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EXPERT REVIEW

Interdisciplinary Science and the Design of a Single-Dose Antibiotic Therapy

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ABSTRACT Azithromycin is a unique antibiotic due to its serum half-life of 69 h. This half-life is long enough to permit administration of an entire course of therapy in a single dose, if the gastrointestinal (GI) side effects of such a high dose can be minimized. A series of exploratory clinical pharmacology studies were carried out to understand the site-specific absorption and toleration constraints involved in delivering a 2 g oral single-dose regimen. These studies demonstrated that (a) GI side effects were locally mediated in the GI tract, (b) the duodenum was more sensitive than the ileocecal region, and (c) colonic absorption was limited. A novel controlled release suspension dosage form was designed to meet these constraints, and was shown to deliver the desired systemic dose with acceptable toleration. This dosage form, Zmax®, is an oral powder-for-constitution which possesses two major features: (a) 200 μm controlled release microspheres which release the drug as they transit down the small intestine, and (b) alkalizing agents which raise the pH of the gastric milieu for ~20 min to minimize gastric release of the drug (which has high solubility at low pH), in order to minimize exposure of the drug to the sensitive duodenal region. The ability to provide a high single dose of azithromycin results in "front-loading" the mononuclear and polymorphonuclear leukocytes which concentrate the drug and carry it to sites of infection. This provides high drug concentrations early on at infection sites, when the bacterial burden is greatest, potentially improving efficacy and potentially overcoming resistant bacterial strains. Finally, this revolutionary single dose formulation gives 100% compliance, which maximizes the likelihood of therapeutic success.

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BACKGROUND

Azithromycin is an azalide antibiotic with a broad spectrum of activity against a variety of gram-positive and gram-negative organisms (Fig. 1), and the clinical indications for this exceptional antibiotic have been widely reviewed (1,2). Due to azithromycin's pharmacokinetic and distributional characteristics, it is a highly unusual drug. This azalide antibiotic exhibits a serum half-life of 69 h and partitions into tissues where its half-life is similar (3–5). The drug partitions exceptionally well into phagocytes, resulting in delivery of the drug to sites of infection (6–9). As a result of its unusual pharmacokinetic properties, convenient and effective oral dosing regimens are enabled. For example, common once-daily dosing regimens are 500 mg on day one followed by 500 mg on days two and three, or followed by 250 mg on days two through five (10). For non-gonococcal urethritis or cervicitis caused by *Chlamydia trachomatis*, a single 1 g dose is effective. A recently approved controlled release suspension (Zmax®), the subject of this review, provides a single 2 g dose for a variety of indications.

Azithromycin is a Biopharmaceutics Classification System (BCS) class III drug, up to a single dose of ~2.85 g. It is moderately well absorbed, with an absolute bioavailability of ~37% (5) and a calculated Maximum Absorbable Dose (MAD) of ~3.4 g (11). The high MAD results from the drug's high solubility.

Azithromycin is a weak base with a high solubility of 440 mg/ml at pH 2.9, 310 mg/ml at pH 6.4, 5 mg/ml at pH 7.4, and 0.005 mg/ml at pH 10.3 (12). pKas have been

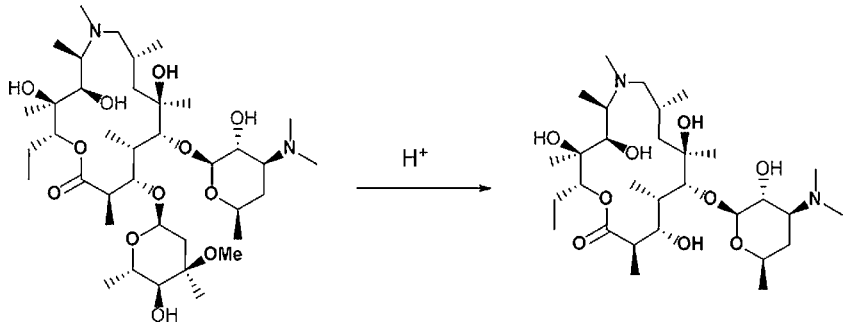

Fig. 1 Azithromycin conversion to des-cladinose-azithromycin (DCA).

reported at 9.16 and 9.37 (13). The drug's solution stability is highly pH dependent, with 10% degradation to des-cladinose-azithromycin (DCA) occurring in about 8 min at pH 1.2 and in about 175 h at pH 4.2 (Fig. 1) (14). 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 (15). Incubation in canine duodenal fluid (pH 6.0) or canine ileal fluid (pH 6.5) resulted in no azithromycin degradation in 3 h (15).

In portally cannulated cynomolgous monkeys, ~64% of an oral azithromycin dose was absorbed, followed by ~46% first-pass elimination, giving an oral bioavailability of ~35% (16). In dogs, a major route of excretion is the feces, resulting from both biliary and transintestinal elimination (17). This excretion route is mechanistically supported by data in rats demonstrating that azithromycin is a substrate for P glycoprotein (PGP) and multidrug resistance-associated protein 2 (Mrp2) (18). In CACO-2 cells, azithromycin is actively effluxed, with a basolateral-to-apical flux which is greater than 10-fold the apical-to-basolateral flux (19). In humans, codosing azithromycin with the PGP inhibitor nelfinavir resulted in a doubling of azithromycin exposure, further supporting the involvement of PGP in azithromycin excretion (20). In human ileostomy subjects, a portion of intravenously dosed azithromycin appeared in intestinal luminal fluid (21). Azithromycin is not significantly metabolized by CYP3A4 (22).

DEFINITION OF PHYSIOLOGICAL TARGETS FOR A CONTROLLED RELEASE (CR) DOSAGE FORM

The exceptionally long azithromycin serum half-life (and tissue half-life) provided the opportunity for a revolutionary antibiotic product—a single dose antibiotic. Typical dosing regimens for azithromycin were (and still are) 500 mg on day one, followed by either 500 mg on days two and three, or 250 mg on days two through five. Oral dosing of azithromycin with the five-day regimen results in gastrointestinal (GI) side effects in some patients. In combined Phase II and Phase III clinical studies involving 3,995 patients (with all dose levels combined), 9.6% of patients reported GI side effects. The most frequent side effects were diarrhea (3.6%), nausea (2.6%) and abdominal pain (2.5%) (23). The Zithromax® capsule package insert indicates that the incidence of these side effects increases with dose.

