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RESEARCH PAPER

Effects of Food on a Gastrically Degraded Drug: Azithromycin Fast-Dissolving Gelatin Capsules and HPMC Capsules

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ABSTRACT

Purpose Commercial azithromycin gelatin capsules (Zithromax®) are known to be bioequivalent to commercial azithromycin tablets (Zithromax®) when dosed in the fasted state. These capsules exhibit a reduced bioavailability when dosed in the fed state, while tablets do not. This gelatin capsule negative food effect was previously proposed to be due to slow and/or delayed capsule disintegration in the fed stomach, resulting in extended exposure of the drug to gastric acid, leading to degradation to des-cladinose-azithromycin (DCA). Azithromycin gelatin capsules were formulated with "super-disintegrants" to provide fast-dissolving capsules, and HPMC capsule shells were substituted for gelatin capsule shells, in an effort to eliminate the food effect.

Methods Healthy volunteers were dosed with these dosage forms under fasted and fed conditions; pharmacokinetics were evaluated. DCA pharmacokinetics were also evaluated for the HPMC capsule subjects. *In vitro* disintegration of azithromycin HPMC capsules in media containing food was evaluated and compared with commercial tablets and commercial gelatin capsules.

Result When the two fast-dissolving capsule formulations were dosed to fed subjects, the azithromycin AUC was 38.9% and 52.1% lower than after fasted-state dosing. When HPMC capsules were dosed to fed subjects, the azithromycin AUC was 65.5% lower than after fasted-state dosing. For HPMC capsules, the absolute fasting-state to fed-state decrease in azithromycin AUC (on a molar basis) was similar to the increase in DCA AUC. *In vitro* capsule disintegration studies revealed extended disintegration times for commercial azithromycin gelatin capsules and HPMC capsules in media containing the liquid foods milk and Ensure®.

Conclusion Interaction of azithromycin gelatin and HPMC capsules with food results in slowed disintegration *in vitro* and decreased bioavailability *in vivo*. Concurrent measurement of serum azithromycin and the acid-degradation product DCA demonstrates that the loss of azithromycin bioavailability in the fed state is largely (and probably entirely) due to gastric degradation to DCA. Capsules can provide a useful and elegant dosage form for almost all drugs, but may result in a negative food effect for drugs as acid-labile as azithromycin.

KEY WORDS azithromycin · capsules · des-cladinose-azithromycin · food effect · HPMC capsules

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INTRODUCTION

Azithromycin is a broad-spectrum azalide antibiotic with unique pharmacokinetics, extensive tissue distribution, and a dosage-form-dependent food effect. Azithromycin is moderately well-absorbed, with an absolute bioavailability of 37% in humans (1). Azithromycin's serum elimination half-life is 69 h, and its half-life in many tissues is similar (2,3), permitting short dosing regimens, *e.g.* once per day for three days or a single-dose regimen (4). Azithromycin is a Biopharmaceutics Classification System (BCS) Class III drug, up to a dose of 2.85 g, and possesses a high predicted Maximum Absorbable Dose of ~3.4 g, due to high solubility (5).

Azithromycin capsules exhibit a negative food effect, *i.e.* a significant decrease in oral bioavailability when dosed with food (6–8). This food effect is not observed when azithromycin is dosed in tablets, sachets, or suspensions (6–8).

A recently reported mechanistic study demonstrated that this capsule food effect is at least partially due to gastric degradation of the drug to des-cladinose azithromycin (DCA) (Fig. 1) (9). *In vitro*, azithromycin's solution stability is highly pH dependent, with 10% degradation to DCA observed in about 8 min at pH 1.2, and in about 175 h at pH 4.2 (10). Furthermore, 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 (G. Foulds and W. Curatolo, unpublished). When healthy human subjects were dosed with commercial azithromycin gelatin capsules (Zithromax®) in the fed and fasted states, the fed-state serum DCA AUC was 243% of the fasted state value (9). The fed-state serum DCA Cmax was 270% of the fasted-state value. This suggested that the azithromycin capsule food effect, in large part, is the result of interaction with food or digested food components with the gelatin capsule shell and/or fill, resulting in slow or delayed disintegration in the stomach and consequent acid degradation to DCA.

In the present work, we set out to eliminate the capsule food effect with two approaches:

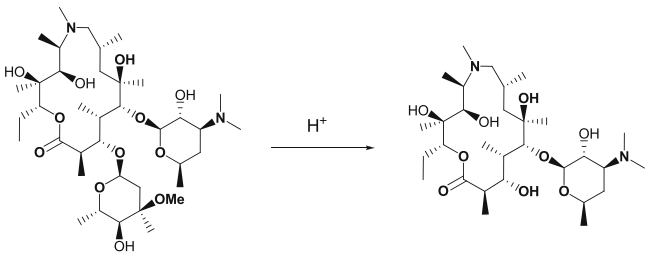

Fig. 1 Azithromycin conversion to des-cladinose-azithromycin (*DCA*).

