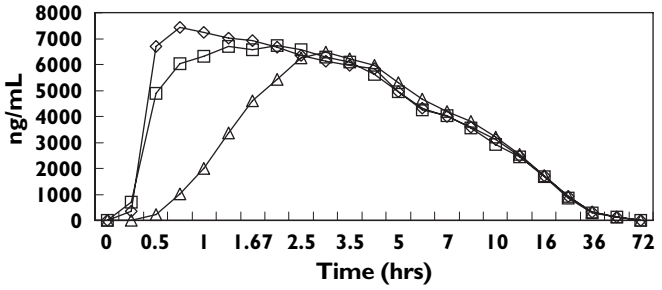

Figure 2

Mean plasma metronidazole concentrations *vs.* time following the administration of the combined BMT capsule in the fasting state (◇), after food (△), and when the drugs were given as separate capsules in the fasting state (□)

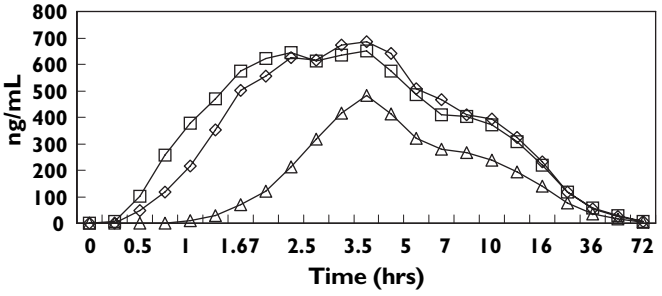

Figure 3

Mean plasma tetracycline concentrations *vs.* time following the administration of the combined BMT capsule in the fasting state (◇), after food (△), and when the drugs were given as separate capsules in the fasting state (□)

occurred during the fasting period and four during the fed period of the BMT capsule. Eleven occurred during the fasting period where the individual drugs were taken. Four events were regarded as severe. Five subjects withdrew from the study: four for reasons unrelated to the medications (phlebitis and pain, bone fracture between study periods, and personal reasons), and one for a reason possibly related to the study medications (worsening of lumbar pain present at onset but not mentioned). All events resolved with time.

Discussion

This study has shown a marked effect of food on the bioavailability of bismuth from the BMT capsule, which resulted in a failure to meet bioequivalence. Given that bismuth is poorly absorbed [1, 2], even a small change in the amount absorbed can largely affect bioequivalence. The observation that the two formulations did not achieve bioequivalence may also be linked to small changes in bioavailability.

The oral absorption of metronidazole is extensive with a bioavailability over 90% [3]. The rate and extent of the absorption of metronidazole from BMT capsules were slightly decreased by food, an observation that is partially in agreement with the product monograph that states that food decreases only the rate but not the extent of absorption of this drug [4]. However, the small magnitude of this decrease supports work indicating that food does not affect the bioavailability of metronidazole [5]. Bioequivalence between the two formulations was observed with respect to metronidazole.

Oral tetracycline has a bioavailability of 60–80% in the fasting state [6, 7]. In the present study significant differences between fasting and fed conditions in C_{max} and AUC but not t_{max} were seen, confirming previous work [8–12]. The effect of formulation may be considered negligible with a difference of less than 10% below the threshold for bioequivalence.

In therapeutic trials, this BMT capsule was administered after meals with eradication rates of *H. pylori* near 90% [13, 16], despite a decreased bioavailability caused by food. The latter is likely to be beneficial since it may be associated with an increased gastric retention time for bismuth, metronidazole and tetracycline, thus prolonging their exposure to *H. pylori* in the gastric mucosa.

In conclusion, food significantly decreases bioavailability of bismuth, metronidazole and tetracycline but their formulation in one capsule had no effect. The administration of the combined BMT capsule given with food has proved to be very effective in the treatment of gastro-duodenal infection by *H. pylori*.

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