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RESEARCH PAPER

Mechanistic Study of the Azithromycin Dosage-Form-Dependent Food Effect

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ABSTRACT

Purpose Azithromycin capsules are known to exhibit a negative food effect, manifest as a decrease in azithromycin bioavailability in the fed state. Azithromycin tablets are known to be bioequivalent to capsules in the fasted state, but do not exhibit a food effect. In the present study, the involvement of gastric degradation of azithromycin to des-cladinose azithromycin (DCA) has been investigated as a possible mechanism for the observed capsule food effect.

Methods Healthy volunteers were dosed with azithromycin tablets and capsules, fasted and fed, in a four-way randomized crossover study. Serum levels of DCA were measured as a function of time post-dose. Natural log-transformed PK parameters were statistically analyzed using an ANOVA model appropriate for the study design.

Results When capsules were dosed to fed subjects, the systemic AUC for DCA was 243% of the value observed after fasted-state dosing, and the DCA C_{max} was 270% of the value observed after fasted-state dosing. When azithromycin tablets were dosed in the fasted and fed states, there was no significant difference in systemic DCA.

Conclusion Gastric degradation of azithromycin to DCA is the likely mechanism for the observed negative food effect observed for azithromycin capsules. This effect is not observed for tablets. These observations suggest that azithromycin capsules exhibit slow and/or delayed disintegration in the fed stomach, resulting in extended gastric residence and degradation of a portion of the gastrically retained azithromycin.

KEY WORDS azithromycin · capsule disintegration · des-cladinose azithromycin · food effect

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INTRODUCTION

Azithromycin is an azalide antibiotic with a broad spectrum of antibacterial efficacy and unusual pharmacokinetics and distribution. The oral bioavailability of azithromycin (based on serum levels) is 37% after a 500 mg oral dose in healthy volunteers, with a peak serum concentration of 0.4 mcg/mL at approximately 2 h after dosing (1). The serum elimination half-life is approximately 69 h (2,3). After oral dosing, the concentrations of azithromycin in a variety of tissues exceed the concentration in serum (1). These unusual pharmacokinetic and distributional characteristics permit convenient dosing regimens: typically 500 mg on day one followed by 250 mg once daily for four more days, or 500 mg once daily for three days (4). For treatment of patients with non-gonococcal urethritis or cervicitis caused by *Chlamydia trachomatis*, a single 1 g dose provides an effective therapeutic regimen. A recently launched controlled release azithromycin formulation, Zmax®, provides therapeutic efficacy without gastrointestinal side effects in a variety of indications after a single 2 gm dose.

Azithromycin is a BCS Class III drug, up to a single dose of ~2.85 gm. The drug is moderately well-absorbed, with a human Maximum Absorbable Dose of ~3.4 gm (5). Studies in portally cannulated cynomolgous monkeys

demonstrated that ~64% of an oral dose was absorbed, followed by ~46% first-pass elimination, giving an oral bioavailability of ~35% (6).

During the development of azithromycin, it was believed that the drug itself had a negative food effect, i.e. administration with food produced a substantial decrease in bioavailability. Consequently, azithromycin labeling indicated that azithromycin should be given at least 1 h before or 2 h after a meal. This belief was based on food-effect studies carried out using azithromycin capsules during Phase II clinical trials, at which time only capsule dosage forms were available. As a result of these studies, Phase III investigations were carried out with capsules in fasted subjects, in order to maximize azithromycin bioavailability and to avoid food-related therapeutic failures. It was subsequently discovered that this food effect was dosage-form-dependent, occurring with capsules but not with tablets, sachets, and suspensions (7–9). This dosage-form dependence suggested that the cause of the food effect with capsules is likely to be preabsorptive. It is known that azithromycin (Fig. 1) loses its cladinose sugar moiety to form des-cladinose azithromycin (DCA) at low pH (10), and this suggested a testable mechanism for the negative food effect seen with capsules.

In the present study, eight young healthy humans were dosed with azithromycin capsules and tablets in the fed and fasted states. Serum levels of DCA were evaluated in order to test the hypothesis that fed state dosing of capsules results in increased gastric conversion of azithromycin to DCA, thus contributing to the food effect observed for capsules but not for tablets.