(a) formulate gelatin capsules with "superdisintegrants" to obtain fast-dissolving capsules which might behave *in vivo* more like azithromycin tablets, which do not exhibit a food effect, and
(b) formulate azithromycin in cellulose-based capsule shells, which may interact with food and food digestion products differently than gelatin capsules.

We also set out to verify the previously proposed mechanism for the azithromycin capsule food effect by measuring both serum azithromycin and serum DCA in the same human PK study.

MATERIALS AND METHODS

All formulation components were pharmaceutical or NF grade. Azithromycin dihydrate was manufactured by Pfizer. Gelatin capsules were obtained from Capsugel, Inc. Hydroxypropylmethylcellulose (HPMC) capsules (containing carrageenan as a gelling agent) were obtained from Shionogi Qualicaps, Inc. Commercial Zithromax® (gelatin) capsules were obtained from Pfizer. Commercial Zithromax® tablets were obtained from Pfizer.

Fast-dissolving gelatin capsule formulations and the HPMC capsule formulation were prepared under GMP conditions at the Pfizer Groton Solid Dosage Manufacturing Facility. The fast-dissolving gelatin capsule formulations (Formulations A and B) were prepared using dry granulation as follows. Drug, lactose, and superdisintegrant were blended, milled, and reblended. A portion of the lubricant (90/10 magnesium stearate/sodium lauryl sulfate) was added, followed by blending. This material was roller-compacted, milled, and then blended with the remainder of the lubricant. This final granulation was encapsulated in Size #0 red opaque hard gelatin capsules.

HPMC capsules were prepared using the same fill formulation as commercial Zithromax® azithromycin capsules. The fill formulation was dry granulated using roller compaction, as described above.

Dissolution Method

In vitro dissolution of various capsule formulations was carried out in a USP-2 dissolution apparatus in 900 ml 0.1 M dibasic sodium phosphate buffer, pH 6.0, at 37°C, with paddles turning at 100 rpm. This is the standard azithromycin capsule dissolution method (11). Azithromycin concentration in dissolution samples was determined by HPLC, utilizing electrochemical detection, as previously described (8). Note that carrying out dissolution tests at gastric pH is problematic for azithromycin, due to rapid degradation to DCA (10).

Disintegration Method

In vitro tablet and capsule disintegration studies were carried out using a PharmaTest Model PTZ-Auto-2 tablet disintegration apparatus, without discs, at 37°C, in 800 ml media. The recorded disintegration time is the time at which the tablet or capsule is visually observed to be completely disintegrated, even if some undissolved pieces of capsule shell remain. While the USP <701> Disintegration Test suggests using 1000 ml medium, 800 ml was chosen to permit visualization of capsule disintegration as the capsule is lifted above the surface of the liquid medium, which is particularly important for opaque media containing milk or Ensure®. Simulated Gastric Fluid (SGF), pH 1.2, is USP SGF, containing NaCl, HCl, and pepsin. pH 5 buffer is 0.1 M sodium acetate buffer. pH 6.8 buffer is 0.1 M sodium phosphate buffer. Whole Milk/SGF media consisted of 250 ml milk diluted to 800 ml with SGF (final pH ~2). Ensure® "Ready-To-Drink Homemade Vanilla" liquid nutritional supplement was from Abbott Labs (lot 06498RNO EV). One 8-fluid-ounce (237 ml) can of Ensure® contains 6 g total fat, 0.5 g saturated fat, <5 mg cholesterol, 9 g protein, 200 mg sodium, 370 mg potassium, 40 g total carbohydrates, 18 g sugar. Ensure®/SGF media consisted of 237 ml Ensure® diluted to 800 ml with SGF (final pH ~2.6). Ensure®/pH 5.6 buffer media consisted of 237 ml Ensure® diluted to 800 ml with acetate buffer (final pH ~5.6).

Study Design of Food Effect Studies in Healthy Subjects

Each formulation was studied in a separate open-label, randomized two-period crossover study with a washout period of 14–16 days between treatments. For the fast-dissolving gelatin capsule formulation A (Study A, $n=12$) and formulation B (Study B, $n=12$), the study design was the same: twelve healthy subjects received a single oral dose of 500 mg (2×250 mg) azithromycin fast-dissolving capsules either under a fasted condition or immediately following a high-fat meal. In each dosing period, serum samples were collected at time 0 (just prior to dosing) and at 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, 24, 48, 72, and 96 h post-dose for azithromycin measurement. For the HPMC capsules (Study C, $n=23$), the study design was similar with the following differences. Serum samples were collected at time 0 (just prior to dosing), and at 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 16, 24, 36, 48, and 72 h post-dose for azithromycin measurement; in addition, serum samples were also collected at the same time points up to 36 h for DCA measurement.