The challenges for developing a single-dose therapy were (a) creating a practical dosage form which can administer a high dose of 1.5 to 2 g, (b) assuring absorption of a high dose, and (c) suppressing the GI side effects elicited by a high dose. Initially, a series of clinical pharmacology studies were carried out to determine whether high-dose CR dosing was physiologically feasible and to understand the mechanism of the GI side effects.

Gastric Degradation

Azithromycin exhibits a dosage-form-dependent negative food effect. When dosed in the fed state, azithromycin capsules give a lower bioavailability than when dosed in the fasted state (24,25). This food effect does not occur for rapidly disintegrating or predisintegrated azithromycin dosage forms such as tablets, suspensions and sachets (24–26). Mechanistic investigations revealed that azithromycin capsules undergo delayed disintegration in the fed stomach, resulting in acid-degradation of the drug to des-cladinose azithromycin (DCA) and lower bioavailability relative to fasted-state dosing (27,28). Thus, it would be undesirable to intentionally or unintentionally design gastric retention into an azithromycin controlled release dosage form due to acid-degradation-related bioavailability concerns.

Divided Dose Study

A pilot study was carried out to determine the effect of spreading the dosing of 2 g azithromycin over a 4 h time period (29,30). The primary goal was to learn whether such a dosing regimen would result in an unacceptable loss in bioavailability. A secondary goal was to determine whether spreading the dosing over 4 h would have an effect on toleration. In this double-blind, randomized, placebo-

Table I Pharmacokinetics for Azithromycin Divided Dose Study, with 2 g Dosed as a Bolus, or Divided into Eight 250 mg Doses, Given Each Half-Hour (29)

Treatment	AUC_{0-144} (μg•h/ml)	Cmax (μg/ml)	Tmax (h)
2 g bolus	18.8	1.69	1.3
2 g over 4 h	18.9	1.13	4.4

controlled, parallel group study, three groups of fasted healthy subjects were dosed as follows:

Group A: eight 250 mg azithromycin capsules at once (bolus dose group)
Group B: eight 250 mg capsules, with the regimen one capsule every half-hour (divided dose group)
Group C: placebo capsules

Each subject received eight capsules of drug or placebo at time zero and a capsule of drug or placebo every half-hour for 3.5 h. Blood samples were collected at intervals out to 240 h.

The serum azithromycin AUCs for Groups A and B were almost identical: 18.8 μg-h/ml for the bolus dose group and 18.9 μg-h/ml for the divided dose group (Table I). Cmax was lower, and Tmax was longer, as expected, for the divided dose regimen. These results indicated that dividing a 2 g dose over 4 h does not result in undersaturation of efflux transporters or first-pass metabolic enzymes. However, this divided dose study is an imperfect model for prediction of successful absorption from a CR dosage form because the entire divided dose is exposed to the upper GI tract, unlike the situation with a CR dosage form which releases drug while transiting through the GI tract. Regardless, the pharmacokinetic results indicated that short duration CR dosing would not necessarily result in a large loss of bioavailability.

Before dosing and before each blood collection, subjects were asked to provide information on a variety of side effects (29). They rated their side effects on "Visual Analogue Scales," on a scale from 0 to 10, where "0" indicated no effect and "10" indicated the worst effect possible. These data, presented in Table II, were analyzed in two ways. First, the incidence of side effects was noted by counting the number of subjects who marked a score of >1 and >4 at any time during the post-dosing period. A score >1 was deemed to be a real side effect, whether mild, moderate or severe. A score >4 was deemed to be a moderate or intense side effect. Second, all scores (out to 240 h) for an individual subject, for a specific side effect, were summed, and then the mean sum was determined for all the subjects for that side effect and reported as a Mean Cumulative Visual Analogue Score (MCVAS). This mean score does not correspond to the 1–10 visual analogue scale, because it is a sum of all non-zero scores for the observation period.

Table II presents Incidence and Mean Cumulative Visual Analogue Scores for the side effects nausea, regurgitation, and abdominal cramping. Regurgitation was not an issue for any of the three treatment groups. For nausea and abdominal cramping, the 2 g bolus dose and 4-h divided dose regimens exhibited similar scores which are higher than those for placebo. These results indicated that the divided dose regimen did not improve side effects, and in the absence of other data would suggest that CR dosing would not ameliorate the side effects of a high single-dose therapy. However, intubation delivery of azithromycin in the duodenal and ileocecal regions (discussed below in "Duodenal and Ileocecal Toleration") indicated that the duodenum is a particularly azithromycin-sensitive region of the intestine. Since divided oral dosing continually bathes the duodenum with drug over the course of dosing (4 h in this case), it is not surprising that divided dosing did not result in improved side effects.

Duodenal and Ileocecal Absorption

A randomized, open-label, four-way cross-over pilot study compared the pharmacokinetics of a 500 mg azithromycin

Table II Side Effect Quantitation in Azithromycin Oral Divided Dose Study (29). "Incidence" Reports the Number of Subjects Who Reported a Visual Analogue Scale (VAS) Score >1 or >4 Out of 10 at Any Time During the 240 h Post-Dose Evaluation Period. To Obtain the Mean Cumulative VAS Score (MCVAS), All VAS Scores Were Summed for All Subjects for a Particular Side Effect, and Divided by the Number of Subjects. n is Number of Subjects

Treatment	n	Nausea			Regurgitation			Abdominal cramping		
		Incidence		MCVAS	Incidence		MCVAS	Incidence		MCVAS
		>1	>4		>1	>4		>1	>4	
Placebo	16	0/16	0/16	0.25	0/16	0/16	0.06	0/16	1/16	1.19
2 g bolus	15	2/15	1/15	1.93	0/15	0/15	0.53	6/15	1/15	4.67
2 g over 4 h	14	3/14	0/14	2.77	0/14	0/14	1.38	4/14	0/14	4.46

Table III Azithromycin Pharmacokinetics After 500 mg Dose Given IV, Orally, and by Intubation ($n = 11$) (31)

Dosing route	AUC_{0-48} (μg•h/ml)	Cmax (μg/ml)	Tmax (h)	F
Intravenous	8.14 ± 1.77	2.82 ± 0.51	0.8 ± 0.2	—
Oral	3.58 ± 1.22	0.347 ± 0.095	1.9 ± 0.9	0.438
Duodenal	4.02 ± 0.96	0.842 ± 0.328	1.2 ± 1.1	0.499
Ileocecal	3.04 ± 1.46	0.407 ± 0.426	0.7 ± 0.5	0.367