MATERIALS AND METHODS

An open-label, randomized four-period crossover study was carried out with four single-dose azithromycin treatments: (a) two 250 mg azithromycin capsules following an overnight fast, (b) two 250 mg azithromycin capsules administered after a high-fat breakfast, (c) two 250 mg azithromycin tablets following an overnight fast, and (d) two 250 mg azithromycin tablets administered after a high-fat breakfast. Dosage forms were commercially available Zithromax® capsules and tablets. Eight subjects were studied, with two subjects assigned to each of the four possible sequences, with a two-week washout period between treatments. The protocol for this study was reviewed by the Ohio State Institutional Review Board. Before entry into the study, all subjects read and signed consent forms. Subjects were healthy young males ($N=5$) and females ($N=3$) with a mean age of 27.1 y (range 19 to 44 y) and a mean weight of 72.0 kg (range 55.3 to 94.3 kg).

The standard high-fat breakfast consisted of two eggs fried in one tablespoon of butter, two strips of bacon, 170 g hashbrown potatoes, two pieces of toast with two teaspoons of butter and two pats of jelly, and 227 mL whole milk, ingested within a 20 min period. This breakfast is essentially identical to the example test meal suggested by the US FDA, containing approximately 150, 250, and 500–600 calories from protein, carbohydrate, and fat, respectively (11). Capsule and tablet doses were administered with 240 mL water.

Blood samples were drawn at 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24, and 36 h post-dose. Serum samples were stored at −70°C until assayed for DCA by HPLC with electrochemical detection (BAS Analytics, West Lafayette, IN, USA) using a modification of the method used for azithromycin (12). DCA and the internal standard (9a-N-desmethyl, 9a-N-propargyl-azithromycin) were extracted from serum by liquid–liquid extraction at alkaline pH. After the addition of 0.06 M sodium carbonate solution, the macrolides were extracted into methyl-t-butyl-ether (MTBE). The ether layer was transferred to a clean tube and evaporated to dryness, and the residue was taken up into a 30% acetonitrile/phosphate buffer mixture at pH 6.0. The reconstituted extract was washed with hexane to eliminate late-eluting peaks on the chromatogram. The washed

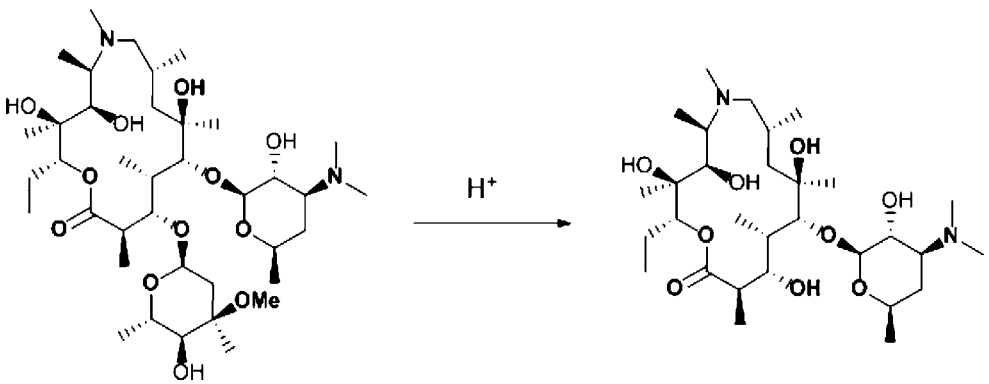

Fig. 1 Azithromycin conversion to des-cladinose-azithromycin (DCA).

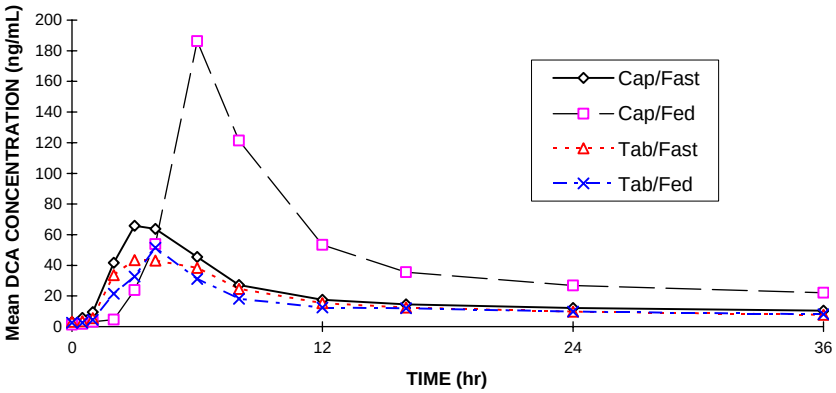

Fig. 2 Des-cladinose azithromycin (DCA) pharmacokinetics after dosing azithromycin in capsules and tablets, fed and fasted (arithmetic mean data).