The high-fat meal 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 is essentially the Food and Drug Administration (FDA) recommended high-fat meal (containing approximately 150 kcal protein, 250 kcal carbohydrate and 500–600 kcal fat) (12). All the azithromycin doses were administered with 240 mL water.

For all studies, subjects signed informed consent forms before the start of each study and were confined in the clinical research unit for at least 24 h following dosing. All study protocols were approved by the local Institutional Review Board (IRB) (Study A: Pharmaco LSR Inc., Austin Texas; Study B: Drug Evaluation Unit, Hennepin County Medical Center, Minneapolis, Minnesota; Study C: Comprehensive Phase One, Ft. Lauderdale, Florida). All three studies were conducted according to the Declaration of Helsinki and with local laws and regulations relevant to the use of new therapeutic agents in the United States.

Sample Size Determination in Food Effect Studies

Since studies A and B were pilot studies, the sample size of 12 subjects was chosen empirically to estimate the relative oral bioavailability of a single 500 mg dose of azithromycin capsule formulation A and B when administered with a standard high-fat meal. Study C was designed to evaluate the effect of a standard high-fat meal on the pharmacokinetics of a single 500 mg dose of azithromycin HPMC capsule compared to a fasted state. Using a historical intra-subject variability of 17% for AUC_{0-72} from a previous azithromycin capsule study (data on file), 20 subjects would provide 90% power to demonstrate that the mean ratio of the fed state to the fasted state is within the standard bioequivalence interval (80%, 125%) (assuming the true ratio is 0.95). This calculation was done using nQuery Advisor v. 4.0. Twenty-three (23) subjects were enrolled in study C.

Pharmacokinetic Assessments for Azithromycin and DCA

Serum samples were analyzed for azithromycin and DCA concentrations at BAS Analytics (West Lafayette, IN) using liquid-liquid extraction followed by liquid chromatography with electrochemical detection methods, which have been reported previously (9,13). The dynamic range of the assay was 0.0104 to 1.000 μg/ml for azithromycin, and the dynamic range of the assay was 0.00313 to 0.100 μg/ml for DCA. Serum azithromycin and DCA concentrations that were below the lower limit of quantitation (BLLQ) were reported as 0 ng/ml for pharmacokinetic analysis.

Pharmacokinetic analyses were carried out using WinNonlin V.3.2 (Pharsight®, Mountain View, CA) using

standard non-compartmental methods. Maximum observed serum azithromycin concentrations (Cmax) were estimated directly from the experimental data. Tmax was defined as the time to the first occurrence of Cmax, which was also estimated directly from the observed data. Area under the serum concentration-time curve from time 0 to 72 h post-dose ($AUC_{0–72}$) and from time 0 to the time of the last quantifiable concentration ($AUC_{0\text{-}Tlast}$) were estimated using the log/linear trapezoidal rule. In studies A and B, truncated $AUC_{0–72}$ was used as the primary endpoint for azithromycin comparison according to the FDA draft guidance on bioequivalence studies for orally administered drug products with a long elimination half-life (14). In study C, since the azithromycin concentrations at 72 h in many subjects were BLLQ, $AUC_{0\text{-}Tlast}$ was used for statistical analyses; similarly, for DCA, $AUC_{0\text{-}Tlast}$ was calculated instead of $AUC_{0–36}$ due to undetectable levels at 36 h. Arithmetic means are reported for all pharmacokinetic parameters except Tmax, for which the median is reported.

For statistical analyses, natural log-transformed azithromycin $AUC_{0\text{-}Tlast}$, $AUC_{0–72}$, and Cmax were analyzed using a mixed effects ANOVA model with a SAS Proc Mixed procedure (SAS Institute Inc., Cary, NC). The sequence, treatment and period were considered fixed effects, and subjects (within sequence) were considered a random effect. The point estimates of the adjusted mean differences (test–reference) and corresponding 90% confidence intervals (CIs) around the differences were calculated. The differences and 90% CIs for the differences were anti-log transformed to derive estimates of the ratios of the adjusted geometric means (test/reference) and the corresponding 90% CIs for these ratios. The fasted condition served as the reference. The absence of a food effect was established if the 90% CIs on the geometric ratios for $AUC_{0\text{-}Tlast}$ or $AUC_{0–72}$ and Cmax were contained in the acceptance interval (80%, 125%).

RESULTS

Formulations

The commercial Zithromax® capsule formulation (Table I) contains the mild disintegrant corn starch. In an attempt to achieve faster dissolving formulations, a variety of capsule formulations were evaluated, and those which exhibited the fastest *in vitro* release were chosen for food effect assessment. Formulation A (Table I) substitutes the "superdisintegrant" sodium starch glycolate (Explotab®) for corn starch. Formulation B (Table I) substitutes the "superdisintegrant" croscarmellose sodium (Ac-Di-Sol®) for corn starch.