F absolute bioavailability

dose given IV, orally, and by intubation to the duodenum and ileocecal region, in fasted individuals (31). The treatments were:

Treatment A: 1 mg/ml 0.9% saline infused intravenously over 1 h
Treatment B: 2 × 250 mg capsules orally
Treatment C: 10 mg/ml solution to duodenum over 5 min
Treatment D: 10 mg/ml solution to ileocecal junction over 5 min

Blood samples were collected out to 96 h, and AUC_{0-48} was reported. Table III provides pharmacokinetic parameters for the four treatments. Oral bioavailability (43.8%) was a little higher than typical historical values. Duodenal bioavailability (49.9%) was also higher than typical oral bioavailability values, perhaps because of avoidance of the stomach, at whose fasting pH azithromycin is unstable (14). The ileocecal bioavailability of 36.7% was encouraging, indicating that a controlled release dosage form would have the potential to experience good absorption in the ileocecal region and perhaps the ascending colon if sufficient water were present to dissolve the drug and to maintain it in solution. In a dog colon absorption model, in which azithromycin was dosed 30 cm proximal to the anal sphincter, the bioavailability relative to oral dosing was 8.7% (32). Thus, it was reasonable to expect poor colonic absorption at some point in the human colon.

Rectal Absorption

A randomized open-label cross-over pilot study was carried out in six healthy subjects, who received a 500 mg azithromycin IV infusion over 60 min and a 500 mg dose (12.5 ml of a 40 mg/ml solution) over 5 min intrarectally (33). $AUC_{0\text{-last}}$ was 10.0 μg•h/ml and 0.31 μg•h/ml for IV and rectal dosing, respectively. The rectal bioavailability was very low, around 3%. The low rectal bioavailability indicated that, at some position in the colon, bioavailability drops significantly.

Duodenal and Ileocecal Toleration

A randomized, double-blind, placebo-controlled pilot study was carried out to compare the toleration of the duodenum and ileocecal region to azithromycin (29,30). A high 2 g dose was chosen to increase the probability of observing a side effect signal. Two parallel groups of six healthy males were given 2 g azithromycin solution via nasogastric tube or by intravenous infusion. IV solutions were delivered at 1 mg/ml over 1 h. Infusions to the duodenum or ileocecal junction were delivered at a concentration of 40 mg/ml within 5 min. All subjects had both an IV line and nasogastric tube placed during all doses. When doses were administered through the nasogastric tube, placebo (normal saline) was administered through the IV line, and vice versa.

Duodenal and ileocecal absorption of a high 2 g dose was moderate, with bioavailabilities similar to the 37% typical for oral dosing of lower doses (Table IV). Side effect incidence and VAS data revealed that nausea, regurgitation, and abdominal cramping were all lower for ileocecal, relative to duodenal, dosing (Table V). This was an important result, because it suggested that, for a high dose, GI side effects could be minimized with a dosage form which minimized exposure of the drug to the duodenum, releasing the majority of the drug load lower in the small intestine. Thus, it would be undesirable to intentionally or unintentionally maximize gastric retention of an azithromycin controlled release dosage form. These side effect results also support an interpretation of the side effect results in the Divided Dose Study. Dividing a 2 g dose over 4 h did not improve side effects, probably because both the bolus and divided dose regimens exposed the sensitive duodenal region to high concentrations of azithromycin and to the total azithromycin dose over a relatively short time period.

Table IV Pharmacokinetics for 2 g Azithromycin Solution Dosed by Intubation in the Duodenum ($n = 5$) and Ileocecum ($n = 6$), and by Intravenous Infusion (29)

Treatment	AUC_{0-96} (μg•h/ml)	Cmax (μg/ml)	Tmax (h)	F
Intravenous	38.7 ± 6.7	10.44 ± 1.38	0.82 ± 0.26	—
Duodenal dosing	17.0 ± 3.9	3.24 ± 1.76	0.3 ± 0.07	0.439
Ileocecal dosing	14.5 ± 7.0	0.77 ± 0.25	1.39 ± 1.42	0.375

F absolute bioavailability

Table V Side Effect Quantitation in Intubated Subjects Dosed with Azithromycin Duodenally or Ileocecally (29). "Incidence" Reports the Number of Subjects Who Reported a Visual Analogue Scale (VAS) Score >1 or >4 Out of 10 at Any Time During the 96 h Post-Dose Evaluation Period. To Obtain the Mean Cumulative VAS Score (MCVAS), All VAS Scores Were Summed for All Subjects for a Particular Side Effect, and Divided By the Number of Subjects. n is Number of Subjects

Treatment	n	Nausea			Regurgitation			Abdominal cramping		
		Incidence		MCVAS	Incidence		MCVAS	Incidence		MCVAS
		>1	>4		>1	>4		>1	>4	
Duodenal	5	2/5	1/5	11.6	3/5	0/5	7.2	5/5	0/5	13.2
Ileocecal	6	2/6	0/6	2.0	0/6	0/6	0	2/6	0/6	3.3

Escalating IV Dose Study

A parallel group escalating IV dose pilot study was carried out to determine whether the GI side effects of azithromycin were locally or systemically mediated. Four groups of healthy subjects were dosed with a 2 h intravenous infusion of 0, 1, 2, or 4 g azithromycin in solution (4,29,30). Because azithromycin has ~37% oral bioavailability, these IV doses are equivalent to oral doses of 2.7, 5.4, and 10.8 g, respectively. The AUCs achieved (and Cmax values) were more than 2-fold higher than equivalent oral doses, as expected (Table VI). Side effect incidence and VAS Scores showed that the lower IV doses were well tolerated (Table VII). For example, the 1 g IV dose (equivalent to a 2.7 g oral dose), elicited little in the way of side effects. A comparison of the scores for a 1 g IV dose (2.7 g equivalent oral dose) with those for duodenal dosing at 2 g (Table V) shows that the IV dose is relatively innocuous with respect to GI side effects. The 2 g IV dose also gives relatively low side effect scores, despite the fact that is the equivalent of an unprecedentedly large oral dose of 5.4 g. These results strongly suggested that the GI side effects of azithromycin are not systemically mediated, but are due to local interaction of the drug with the GI tract. At the very high IV dose of 4 g (10.8 g equivalent oral dose), more extensive GI side effects are observed (Table VII). This may indicate a minor systemic component to the mechanism of the studied side effects. However, in a separate study in ileostomy subjects, 13% of a 500 mg IV dose was recovered in ileal fluid, indicating biliary and/or transintestinal excretion (21). Thus, the GI side effects reported at the 4 g IV dose may be due to a portion of this IV dose which was excreted into the lumen of the small intestine, resulting in GI side effects which are locally mediated.