extract was injected onto an HPLC-EC system (BAS 200 or BAS 201 with dual electrode electrochemical detection) with a hydrocarbon-coated zirconium oxide stationary phase (3 M Zirstar Z18 column—Z-RP Reverse Phase Column, 50 mm×4.6 mm, 7 micron, 100 angstrom—ANSPEC part number L4820) and eluted with a 30% acetonitrile/phosphate buffer mobile phase at pH 9.8. The detector voltages were set at 600 mV and 850 mV.

Standard curves were run in duplicate in seven steps over the range of 3.1 ng/mL to 100 ng/mL. Accuracies of back-calculated concentrations ranged from 97.6% to 102.6% for the 10 assay runs that passed quality control criteria and produced data utilized in the data analysis. Precision was ±8.7% or better. The interday accuracies of the mean values for the 3 quality control concentrations were 108.7% at 7.5 ng/mL, 103.0% at 40 ng/mL, and 103.5% at 80 ng/mL. Samples measured at greater than 100 ng/mL were diluted and reassayed, and standards were similarly diluted and reassayed.

Standard non-compartmental analysis was performed on the serum concentration *vs.* time profiles to determine the following PK parameters. Maximum observed des-cladinose-azithromycin concentrations (C_{max}) were determined by inspection of the data, T_{max} was defined as the time of first occurrence of C_{max}, and area under the serum concentration-*vs.*-time curves (AUC) was calculated for the interval of predose to 36 h by the linear trapezoidal method.

Natural log-transformed AUC and C_{max} were analyzed utilizing an ANOVA model (implemented using Proc Mixed in SAS v8.2) with sequence, period and treatment as fixed effects and subject within sequence as a random effect. Estimates of the adjusted mean differences (Test-Reference) and corresponding 90% confidence intervals were obtained from the model. The adjusted mean differences and 90% confidence intervals for the differences were exponentiated to provide estimates of the ratio (Test/Reference) of adjusted geometric means and 90% confidence intervals for the ratios.

RESULTS

Mean des-cladinose-azithromycin (DCA) pharmacokinetic profiles for the four treatment regimens are presented in Fig. 2, and pharmacokinetic parameters are presented in Tables I, II and III. Fig. 2 clearly demonstrates that dosing azithromycin capsules in the fed state results in higher serum DCA levels than observed when capsules are dosed fasted, or when tablets are dosed fed or fasted.

For capsules, the DCA $AUC_{0\text{-}36}$ in the fed state was 2.4-fold the $AUC_{0\text{-}36}$ in the fasted state (Table I). Similarly, DCA C_{max} after dosing azithromycin capsules in the fed state was 2.7-fold the C_{max} for capsules dosed in the fasted state (Table II). The calculated 90% confidence limits for both AUC and C_{max} ratios did not include 1.0 (100%),

Table I Des-Cladinose-Azithromycin $AUC_{0\text{-}36}$ After Dosing Azithromycin Capsules and Tablets, Fed and Fasted, and $AUC_{0\text{-}36}$ Ratios for Capsule Fed *vs.* Capsule Fasted, Capsule Fed *vs.* Tablet Fed, and Tablet Fed *vs.* Tablet Fasted

Comparison	Adjusted Geometric Means (ng·hr/ml)		Ratio of Means (%)	90% Confidence Limits on Ratio of Means	
	Test	Reference		Lower (%)	Upper (%)
Capsule Fed (test) *vs.* Capsule Fasted (ref)	1,588.14	653.75	242.93	101.84	579.50
Capsule Fed (test) *vs.* Tablet Fed (ref)	1,588.14	302.21	525.50	220.29	1,253.6
Tablet Fed (test) *vs.* Tablet Fasted (ref)	302.21	291.33	103.74	43.49	247.46

Table II Des-Cladinose-Azithromycin C_{max} After Dosing Azithromycin Capsules and Tablets, Fed and Fasted, and C_{max} Ratios for Capsule Fed *vs.* Capsule Fasted, Capsule Fed *vs.* Tablet Fed, and Tablet Fed *vs.* Tablet Fasted

Comparison	Adjusted Geometric Mean (ng/ml)		Ratio of Means (%)	90% Confidence Limits on Ratio of Means	
	Test	Reference		Lower (%)	Upper (%)
Capsule Fed (test) *vs.* Capsule Fasted (ref)	181.31	67.06	270.35	134.94	541.63
Capsule Fed (test) *vs.* Tablet Fed (ref)	181.31	44.32	409.09	204.19	819.60
Tablet Fed (test) *vs.* Tablet Fasted (ref)	44.32	35.69	124.19	61.98	248.80

indicating a significant treatment effect, i.e. a significant fed/fasted effect.