In vitro capsule dissolution is presented in Table II. The fast-dissolving formulations differ from the commercial Zithromax® capsule at the 5 min time point. Formulations A and B released about 39–62% and 63–71% of their azithromycin at 5 min, respectively, while the commercial formulation releases about 17–29%.

The azithromycin formulation in HPMC capsule shells is identical to the commercial gelatin capsule formulation (Table I). This HPMC capsule formulation dissolves *in vitro* similarly to the commercial gelatin capsule formulation (Table II). For the HPMC capsule, timepoints before 15 min were not evaluated because the standard release test for azithromycin capsules has 15 min as the first timepoint, and the intent of the HPMC formulation was to directly replace HPMC for gelatin, with the commercial fill formulation.

Pharmacokinetic Studies

Study A evaluated the effect of a high-fat meal on the absorption of 500 mg azithromycin fast-dissolving gelatin capsule Formulation A (2×250 mg) in 12 subjects. The high-fat meal significantly decreased the rate and extent of absorption of azithromycin from Formulation A as

Table I Azithromycin Capsule Formulations

Component	Commercial Zithromax® gelatin capsule (mg)	Fast-dissolving gelatin capsule formulation A (mg)	Fast-dissolving gelatin capsule formulation B (mg)	HPMC capsule formulation (mg)
Azithromycin dihydrate	262.05	262.05	262.05	262.05
Lactose, anhydrous	151.55	170.35	179.75	151.55
Corn starch, hydrous	47.0	0	0	47.0
Sodium starch glycolate (Explotab®)	0	28.20	0	0
Croscarmellose sodium (Ac-Di-Sol®)	0	0	18.80	0
Magnesium stearate/sodium auryl sulfate (90/10)	9.40	9.40	9.40	9.40
Total	470.0	470.0	470.0	470.0

Table II *In vitro* Dissolution of Azithromycin Capsule Formulations (average of 6 capsules; range in parentheses; details in Materials and Methods)

Formulation	% dissolved				
	5 min	10 min	15 min	30 min	60 min
Commercial Zithromax® Gelatin Capsule	21 (17–29)	87 (75–99)	100 (98–102)	100 (98–102)	100 (99–101)
Fast-Dissolving Gelatin Capsule Formulation A	52 (39–62)	101 (96–103)	104 (103–105)	103 (100–105)	ND
Fast-Dissolving Gelatin Capsule Formulation B	67 (63–71)	101 (100–102)	103 (101–105)	101 (99–104)	ND
HPMC Capsule	ND	ND	98 (88–102)	101 (99–102)	101 (99–102)

ND not done

evidenced by 48.3% decrease in mean Cmax and 38.9% decrease in mean $AUC_{0–72}$ (Fig. 2, Table III). The Tmax was prolonged (~ 2 h later). The 90% CI for the Cmax and $AUC_{0–72}$ ratios fell out of the 80%–125% acceptance interval for bioequivalence, indicating a significant food effect. Intra-subject variability for Cmax and $AUC_{0–72}$ were 43% and 25%, respectively.

Study B evaluated the effect of a high-fat meal on the absorption of 500 mg azithromycin fast-dissolving gelatin capsule Formulation B (2×250 mg) in 12 subjects. The high-fat meal significantly decreased the rate and extent of absorption of azithromycin from Formulation B as evidenced by 49.2% decrease in mean Cmax and 52.1% decrease in mean $AUC_{0–72}$ (Fig. 2, Table III). The Tmax was prolonged (~ 2 h later). The 90% CI for the Cmax and $AUC_{0–72}$ ratios fell out of the 80%–125% acceptance interval for bioequivalence, indicating a significant food effect. Intra-subject variability for Cmax and $AUC_{0–72}$ were 34% and 26%, respectively.

Study C evaluated the effect of a high-fat meal on the absorption of 500 mg azithromycin HPMC capsules (2× 250 mg) in 23 subjects. The high-fat meal also significantly decreased the rate and extent of absorption of azithromycin from HPMC capsules as evidenced by 53.0% decrease in mean Cmax and 65.5% decrease in mean $AUC_{0\text{-Tlast}}$ (Fig. 3, Table III). The Tmax was prolonged (~ 2 h later). The 90% CI for the Cmax and $AUC_{0\text{-Tlast}}$ ratios fell out of the 80%–125% acceptance interval for bioequivalence, indicating a significant food effect. Intra-subject variability for Cmax and $AUC_{0\text{-Tlast}}$ were 60% and 88%, respectively.

In Study C, serum concentrations of the acid-degradation product DCA were also measured, and Fig. 3 presents mean pharmacokinetic profiles for DCA. While azithromycin systemic exposure decreased with food, DCA systemic exposure increased (Fig. 3, Table III). In the fed state, the median DCA Tmax was also prolonged by 2 h (Table III).