A side effect signal was reported in the placebo group (Table VII), due to one subject. This underscores the fact that such small studies are useful for providing project guidance, but must be viewed in the context of other studies.

Table VI Azithromycin Pharmacokinetics for a 2 h Intravenous Infusion of 1 g ($n=6$), 2 g ($n=6$), or 4 g ($n=5$) Azithromycin (4,29)

IV Dose (g)	$AUC_{0\text{-}inf}$ (μg•h/ml)	Cmax (μg/ml)	Tmax
1.0	23 ± 4	3.11 ± 0.38	1.9
2.0	46 ± 9	6.84 ± 2.00	1.8
4.0	82 ± 15	9.91 ± 0.73	1.05

SUMMARY OF TARGET-SETTING PRECLINICAL AND CLINICAL PHARMACOLOGY STUDIES

The studies described above were critical for setting targets for controlled release dosage form design. These studies indicated that

(a) azithromycin GI effects are locally mediated;
(b) azithromycin GI effects are more severe in the duodenal region than the ileocecal region;
(c) doses as high as 2 g are well absorbed down to the ileocecal region, but absorption falls off at some point in the colon;
(d) prolonged gastric retention of a dosage form is likely to result in bioavailability loss due to acid degradation to des-cladinose azithromycin, and in GI side effects due to duodenal sensitivity.

In addition to the physiological constraints described above, a dosage form design must recognize other critical practical constraints. A 2 g dose is high for conventional CR tablets; thus, the design almost certainly had to involve some sort of CR beads. Again, it is impractical to place these beads in a capsule or tablet because of the dose size, and the logical conclusion was to utilize a powder containing controlled release beads which can be constituted with water or a beverage. A suspension generally should have beads of <300 μm diameter to minimize a feeling of grittiness in the mouth. Finally, azithromycin is a very bitter drug. The marketed azithromycin immediate release pediatric oral suspension contains sodium phosphate base to raise

Table VII Side Effect Quantitation in Subjects Dosed With Azithromycin Intravenously (29). "Incidence" Reports the Number of Subjects Who Reported a Visual Analogue Scale (VAS) Score >1 or >4 Out of 10 at Any Time During the 240 h Post-Dose Evaluation Period. To Obtain the Mean Cumulative VAS Score (MCVAS), All VAS Scores Were Summed for All Subjects for a Particular Side Effect, and Divided By the Number of Subjects. n is Number of Subjects

IV Dose and [Equivalent Oral Dose][a]	n	Nausea			Regurgitation			Abdominal cramping		
		Incidence		MCVAS	Incidence		MCVAS	Incidence		MCVAS
		>1	>4		>1	>4		>1	>4	
0 g [0 g]	5	1/5	0/5	3.2	0/5	0/5	2.6	1/5	0/5	3.4
1 g [2.7 g]	6	0/5	0/5	0	0/5	0/5	0	0/5	0/5	0.5
2 g [5.4 g]	6	4/6	2/6	13.2	1/6	0/6	0.5	1/6	1/6	3.8
4 g [10.8 g]	5	3/5	2/5	10.6	1/5	1/5	3.8	4/5	2/5	11.8

[a] based on oral bioavailability of 37%

the pH of the constituted suspension to around pH 10, at which pH azithromycin has low solubility, and thus minimized taste. The marketed immediate release pediatric suspension also contains sucrose and flavorings (34).

AZITHROMYCIN CR DOSAGE FORM

Initial formulation work utilized coated extruded spheronized bead formulations which did not satisfy one or more of the above constraints (Curatolo, LeMott, and Korsmeyer, unpublished). This was followed by a collaboration between Pfizer and the drug delivery company Bend Research Inc. (Bend, OR), resulting in a dosage form which met the difficult physiological and practical constraints (35). This review will not cover the history of failed formulations, but will describe the progress to the successful formulation which has been approved by FDA and other regulatory bodies. This complex formulation is a suspension which consists of ~200 μm drug-containing CR microspheres, and alkalizing agents which temporally control gastric pH to minimize drug release in the upper GI tract.

CR Microspheres

Spherical CR microspheres were manufactured using a melt-congeal process (36), schematized in Fig. 2 (35). The bead matrix material was the water-insoluble trigyceride glyceryl behenate (Compritol®), which is solid at body temperature. The bead formulation also contained the water-soluble polymer poloxamer 407 (Lutrol®) as a porosigen, that is, as a component which leaves pores behind as it dissolves out of the bead in the use environment. A mixture of azithromycin dihydrate, glyceryl behenate, and poloxomer 407 was fed into a heated extruder to form a suspension of crystalline drug in molten glyceryl behenate and poloxamer, which was then fed onto a heated spinning disk that converted the suspension into droplets that formed small beads with a narrow size distribution as they rapidly cooled (37). In order to maintain the crystallinity of azithromycin dihydrate in the microspheres, two process steps were included after much experimentation. First, water was added during the heated extrusion to prevent loss of water from the crystalline azithromycin dihydrate (38). Second, after manufacture, the microspheres were annealed by storing in a heated controlled-humidity environment for five days (39). Powder x-ray diffraction of the final beads demonstrated that the drug maintained its form as crystalline azithromycin dihydrate. The maintenance of this crystalline dihydrate form is highly desirable because of its excellent chemical stability.

Release of a high solubility drug like azithromycin is expected to be very rapid from such small beads (~200 μm), and the effects of porosigen content and dissolution medium pH were studied. Figure 3 presents dissolution at pH 6.0 of microspheres containing three different levels of the poloxamer porosogen (35). It is clear that the azithromycin release rate is rapid and increases with increasing poloxamer content. Because azithromycin exhibits pH-dependent solubility, the effect of pH was determined, and Fig. 4 presents the effect of pH on *in vitro* release of azithromycin from microspheres containing 4% poloxamer (35). It is clear that drug release from these small microspheres would be variable as they transit the varying pH environments of the GI tract and that release would be particularly rapid at pH 6 and below. As described in the Background above, azithromycin is highly soluble at low and moderate pHs and becomes poorly soluble at high pH. Figure 5 presents the proposed mechanism of drug release, which involves dissolution of azithromycin and passage through narrow paths formed as the water-soluble poloxamer polymer dissolves and exits (35). Glyceryl behenate is water insoluble and does not melt or dissolve at 37°C.

