Influence of Study Design in Assessing Food Effects on Absorption of Erythromycin Base and Erythromycin Stearate

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We performed a series of six single-dose and multiple-dose studies to evaluate the effect of food on the absorption of erythromycin base and erythromycin stearate. When we used a single-dose design, we found that an unprotected erythromycin base preparation was absorbed extensively if a prolonged fast preceded administration of the drug. A shorter fasting period (as occurs in clinical settings) dramatically reduced the absorption of unprotected base; however, film-coated tablets seemed to be as well protected as and were absorbed more rapidly than enteric-coated tablets when they were evaluated by single-dose testing procedures. In contrast, when a commercially available film-coated preparation of erythromycin base was evaluated in multidose fashion between meals (fasting), the drug was about 25% less well absorbed than commercially available enteric-coated tablets. Finally, when commercially available film-coated erythromycin base and stearate formulations were administered with meals, both film-coated preparations were 43 to 59% less well absorbed than the enteric-coated base formulation. Furthermore, the enteric-coated formulation performed equally well when administered either every 6 h between meals (fasting) or four times a day (immediately after meals and at bedtime). These studies document the need for multidose bioavailability techniques when the bioavailabilities of acid-labile drugs are evaluated.

The interference of food with drug absorption is being recognized increasingly (5, 8). The presence of food in the gastrointestinal tract can decrease the rate of gastric emptying. For erythromycin base and erythromycin stearate, which are acid labile (1, 3, 7, 9), prolonged exposure to the acidic milieu of the stomach resulting from food ingestion could result in inactivation and consequent reduction in the extent of absorption of active drug. For example, in vitro studies have demonstrated that both erythromycin base and erythromycin stearate dissolve in gastric acid within 5 min and retain only 3.5 and 2% antibiotic activity, respectively (7). For many years, erythromycin base has been formulated with a protective enteric coating that is resistant to the effects of gastric acid. When slowed gastric emptying resulting from food ingestion increases the exposure of erythromycin tablets to acid, the enteric-coated tablets are protected in the stomach and are allowed to pass intact into the alkaline intestinal contents, where they can dissociate and be absorbed. Thus, this coating produces a lag in initiation of absorption but no decrease in extent of absorption. Since clinical use of erythromycin almost always requires multiple dosing, a lag in initiation of absorption should not be of ultimate clinical concern. Because erythromycin stearate is less water soluble than erythromycin base, the stearate has not been formulated with an acid-resistant coating.

To provide clinically relevant information, erythromycin should be studied under conditions frequently encountered in clinical situations. Although erythromycin has been studied extensively, many of the bioavailability studies have produced conflicting reports on blood levels, probably because of the various effects of single dosing, multiple dosing, fasting, and food ingestion.

MATERIALS AND METHODS

In each of the studies described here normal adult volunteers gave informed consent before participating. When fasting was required, subjects were permitted water only ad libitum. Because the volume and caloric value of a meal affect the rate of gastric emptying (2), meals were standardized for each subject throughout the studies of food effects on bioavailability. After blood samples were drawn according to the study design, the serum was separated from each sample and frozen immediately; later, sera were assayed for erythromycin by a microbiological assay.

The statistical method used in these studies, which all followed a randomized complete crossover design, was analysis of variance. In the studies with a three-way crossover design, we also used Tukey's allowable difference between treatments.
Study 1. Serum erythromycin levels were determined in 21 volunteers after each was given 500 mg of erythromycin base as two 250-mg tablets orally; each volunteer received either unprotected erythromycin base tablets (E-Mycin core tablets without coating; research no. 16,268-1), film-coated erythromycin base tablets (E-Mycin core tablets with a film coating; research no. 16,268-2), or enteric-coated erythromycin base tablets (E-Mycin core tablets; The Upjohn Co.; research no. 16,268-3). The core tablets for all three preparations were identical in that they were taken from the same manufacturing lot. The subjects were required to fast for 10 h before and 2 h after they took the medication. Blood samples were drawn at 0, 0.75, 1.5, 3, 4.5, 6, 8, 10, 12, and 14 h after treatment. This study followed a randomized three-way crossover design.