In Vitro Dosage Form Disintegration

To probe the interaction of food with azithromycin dosage forms, disintegration of commercial Zithromax® gelatin capsules, commercial Zithromax® tablets, and HPMC capsules was studied in a variety of dissolution media (Table IV). In SGF, the capsule formulations disintegrated more slowly than the tablet formulation, but all were within the generally accepted bounds for an immediate release dosage form. Azithromycin tablets, which do not exhibit a

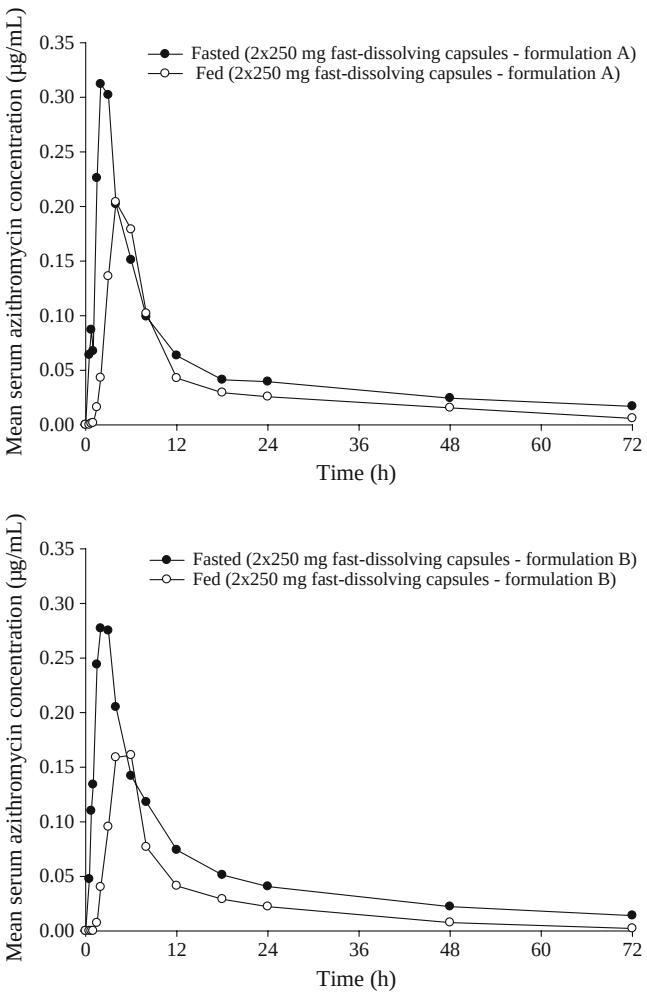

Fig. 2 Mean serum azithromycin concentration-time profiles (72 h) following a single 500 mg dose of azithromycin fast-dissolving gelatin capsules [upper panel—formulation A (*study A*), lower panel—formulation B (*study B*)] under fasted and fed conditions.

Table III Summary of Statistical Analysis of Azithromycin and DCA Pharmacokinetic Parameters Following Administration of Single 500 mg (2 × 250 mg) Doses of Azithromycin Capsules in Food Effect Studies A, B and C

Pharmacokinetic parameter	Arithmetic mean (SD)		Geometric mean ratio (%) (90% CI)[d]
Study A (fast-dissolving gelatin capsule formulation A, $N = 12$)			
Azithromycin	Fasted	Fed	
$AUC_{0\text{-}Tlast}$ (μg·h/mL)	3.76 (0.84)	2.32 (1.39)	55.19 (43.48, 70.06)
$AUC_{0–72}$ (μg·h/mL)	3.51 (0.69)	2.32 (1.15)	61.10 (50.62, 73.76)
C_{max} (μg/mL)	0.476 (0.17)	0.251 (0.11)	51.71 (37.70, 70.91)
T_{max} (h)[a]	2.0 (1.5–6.0)	4.0 (3.0–8.0)	–
Study B (fast-dissolving gelatin capsule formulation B, $N = 12$)			
Azithromycin	Fasted	Fed	
$AUC_{0\text{-}Tlast}$ (μg·h/mL)	3.78 (0.95)	1.69 (0.86)	41.32 (32.83, 52.01)
$AUC_{0–72}$ (μg·h/mL)	3.58 (0.80)	1.81 (0.75)	47.85 (39.56, 57.89)
C_{max} (μg/mL)	0.395 (0.13)	0.206 (0.08)	50.75 (39.50, 65.19)
T_{max} (h)[a]	2.0 (1.0–3.0)	4.0 (2.0–6.0)	–
Study C (HPMC capsule, $N = 23$)			
Azithromycin	Fasted	Fed	
$AUC_{0\text{-}Tlast}$ (μg·h/mL)[b]	3.37 (1.50)	1.47 (1.05)	34.50 (22.11, 53.82)
C_{max} (μg/mL)	0.455 (0.23)	0.232 (0.12)	46.95 (34.66, 63.60)
T_{max} (h)[a]	3.0 (1.0–5.0)	5.0 (3.0–8.0)	–
DCA	Fasted	Fed	
$AUC_{0\text{-}Tlast}$ (μg·h/mL)[c]	0.638 (0.57)	1.26 (0.61)	–
C_{max} (μg/mL)	0.127 (0.13)	0.261 (0.15)	–
T_{max} (h)[a]	3.0 (1.0–5.0)	5.0 (3.0–8.0)	–