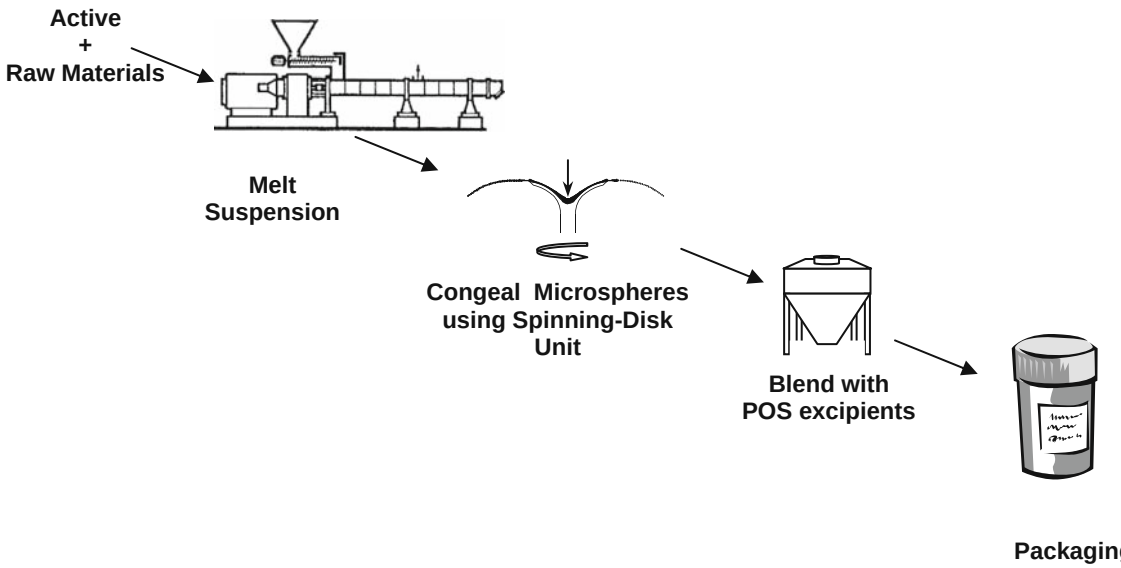

Fig. 2 Schematic of the manufacturing process for microspheres (35).

Alkalizing Agents to Control Azithromycin Release Rate

Azithromycin CR microspheres were mixed with alkalizing agents in order to prevent release of azithromycin from the microspheres when constituted with water and to prevent rapid release of the drug in the low pH environment of the stomach. Various alkalizing agents and mixtures of alkalizing agents were titrated *in vitro* with HCl to estimate which alkalizers and which quantities would potentially be useful for raising stomach pH, given assumptions about the gastric acid secretion rate (12). With these data as backdrop, a study of the effect of alkalizers on human gastric pH was carried out (12).

Eighteen healthy volunteers were divided into three groups ($n=6$), each of which received two alkalizer formulations and a placebo formulation in a three-way open-label randomized cross-over study (12). A washout period of at least one day occurred between treatments. Each subject was intubated with a Syntetics Digitrapper pH probe placed in the stomach approximately 30 min before formulation administration, and baseline pH was measured. Six formulations were tested (Table VIII). pH was recorded continuously for 2 h post-dose, with the subjects in a sitting position. With the exception of Formulation 1, all formula-

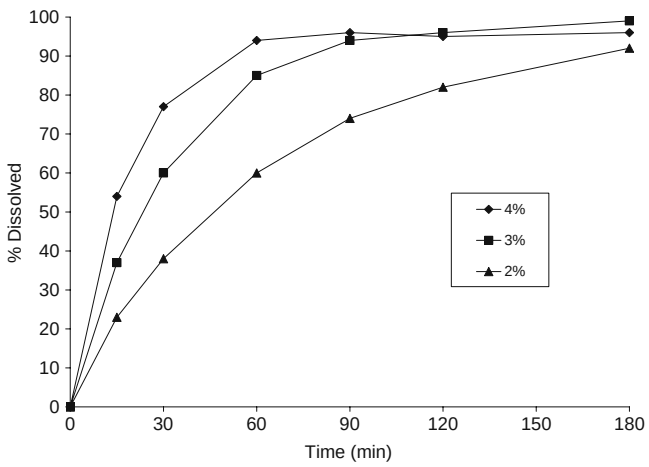

Fig. 3 Comparison of dissolution profiles from azithromycin microspheres containing 2%, 3%, and 4% poloxamer (Dissolution conditions: USP-2, 50 rpm, 900 mL phosphate buffer, pH ~6.0) (35).

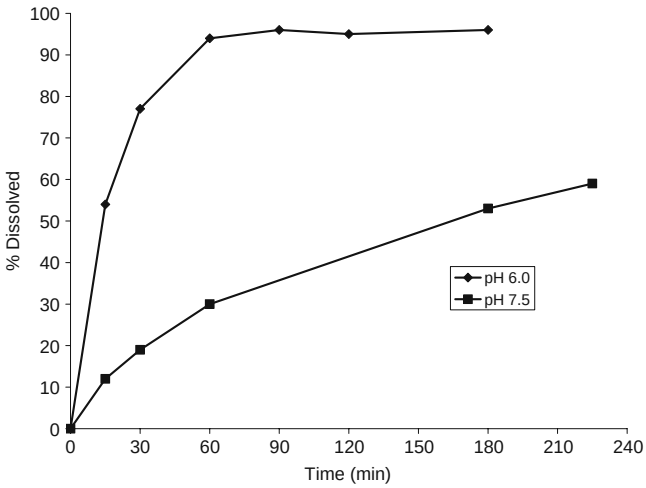

Fig. 4 Comparison of dissolution profiles from azithromycin microspheres containing 4% poloxamer as a function of pH of the dissolution media. In the case of dissolution in pH 7.5 media, the paddle speed was increased to 150 rpm after 180 min (35).