Additionally, when azithromycin capsules were dosed in the fed state, the DCA $AUC_{0\text{-}36}$ was 5.3-fold the DCA $AUC_{0\text{-}36}$ after dosing tablets in the fed state (Table I). DCA C_{max} after dosing azithromycin capsules in the fed state was 4.1-fold the DCA C_{max} after dosing tablets in the fed state (Table II). The calculated 90% confidence limits for both AUC and C_{max} ratios did not include 1.0 (100%), indicating a significant difference between capsules and tablets when dosed fed.

For azithromycin tablets dosed in the fed state, the DCA $AUC_{0\text{-}36}$ was 1.04-fold the value observed after dosing tablets in the fasted state (Table I). Similarly, DCA C_{max} after dosing azithromycin tablets in the fed state was 1.24-fold the C_{max} for tablets dosed in the fasted state (Table II). The calculated 90% confidence limits for both AUC and C_{max} ratios included 1.0 (100%), indicating no treatment effect, i.e. no fed/fasted effect for tablets.

DCA T_{max} for capsules was 6 h in the fed state and 3.5 h in the fasted state. DCA T_{max} for tablets was 3.5 h in both fed and fasted states (Table III).

DISCUSSION

It is well-known that azithromycin capsules exhibit a negative food effect, i.e. provide lower azithromycin bioavailability when dosed in the fed state. The results reported here clearly demonstrate that dosing azithromycin capsules in the fed state results in formation of significantly more of the acid-degradation product DCA than does dosing capsules in the fasted state.

Table III Des-Cladinose-Azithromycin T_{max} After Dosing Azithromycin Capsules and Tablets, Fasted and Fed

Treatment	Median T_{max} (hr) [range]
Capsule Fasted	3.5 [2–4]
Capsule Fed	6 [4–8]
Tablet Fasted	3.5 [2–6]
Tablet Fed	3.5 [2–6]

A published *in vitro* study indicated that azithromycin solution stability is highly pH dependent, with 10% degradation to DCA observed in about 8 minutes at pH 1.2, and in about 175 h at pH 4.2 (10). In addition, we have observed that incubation of azithromycin with pH 1.5 human gastric fluid (or boiled human gastric fluid) results in degradation of azithromycin with a half-life of about 25 min (Foulds and Curatolo, unpublished).

In a study by Dressman *et al.* of the effect of food on human gastric pH, median fasting gastric pH was 1.7, with approximately 50% of the subjects having a gastric pH of 1.2 or lower (13). Upon feeding, median gastric pH rose to 6.7. After feeding, the pH declined to pH 2 over a period which ranged from 8 min to 240 min (median 96 min) for 24 healthy volunteers.

We previously reported a study of azithromycin and DCA intestinal/biliary excretion in ileostomy subjects (14). After dosing azithromycin, recovery of DCA in ileostomy fluid over 24 h post-dose was 13% of dose after fasted oral dosing and 0.5% of dose after IV dosing. These data strongly suggested that significant degradation of azithromycin to DCA, likely due to gastric acid, occurs preabsorptively in orally dosed humans.

Fig. 2 and Table III clearly demonstrate that appearance of DCA in the systemic circulation is delayed in the fed state relative to the fasted state for capsules. A reasonable hypothesis is that the residence time of azithromycin in the capsule-dosed fed stomach is longer than in the fasted stomach, resulting in more extensive acid-induced conversion to DCA. The large increase in DCA T_{max} in the capsule-dosed fed state is somewhat surprising (3.5 h fasted, 6 h fed). In a separate unpublished study, T_{max} for azithromycin after azithromycin capsule dosing was observed to be 2.1 h in the fasted state and 3.8 h in the fed state. Thus, both azithromycin and DCA absorption are delayed in the fed state after capsule dosing, consistent with the idea that the residence time of capsule-dosed azithromycin is longer in the fed stomach. Although on feeding, gastric pH increases to a pH at which azithromycin is stable, the observed fed-state capsule DCA T_{max} of 6 h suggests that a portion of the azithromycin in capsules is retained in the fed stomach for a period of time longer than the post-prandial time needed for the pH to decrease to