DCA des-cladinose-azithromycin, *SD* standard deviation, *CI* confidence interval, *BLLQ* below the lower limit of quantification

[a] Median (range)

[b] The azithromycin concentrations at the last collection time point (72 h) were BLLQ in 16 subjects

[c] The DCA concentrations at the last collection time point (36 h) were BLLQ in six subjects

[d] Ratio = fed/fasted

food effect *in vivo*, disintegrated more slowly in the presence of food components (Ensure®, whole milk), but in all studied conditions disintegration time was under 9 min. Commercial Zithromax® gelatin capsules, which exhibit a food effect *in vivo*, exhibited delayed *in vitro* disintegration in media containing food components. HPMC capsules, which were shown to exhibit a food effect *in vivo* in the current work, exhibit the most delayed *in vitro* disintegration of the three tested dosage forms in the presence of food components, with disintegration times greater than 20 min under the conditions of the *in vitro* test. The effect of food on *in vitro* disintegration of fast-dissolving gelatin capsules was not studied.

DISCUSSION

Early in the development of azithromycin, a negative food effect was observed for azithromycin capsules, which provided the only dosage form available at that time. The observed decrease in bioavailability in the fed state was thought to be an intrinsic property of the drug, and in order to avoid food-related therapeutic failures, Phase III efficacy studies were carried out with the admonition to take the drug one hour before or two hours after a meal. When the capsules were launched after marketing approval by FDA and other agencies, the package label included this admonition. Later, tablet, sachet and pediatric suspension dosage forms were developed and were surprisingly found to exhibit no food effect (6–8).

Previously, a mechanistic pharmacokinetic study of commercial azithromycin gelatin capsules (Zithromax®) demonstrated increased serum levels of the acid-degradation product DCA after fed-state dosing (9). It was proposed that, in the fed stomach, azithromycin capsules undergo slow and/or delayed disintegration, resulting in acid-degradation of azithromycin retained in the capsules in the stomach as the post-prandial pH increases then decreases. A quantitative comparison of systemic azithromycin loss and systemic DCA increase was not possible due to study design. In that study, an increase in serum DCA was not observed when commercial azithromycin tablets (Zithromax®) were dosed to fed subjects, relative to fasted-state dosing (9). The lack of an azithromycin food effect and lack of fed-state serum DCA increase for tablets likely result from fast disintegration of tablets and subsequent movement of azithromycin into the duodenum, where the pH is such that the drug is chemically stable in solution. The lack of a food effect for the pediatric suspension and for a sachet dosage form can be similarly explained since these dosage forms are essentially "predisintegrated" at dosing.

In Studies A and B, azithromycin gelatin capsules were formulated with the goal of decreasing capsule dissolution time in order to achieve capsule behavior like that of tablets

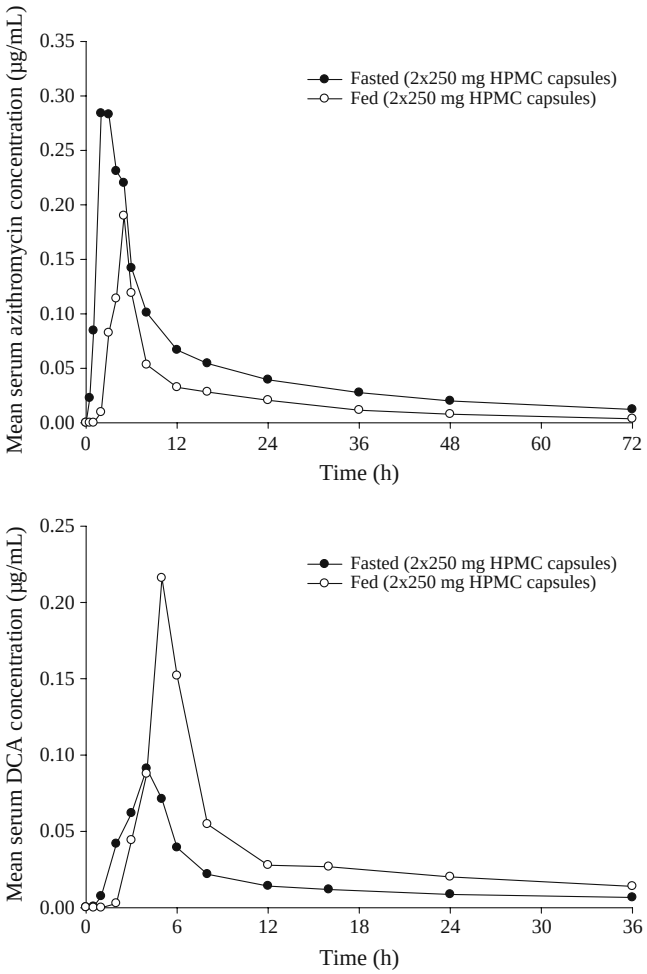

Fig. 3 Mean serum azithromycin (*upper panel*) and DCA (*lower panel*) concentration-time profiles following a single 500 mg dose of azithromycin HPMC capsules (*study C*) under fasted and fed conditions.