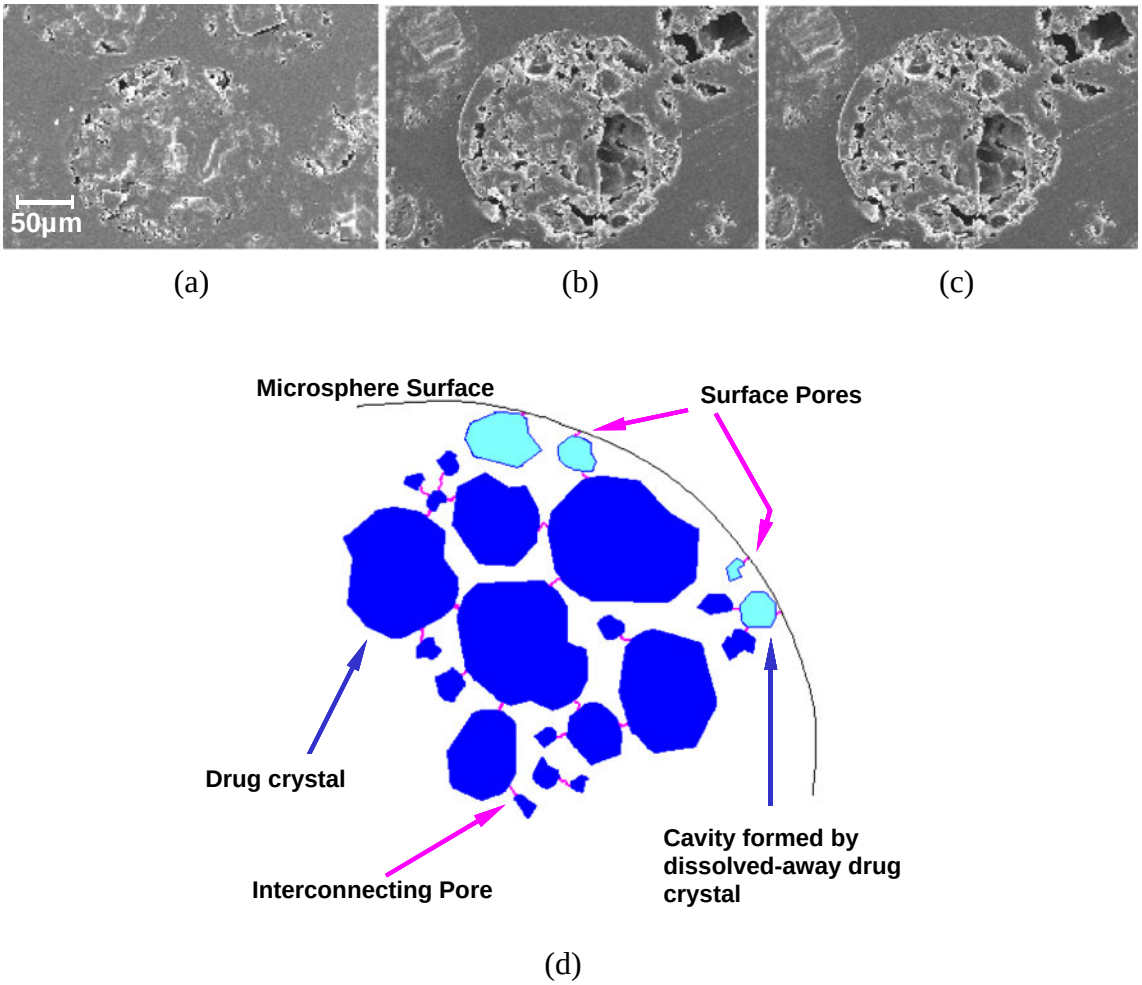

Fig. 5 Cross-section of microspheres after (**a**) 5-min exposure to water, (**b**) 30 min exposure to water, (**c**) 60 min exposure to water. (**d**) Schematic showing larger pores corresponding to areas which were occupied by azithromycin and interconnected areas which were occupied by poloxamer (35).

tions raised the gastric pH to 6 or above, on average, and maintained an elevated gastric pH for around 20 min. Figure 6 presents typical pH traces for the trisodium phosphate/magnesium hydroxide formulation for six subjects (35). Thus, these alkalizer formulations had the capacity to raise the pH of the stomach for a period of time required to minimize release of azithromycin from microspheres, which would then leave the fasted stomach into the pH 6.5 duodenum with a half-emptying time of about 8 min (40).

Table VIII Alkalizing Agents Tested in Human Gastric pH Study (12)

Formulation	Alkalizing agent
1	176 mg anhydrous TSP
2	352 mg anhydrous TSP
3	352 mg anhydrous TSP plus 500 mg calcium carbonate
4	352 mg anhydrous TSP plus 250 mg magnesium hydroxide
5	352 mg anhydrous TSP plus 500 mg tromethamine (TRIS)
6	352 mg anhydrous TSP plus 1000 mg tromethamine (TRIS)
Placebo	water

TSP trisodium phosphate

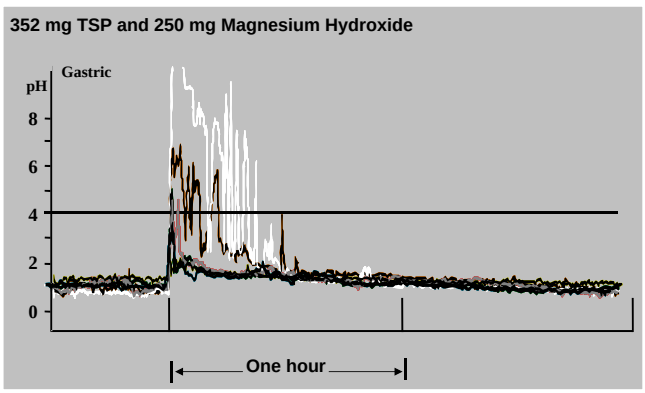

Fig. 6 *In vivo* gastric pH traces from subjects dosed with 352 mg trisodium phosphate (TSP) and 250 mg magnesium hydroxide, demonstrating an elevated pH maintained for about 20 min (12,35).

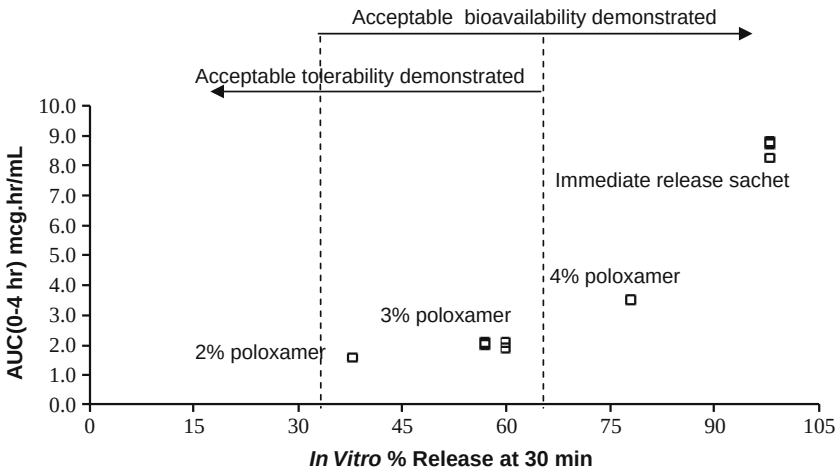

Fig. 7 Relationship between *in vivo* $AUC_{0\text{-}4\ h}$ (a surrogate for exposure in the upper GI tract, which is undesirable) and *in vitro* azithromycin release from microspheres containing 2%, 3%, and 4% poloxamer (porosigen) content, compared with an immediate release azithromycin sachet dosage form (35).