Study 2. In this study we compared the serum erythromycin levels achieved after oral administration of single 500-mg doses (two 250-mg tablets) of the same three preparations used in study 1, but the doses were administered 2 h after a standardized meal instead of after a prolonged fast. The 12 volunteers were required to fast for 2 h after they took the medication. Blood samples were drawn at 0, 0.75, 1.5, 3, 4.5, 6, 8, 10, and 12 h after treatment. This study followed a randomized three-way complete crossover design.

Study 3. In this study we compared serum levels of erythromycin during and after oral administration of 12 doses of 250-mg enteric-coated erythromycin base tablets (E-Mycin; lot no. 364FW; The Upjohn Co.) and film-coated erythromycin base tablets (erythromycin base Filmtab; lot no. 80-853-4; Abbott Laboratories) (i.e., commercially available preparations from different manufacturers); these tablets were given every 6 h and there was a fast of at least 2 h before and after each treatment. Blood samples were drawn every 1.5 h during the first 24 h after treatment was begun and again at 1.5 h intervals during day 3 (48 to 72 h). A total of 18 volunteers participated in this study, which followed a two-way crossover design.

Study 4. This study was similar to study 3, except that commercially available film-coated erythromycin stearate tablets were used instead of film-coated erythromycin base tablets. The volunteers were given, in crossover fashion, either film-coated erythromycin stearate tablets (E-Mycin; erythromycin base; Abbott Laboratories; research no. 16,274-2) or enteric-coated erythromycin base tablets (Erythrocin; erythromycin stearate; lot no. 837-1982-21; The Upjohn Co.); each volunteer was given 250 mg every 6 h for a total of 10 doses, and there was a fast of at least 2 h before and after each treatment. Blood samples for determinations of serum concentrations of erythromycin were drawn every 1.5 h for the first 12 h after treatment was begun on day 1 and then again every 1.5 h during the 48- to 60-h period on day 3.

Study 5. Serum levels of erythromycin were determined in 21 volunteers after oral administration four times daily (immediately after meals) of 250-mg enteric-coated erythromycin base tablets (E-Mycin; lot no. 082-FM; The Upjohn Co.), 250-mg film-coated erythromycin base tablets (erythromycin base Filmtab; lot no. 70-716-AF; Abbott Laboratories), and 250-mg film-coated erythromycin stearate tablets (Erythrocin Stearate; lot no. 64,984-AF; Abbott Laboratories). A total of 12 doses were administered at intervals approximating breakfast, lunch, supper, and bedtime (i.e., at 5-, 5-, 6-, and 8-h intervals). Blood samples were drawn at 0, 1, 2, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 48, 49, 50, 52, 53, 54, 56, 58, 60, 62, 64, 66, 68, 70, and 72 h after treatment was begun. In this study we used a three-way complete crossover design.

Study 6. In this study we compared the serum concentrations of erythromycin in 18 volunteers during and after oral administration of 12 doses of a commercially available lot of 250-mg enteric-coated erythromycin base tablets (E-Mycin; lot no. 081-FM; The Upjohn Co.) either four times daily immediately after meals or every 6 h with fasting (2-h fast before and 4-h fast after treatment). Blood samples were drawn hourly for the first 24 h after treatment was begun (day 1) and again at hourly intervals during day 3 (48 to 72 h). A randomized two-way complete crossover design was used.

Analytical methods. Serum erythromycin concentrations were determined microbiologically by using Sarcina lutea as the assay organism (4). The assay medium used was Penassay Seed Agar (Difco Laboratories) adjusted with sodium hydroxide. The cylinder plate method was employed, with a six-point standard curve on eight replicate plates. There were five samples and one assay control standard per plate, and there were four replicate sample plates. A primary standard was also used, which consisted of the zero-time or predose serum specimen for each subject.

The serum specimens were assayed undiluted and were compared with their respective secondary standards. All samples from each subject were assayed on the same day.

RESULTS

Study 1. When unprotected core tablets were ingested after prolonged fasting, rapid increases in serum erythromycin levels were observed (Fig. 1), which reached a maximum of 1.57 μg/ml at 3 h. Lower peak concentrations were achieved after ingestion of the film-coated preparation (0.97 μg/ml at 3.0 h) and the enteric-coated preparation (1.01 μg/ml at 4.5 h). The areas under the serum concentration-time curves (AUCs) are shown in Table 1. Although the AUC observed for the unprotected tablets was 50% larger than the AUC obtained with the enteric-coated tablets and 47% larger than AUC observed for the film-coated preparation, only the AUCs for the unprotected core tablets and the enteric-coated tablets were significantly different (P < 0.05). The average serum concentrations for the three preparations were significantly different (P < 0.01) at all sampling times except 0, 4.5, and 6 h.