in vivo. The fast-dissolving gelatin capsule formulations A and B exhibited 52% and 67% mean *in vitro* dissolution at 5 min, respectively, compared to 21% for commercial Zithromax® gelatin capsules. The commercial Zithromax® capsules are certainly adequate immediate release capsules, with 100% dissolution in 15 min under standard conditions (Table II). Furthermore, commercial Zithromax® capsules have been shown to be bioequivalent to commercial Zithromax® tablets, *when dosed in the fasted state*. It must be recognized that capsule formulation can be complicated, and dissolution rate can be affected by a variety of capsule process parameters, in addition to capsule formulation. For example, it has been demonstrated that the number and force of tamping steps on a commercial capsule filling machine can affect the dissolution rate of the manufactured capsules, for a low-dose poorly soluble drug (29). However, this effect is not observed for formulations containing high disintegrant levels (29) equivalent to those in our fast-dissolving azithromycin capsules. Furthermore, azithromycin is a high-dose, high-solubility drug. It is our opinion that the fast-dissolving formulations A and B represent the practical limit in azithromycin capsule dissolution rapidity, *i.e.* it is unlikely that significantly faster-dissolving azithromycin capsules can be formulated. If it were desired to continue to attempt to formulate azithromycin capsules without a food effect, a reasonable approach would be to screen prototype formulations in the milk-containing and Ensure®-containing disintegration tests described above.

Fast-dissolving capsule formulations A and B exhibited large food effects (Fig. 2, Table III), demonstrating that faster *in vitro* dissolution did not result in *in vivo* food effect behavior similar to tablets. The inescapable conclusion is that *in vivo* fed-state capsule disintegration and/or dissolution for these "fast-dissolving" gelatin capsules is significantly slower than observed in the standard *in vitro* dissolution test. This is supported by the observed increased Tmax in the fed state. Surprisingly, the magnitude of the food effect observed for the fast-dissolving gelatin capsule formulations A and B (38.9% and 52.1% decrease, respectively) is larger than that reported for commercial Zithromax® capsules (20% decrease (7)).

HPMC has been proposed as an alternative to gelatin for use as a capsule shell material (15). In evaluating reports on HPMC capsules, care must be taken to note that some HPMC capsule shells contain carageenan as a gelling agent

Table IV *In Vitro* Disintegration Times for Commercial Azithromycin Gelatin Capsules (Zithromax®), Commercial Azithromycin Tablets (Zithromax®), and Azithromycin HPMC Capsules, in Various Media (details in Materials and Methods)

Medium	Disintegration time (min)[a]		
	Tablet	Gelatin Capsule	HPMC Capsule
Water	3.6 (0.63) [6]	10.6 (0.88) [3]	11.7 (1.9) [3]
SGF, pH 1.2	2.8 (0.65) [6]	6.6 (0.61) [6]	11.2 (0.56) [3]
pH 5 buffer	3.1 (0.56) [6]	9.4 (0.76) [6]	11.5 (2.2) [3]
pH 6.8 buffer	4.6 (0.26) [3]	10.2 (1.4) [3]	11.7 (1.4) [3]
Whole milk	7.7 (0.49) [6]	12.3 (1.3) [6]	25.0 (3.2) [6]
Whole milk/SGF	5.1 (1.0) [3]	12.9 (4.3) [3]	22.2 (1.8) [3]
Ensure/SGF	5.4 (0.23) [3]	14.8 (3.7) [3]	20.0 (2.6) [3]
Ensure/pH 5.6 buffer	8.0 (0.41) [3]	16.8 (1.7) [3]	26.1 (1.3) [3]

[a] Standard deviation in parentheses; number of replicates tested in brackets

necessary for manufacture, and some use gellan gum as a gelling agent; both also utilize potassium salts to set the gel. These two types of HPMC capsules may differ in their performance, both *in vitro* and *in vivo*, because of the differences between these gelling agents with respect to hydration and dissolution in various dissolution media (16). Honkanen *et al.* reported that orally administered HPMC (carageenan) capsules were bioequivalent to gelatin capsules for the BCS Class II drug ibuprofen, when dosed to fasted humans (17). Honkanen *et al.* also reported similar fasted state bioequivalence when the drug was the BCS Class III drug metoclopramide (18). Cole *et al.* reported that orally administered ibuprofen HPMC (gellan) capsules were bioequivalent to ibuprofen gelatin capsules when dosed in the fasted state, or when dosed in the fed state, but fed bioavailability was lower (16).