Arriving at the Final Azithromycin CR Formulation—Zmax®

A series of clinical investigations explored the relationship of formulation variables to bioavailability and tolerability. This involved exploration of both the formulation of the CR microspheres and the nature and quantity of alkalizing agents. For example, a pharmacokinetic evaluation was carried out on microspheres containing 4% poloxamer porosigen (a relatively high content), dosed with 352 mg trisodium phosphate (TSP) as alkalizing agent (12). This formulation did not exhibit delayed serum azithromycin levels relative to azithromycin immediate release tablets. Furthermore, this formulation did not show any advantage in ameliorating the side effects diarrhea, nausea, or vomiting.

Another pharmacokinetic study was carried out on microspheres containing less poloxamer porosigen (2% and 3%), dosed with 352 mg TSP and in one leg additionally 250 mg magnesium hydroxide (12). Relative to azithromycin immediate release sachet controls, these formulations exhibited a lower Cmax, a longer Tmax, and reasonable relative bioavailability ranging from 73% to 89%. The three tested formulations demonstrated a considerable improvement in the side effects nausea and vomiting, with no improvement in diarrhea. While diarrhea is certainly undesirable, it was deemed less important than vomiting because vomiting could potentially result in uncertainty about whether the single dose therapy was actually received in the bloodstream.

The knowledge gained about porosigen content in these developmental pharmacokinetic studies is summarized in Fig. 7 (35). Microspheres containing 3% poloxamer porosigen appeared to be the best choice for reproducibly achieving both acceptable bioavailability and toleration.

Based upon these studies, a lead formulation was chosen for extensive pharmacokinetic and side effect profiling. This formulation consisted of glyceryl behenate microspheres containing azithromycin dihydrate and 3% poloxamer porosigen, mixed with the alkalizing agents TSP and magnesium hydroxide, in addition to the sugar, suspending agents, and flavors needed to make a useful palatable suspension. Figure 8 presents pharmacokinetic profiles for the microsphere formulation and an immediate release sachet control (41). The early high serum azithromycin peak is muted as a result of the controlled release of the drug. $AUC_{0\text{-}96hr}$ and Cmax for the microsphere formulation were 82.8% and 43.2% of the values for the immediate release sachet, respectively (41). The 82% relative bioavailability indicated that a 2 g CR dose would be capable of effectively delivering at least the 1.5 g total azithromycin dose required for effective therapy with previous multiday regimens.

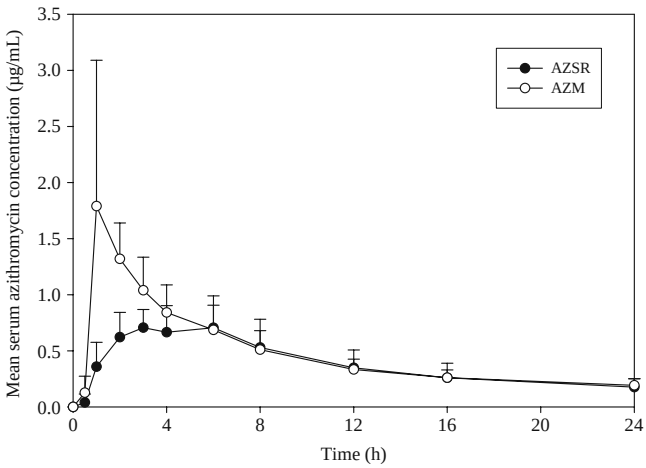

Fig. 8 Mean serum pharmacokinetic profiles for a single 2 g dose of formulation (AZSR) composed of azithromycin CR microspheres containing 3% poloxamer porosigen, with TSP and magnesium hydroxide alkalizers (41). The comparison formulation is two commercial 1 gm azithromycin immediate release sachets (AZM). Error bars are standard deviation; $n = 16$.

Table IX Adverse Event Incidence for 2 g Azithromycin CR Microsphere Dosage Form (AZSM), Compared to 2 g Immediate Release Sachet (AZM) (41)

Side effect	Incidence	
	AZM*	AZSM**
Abdominal pain	35/108	38/106
Diarrhea	30/108	19/106
Nausea	59/108	18/106
Vomiting	28/108	4/106

* $n = 108$

** $n = 106$

A large adverse event study was carried out comparing the lead CR microsphere formulation and the commercial immediate release sachet dosage form, with approximately 100 subjects per leg (41). The incidences of nausea and vomiting were shown to be significantly less for the microsphere formulation, at the $p < 0.0001$ level (Table IX). The incidence of diarrhea was less for the microsphere formulation, at the $p = 0.04$ level. Abdominal pain incidence was similar for both formulations.

These studies demonstrated that a suspension of 3% poloxamer-containing ~200 μm microspheres, combined with the alkalizing agents TSP and magnesium hydroxide, met the complex multidimensional physiological and practical constraints for delivery of a single high dose of azithromycin.

EFFICACY OF SINGLE-DOSE AZITHROMYCIN TREATMENT

The azithromycin CR microsphere formulation has been evaluated in a series of Phase III studies. For example, in a comparative study with seven-day levofloxacin therapy for mild-to-moderate community-acquired pneumonia (CAP) in adults, a 2 g single dose of azithromycin microspheres was at least as effective as the levofloxacin course of therapy (42). In another study of adult CAP, 2 g azithromycin microspheres were as effective and well-tolerated as a seven-day course of extended-release clarithromycin (43). In a study of acute exacerbation of chronic bronchitis, the single-dose azithromycin formulation was as effective as a seven-day levofloxacin course of therapy (44). The efficacy of the azithromycin microsphere formulation in respiratory tract infections has been reviewed (45,46).