basal fasting levels, as reported by Dressman (13). As the fed gastric pH returns to low basal levels, azithromycin in the gastrically retained capsules is degraded by protons which cross the hydrated capsule shell. The slowed or delayed disintegration of azithromycin capsules in the fed state may be related to capsule shell disintegration time, to the possible formation of a slowly disintegrating azithromycin "plug," or to some combination of both.

Published reports demonstrate that food does not affect the bioavailability of commercial 250 mg azithromycin tablets: azithromycin AUCfed/AUCfasted is 0.97, and $C_{max,\ fed}/C_{max,\ fasted}$ is 1.13 (7–9). Thus, azithromycin tablets may be dosed without regard to food. The current study demonstrates that dosing azithromycin tablets in the fed state does not result in formation of additional DCA, relative to fasted-state dosing.

The standard dissolution release test for azithromycin dosage forms (rotating paddles, 100 rpm, 900 mL pH 6.0 phosphate buffer, 37°C) shows >97% azithromycin dissolved in 15 min for both capsules and tablets. In a standard tablet disintegration apparatus, azithromycin tablets completely disintegrate in 2–5 minutes, and capsules in 6–10 minutes, over the pH range 1.2–6.8 (Barbara A. Johnson and Neha Vatsaraj, personal communication). In a preliminary evaluation of the effect of food on azithromycin dosage form disintegration, the inclusion in the disintegration media of milk, Ensure®, or blended FDA breakfast resulted in an increase in disintegration time to 5–12 minutes for tablets and to 11–20 minutes for capsules (Barbara A. Johnson and Neha Vatsaraj, personal communication). While this suggests that food components may have a larger effect on azithromycin capsules than tablets, quantitative extrapolation of this type of data to the *in vivo* situation is difficult, given that the relationship of the agitation in the tablet disintegration apparatus to the agitation in the fed and fasted stomach is unknown.

A published scintigraphy study of *in vivo* capsule disintegration demonstrated that initial release of non-dissolving particles from capsules in the human stomach was slower in the fed state than in the fasted state (93–120 min delay fed *vs.* 30 min delay fasted) (15). Thus, some interaction of the gelatin shell with contents of the fed stomach may be involved in delayed disintegration of capsules in the fed state in general.

Recently, attempts have been made to identify *in vitro* dissolution conditions for prediction of complex dosage form *in vivo* performance. While not relating directly to capsules, it has been reported that the degradation products of dietary triglycerides (more than the dietary triglycerides themselves) can foul thin controlled-release ethylcellulose membranes by partitioning into these membranes and making them more hydrophobic (16). The digestion of dietary materials has been shown to be relevant in *in vitro* assessment of a variety of other dosage-form types (17,18). While the current work reveals a significant effect of food on capsules, previously published work indicates that tablet disintegration may also be delayed by food components (19–21) and that this delay is strongly dependent on tablet formulation (19).

In summary, the loss in azithromycin bioavailability when capsules are dosed in the fed state is at least partially due to gastric degradation of azithromycin to DCA in the fed stomach. This degradation is minimal when tablets are dosed in the fed state, consistent with the published observation that azithromycin tablets do not exhibit a food effect. A reasonable explanation, which is strongly supported by the data presented here, is that commercial azithromycin capsules disintegrate more slowly than tablets in the fed stomach, resulting in increased contact time with gastric acid and increased degradation to DCA. Azithromycin tablets contain a disintegrant (as is almost universally the case for tablets), and these tablets are capable of disintegrating to a powder relatively quickly in the fed or fasted stomach, allowing relatively fast passage of particles through the pylorus to the neutral pH environment of the duodenum, where degradation to DCA will not occur.

The current work cannot distinguish between slow and delayed capsule disintegration in the fed stomach, both of which are consistent with our observations. In the stomach, fed or fasted, the capsule gelatin shell hydrates and becomes permeable to water and protons. Thus, even if the capsule remains relatively intact in the fed stomach for a period of time, acid can enter the capsule and cause degradation of azithromycin to DCA.

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