The results of this study appeared to indicate that unprotected tablets were the formulation that was absorbed the best and, therefore, the ideal preparation of erythromycin base.

Study 2. Figure 2 shows that, when unpro-
tected erythromycin core tablets were administered with a fast of only 2 h, the average serum concentrations obtained were markedly reduced compared with the levels obtained in study 1. In this study the AUC for the film-coated tablets was 58% larger than the AUC for the unprotected erythromycin base tablets. A higher peak serum concentration was found (1.19 \mu g/ml) with the film-coated tablets than with either the enteric-coated tablets (0.79 \mu g/ml) or the unprotected tablets (0.72 \mu g/ml). The AUCs for the enteric-coated tablets and the film-coated tablets were essentially identical, and the absorption values appeared to be similar to the values observed when these same formulations were administered fasting (see above) (Table 1).

The information gained from this study

![Fig. 1. Average serum erythromycin concentrations in 21 healthy subjects after a single 500-mg dose of unprotected erythromycin base, film-coated erythromycin base, or enteric-coated erythromycin base with a 10-h fast before treatment and a 2-h fast after treatment. Symbols: ○, two 250-mg erythromycin core tablets with no film or enteric coating (research no. 16,268-1); Δ, two 250-mg film-coated erythromycin core tablets (research no. 16,268-2); ■, two 250-mg enteric-coated erythromycin tablets (research no. 16,268-3).]

![Fig. 2. Average serum erythromycin concentrations in 12 healthy subjects after a single 500-mg dose of unprotected erythromycin base, film-coated erythromycin base, or enteric-coated erythromycin base with a 2-h fast before treatment and a 2-h fast after treatment. Symbols: ○, two 250-mg erythromycin core tablets with no film or enteric coating (research no. 16,268-1); Δ, two 250-mg film-coated erythromycin core tablets (research no. 16,268-2); ■, two 250-mg enteric-coated erythromycin tablets (research no. 16,268-3).]

### Table 1. Mean AUC values for single- and multiple-dose studies

<table>
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<tr>
<th>Study</th>
<th>Time</th>
<th>Unprotected erythromycin base</th>
<th>Film-coated erythromycin base</th>
<th>Commercial enteric-coated erythromycin base</th>
<th>Commercial film-coated erythromycin stearate</th>
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* As determined by analysis of variance among treatments for complete crossover design. The results were also tested at the 95% confidence level by the Tukey allowable difference method. The following pairs were statistically significant (P < 0.05): study 1, unprotected erythromycin base and enteric-coated erythromycin base; study 5 at 0 to 24 h, enteric-coated erythromycin base and film-coated erythromycin base, and enteric-coated erythromycin base and film-coated erythromycin stearate; study 5 at 48 to 72 h, enteric-coated erythromycin base and film-coated erythromycin base, enteric-coated erythromycin base and film-coated erythromycin stearate, and film-coated erythromycin base and film-coated erythromycin stearate. Tukey's allowable difference test was not applicable to studies 3 and 4.

b In studies 1 and 2 we used core tablets from the same lot (research product) for all preparations.

c NS, Not statistically significant at the 95% confidence level (P > 0.05).
seemed to indicate that the film-coated erythromycin preparation offered faster absorption than the base preparation and must be the ideal formulation.

**Study 3.** Figure 3 shows the results of study 3, in which enteric-coated and film-coated erythromycin base preparations were administered in multiple doses to fasting volunteers. This study was designed to demonstrate the maximum attainable blood levels for erythromycin since treatment occurred under rigid fasting conditions.