In Study C, replacement of the gelatin capsule shell with the carbohydrate-based HPMC (carageenan) capsule shell did not ameliorate the food effect (Fig. 3, Table III). In this study, the food effect mechanism was probed by measuring serum concentrations of the acid degradation product DCA. Using a MW of 749 g/mole for azithromycin and 574 g/mole for DCA, the mean $AUC_{0\text{-Tlast}}$ fed-fasted difference values were converted to nmole·hr/ml. The loss of azithromycin exposure due to fed-state dosing was 2.52 nmole·hr/ml, and the increase in DCA exposure due to fed state dosing was 1.09 nmole·hr/ml. While the subtleties of the absorption and distribution of DCA are not known, it is clear that the fed-state loss of azithromycin exposure and the fed-state gain in DCA exposure are of the same order of magnitude. Thus, the loss of azithromycin bioavailability in the fed state is largely due (and probably entirely due) to acid degradation to DCA. A reasonable proposal is that in the fed stomach (relative to the fasted stomach), disintegration of azithromycin HPMC capsules is slowed and/or delayed, resulting in exposure of the gastrically retained azithromycin to gastric acid, resulting in degradation to DCA. While gastric pH has been shown to increase with food intake to a pH at which azithromycin is stable (~ pH 6.7), the gastric pH subsequently declines to pH 2, a pH at which azithromycin is unstable, over a period ranging from 8 min to 240 min (median 96 min) for 24 healthy volunteers (19). If the capsule shell remains fully or partially intact for a period of time in the fed stomach, acid degradation can still occur, because the hydrated HPMC/carrageenan shell is certainly permeable to protons. The observed fed-state median Tmax of 5 h (300 min) for azithromycin and DCA (Table III) is consistent with this proposal.

The proposed increased disintegration time for azithromycin gelatin and HPMC capsules in the fed stomach is consistent with published scintigraphy studies of *in vivo* capsule disintegration. Casey *et al.* demonstrated that initial release of non-dissolving particles from gelatin capsules in the human stomach was slower in the fed state (93–120 min delay) than in the fasted state (30 min delay) (20). Cole *et al.* reported that gelatin capsules disintegrated more slowly in the fed stomach, and HPMC (gellan) capsules disintegrated more slowly than gelatin capsules in both fed and fasted states (16). The *in vivo* disintegration time for HPMC (gellan) capsules was quite long in the fed state, with initial disintegration in 1.00 ± 0.37 h and complete disintegration in 1.61 ± 0.65 h (16). Given these observations, it is not surprising that some drugs exhibit food effects when dosed in gelatin or HPMC capsules. However, if food causes only a delay in disintegration, it is likely that for many or most drugs, only a shift in Tmax will occur, which does not cause formal bioinequivalence. For chronic drugs, this is generally not a serious issue.

Mechanistically, it is reasonable to propose that food or food digestion products interact with the capsule shell in the stomach, resulting in delayed disintegration. These food components may also affect the disintegration of the azithromycin/excipient plug. Both effects were visually observed during the *in vitro* disintegration tests, and it is difficult to assign the relative magnitude of each. The *in vitro* disintegration data in Table IV demonstrate that food components in milk and in Ensure® cause increased disintegration time for azithromycin tablets, gelatin capsules, and HPMC capsules. The disintegration time in each medium is rank ordered HPMC > gelatin > tablets. This is the same rank order as the magnitude of the food effect for these dosage forms. The fed state azithromycin AUC, relative to the fasted state AUC, is decreased 65.5% for HPMC capsules (Study C), is decreased 20% for commercial Zithromax® gelatin capsules (7), and is bioequivalent for commercial Zithromax® tablets (7). The increased *in vitro* disintegration times in the presence of food components are particularly large for azithromycin HPMC capsules, which exhibited disintegration times greater than 20 min in the presence of whole milk or Ensure®. It is important to note that quantitative *in vitro/in vivo* comparisons are inappropriate because the relationship of the agitation in the *in vitro* disintegration apparatus to the agitation in the fed and fasted stomach is unknown. The effect of food components on *in vitro* dosage form disintegration and drug dissolution is an active research field, and many examples of such effects have been reported (21–28).

In conclusion, gelatin and HPMC capsules are elegant dosage forms with many advantages, particularly ease of identification of dose strengths via coloring. Furthermore, HPMC capsules provide the advantages of low moisture content, resistance to crosslinking, and mechanical integrity under low moisture conditions (30). However, the current work suggests that gelatin and HPMC capsule

formulations of gastrically degraded drugs may exhibit a significant bioavailability decrease when dosed with high fat meals.

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