The azithromycin CR microsphere formulation is approved and available as Zmax® in the U.S. and many other countries, and is under continued testing in additional indications.

DISCUSSION

The azithromycin CR microsphere dosage form Zmax® was the result of an early investment in understanding the site-specific absorption and toleration of azithromycin, and the application of unusual and novel technologies to design a dosage form which met the haiku-like physiological and practical constraints. It is interesting to consider the potential therapeutic advantages of this single-dose antibiotic therapy, although some of these potential advantages have not yet been demonstrated with statistical significance.

Early in the development of azithromycin, it was recognized that the drug partitioned extensively into tissues, achieving much higher tissue concentrations than serum concentrations (5). It was also recognized early that azithromycin concentrates in phagocytes, which act as "Trojan horses" to carry the drug to sites of infection (6–9). Recently, a study in 24 healthy adults addressed the ability of the 2 g single-dose microsphere formulation to "front-load" white blood cells with azithromycin, compared to traditional three-day therapy (500 mg on days 1, 2, and 3) (47). For both regimens, the total azithromycin exposures in mononuclear leukocytes (MNL) and polymorphonuclear leukocytes (PMNL) were approximately 300-fold and 600-fold higher than serum exposure, respectively. It is very interesting to note that the first-day exposure ($AUC_{0\text{-}24}$) for the single-dose therapy was approximately three-fold higher in MNLs than for the traditional three-day therapy (Fig. 9). Measurements in PMNLs were similar. Thus, the single-dose therapy has the potential to maximize drug exposure at infection sites early in therapy when the bacterial burden is greatest. This potential is supported by preclinical experiments in which mice and gerbils were

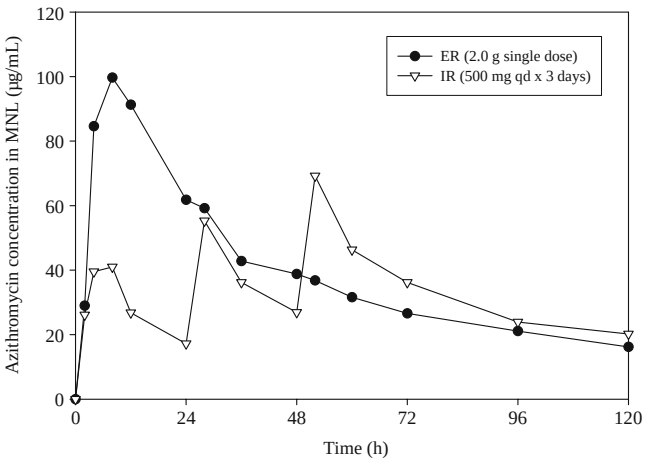

Fig. 9 Mean azithromycin concentration *versus* time profiles in mononuclear leukocytes (MNL) following administration of a 2 g single-dose CR microsphere regimen (ER) *versus* a three-day immediate release regimen (500 mg QD x 3 Days) (IR) to healthy subjects ($n = 12$ per group) (47).

dosed with azithromycin in a high single dose on the first treatment day, or as two divided doses over two days, or as three divided doses over three days (48). Efficacy in mouse pneumonia, acute peritonitis, and neutropenic thigh infection models, and in a gerbil model of *Haemophilus influenzae* acute otitis media, were studied. In the mouse models, a high single oral dose resulted in superior rates of survival and bacterial clearance. In the gerbil, a high single dose sterilized the middle ear and cleared *H. influezae* more rapidly than the multiday regimens. This unusual azithromycin dose regimen-dependent biology and its medical antibacterial implications have recently been reviewed (49).

There is also the possibility, as yet undemonstrated in humans, that azithromycin-resistant bacteria may be more effectively killed with a single-dose "front-loaded" regimen. Demonstration of such an advantage would require a clinical study in a setting where a significant population of resistant pathogens was present.

A very obvious feature of single-dose azithromycin therapy is 100% compliance. General drug-dosing non-compliance rates have been reported to range from 13% to 93% (50,51), and compliance rates of 80%, 69%, and 38% have been reported for once-daily, twice-daily, and thrice-daily administration, respectively (52). Compliance is particularly problematic in children, and one study reported that half the studied outpatients ceased ten-day oral penicillin therapy by the third day (53). While infections in some non-compliant patients resolve, compliant patients have a significantly higher incidence of resolution (52). In addition, poor compliance is potentially a factor in growing antibiotic resistance. The unusual single-dose therapy provided by the azithromycin CR microsphere formulation may be particularly useful in charitable clinics where large numbers of patients must be dosed, and where patient understanding of compliance issues may be minimal.

Finally, the exploratory clinical pharmacology studies which defined the constraints for CR dosing were particularly useful in the azithromycin case, and it is interesting to consider whether such studies would be broadly useful. For example, a divided dose study is a straightforward way to predict whether bioavailability will drop for an extended release dosage form, due to first-pass undersaturation effects. The site-specific absorption intubation studies may also be useful for some drugs, and while some of this information may be obtainable (with assumptions) by dosing prototype CR dosage forms with varying delivery durations, the intubation approach gives clean pharmacokinetic conclusions. The dog colonoscopy model is also generally useful (28). A useful detailed technical review of human intubation/pharmacokinetic studies has recently appeared (54).

The exploratory side effect studies utilizing intubations and Visual Analogue Scales may or may not be generally applicable. In the case of azithromycin, it is possible that GI side effects are pharmacologically mediated in the GI wall because the drug has been reported to bind to the human gastric antrum motilin receptor (55). The macrolides clarithromycin and erythromycin also bind to this receptor (55) and are known to affect GI motility (56,57). However, the motilin receptor is expressed throughout the GI tract (58,59), and the relationship is not clear between motilin receptor density and the observed site-specific GI sensitivity to azithromycin. For drugs with side effects which are not tightly pharmacologically defined, pleitropic drug effects may confound interpretation of pilot side effect studies carried out with small numbers of subjects.

For azithromycin, intravenous dosing studies were particularly useful for clear demonstration that GI side effects are locally mediated. If an IV dosage form and appropriate resources are available, this approach should be generally useful when there is suspicion that side effects may be locally mediated, and verification is needed to drive dosage form design. When side effects are systemically mediated, IV studies may potentially be useful to define whether these side effects are absorption rate-dependent, Cmax-dependent, or AUC-dependent.

In the end, each case is different, and the important message is to take an appropriate scientifically based approach to mapping out the physiological and practical constraints to be overcome to achieve an optimal dosage form design.

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