On day 1 of the study (0 to 24 h), as determined by the AUCs (Table 1), the film-coated preparation was 25% less well absorbed ($P < 0.025$) than the enteric-coated preparation. The average serum concentrations at 10.5, 12, and 24 h were also significantly less ($P < 0.05$) for the film-coated formulation. On day 3 of the study, the same lower level of absorption of the film-coated preparation was observed, with statistically significant differences ($P < 0.05$) in serum concentrations observed at 48, 51, 52.5, 54, 60, 66, and 72 h. The AUC for the film-coated tablets was 27% less ($P < 0.001$) than the AUC for the enteric-coated preparation (Table 1).

This multidose study changed the conclusion based on the results of the second single-dose study (study 2). Here, the enteric-coated preparation delivered consistently and substantially higher serum erythromycin levels than the film-coated preparation, even when the tablets were administered under fasting conditions.

**Study 4.** Figure 4 shows the results of a comparison of commercially available enteric-coated erythromycin base tablets and film-coated erythromycin stearate tablets administered under rigid fasting conditions every 6 h. During the first 12 h of day 1, the average serum erythromycin concentrations from the two treatments were essentially identical. However, after continued treatment the erythromycin levels from the film-coated stearate tablets fell significantly below the levels from the enteric-coated erythromycin base tablets. This decrease in serum erythromycin levels of erythromycin stearate was reflected by a 37% lower AUC (Table 1).

**Fig. 3.** Average serum erythromycin concentrations in 18 healthy subjects after 12 250-mg doses of entericoated erythromycin base or film-coated erythromycin base given every 6 h with a 2-h fast before treatment and a 2-h fast after treatment. Symbols: □, 250-mg enteric-coated erythromycin base tablets (lot no. 364FW); ○, 250-mg film-coated erythromycin base tablets (lot no. 837-1982-21).

**Fig. 4.** Average serum erythromycin concentrations in 12 healthy adults after 10 250-mg doses of either enteric-coated erythromycin base or film-coated erythromycin stearate administered every 6 h with a 2-h fast before treatment and a 2-h fast after treatment. Symbols: □, 250-mg enteric-coated erythromycin base tablets (research no. 16,274-2); △, 250-mg film-coated erythromycin stearate tablets (lot no. 837-1982-21).
Study 5. Figure 5 shows the results of a study in which we compared the absorption characteristics of commercially available film-coated erythromycin base tablets and film-coated erythromycin stearate tablets produced by one manufacturer with the absorption characteristics of a commercially available entericoated erythromycin base preparation produced by a second manufacturer. When both film- and entericoated erythromycin base tablets were administered immediately after meals, the relative reduction in erythromycin absorption of the film-coated tablets compared with the entericoated base tablets was even greater than was observed in study 3, in which these formulations were administered in a relative fasting state. The entericoated erythromycin base tablets produced higher serum levels than the film-coated erythromycin base tablets in 27 of the 30 sampling periods. Based on AUCs (Table 1), the film-coated erythromycin base preparation was only 57% as available (P < 0.001) as the entericoated product on day 1 of treatment (0 to 24 h) and 62% as available (P < 0.001) on day 3 (48 to 72 h).

Furthermore, the film-coated erythromycin base tablets also showed a greater variability in absorption. The coefficients of variation for the AUCs of the film-coated tablets were 82% at 0 to 24 h and 63% at 48 to 72 h, whereas for the entericoated tablets the coefficients of variation were 55 and 31%, respectively.

In this study we also examined the effects of concomitant food administration on the absorption of film-coated erythromycin stearate and film-coated and entericoated erythromycin base. For the first 4 h of day 1, we found little difference in the serum concentrations of the three formulations. However, after the first 4 h of treatment, both film-coated erythromycin preparations (base and stearate salt) produced consistently lower serum levels than the entericoated erythromycin base preparation. We found no substantive differences in AUCs between the two film-coated preparations during day 1 (0 to 24 h), but there were substantial differences (P < 0.05) (43 and 48%) in the AUCs between the entericoated preparation and both film-coated preparations.

On day 3 of the study (48 to 72 h), there were substantive and statistically significant differences (P < 0.001) in AUC among the three preparations. The film-coated base preparation was absorbed only 62% (P < 0.05) as well as the entericoated tablets, and the film-coated stearate preparation was absorbed only 41% (P < 0.05) as well as the entericoated base tablets. The film-coated stearate preparation was absorbed 66% as well as the film-coated base preparation.

The erythromycin stearate preparation produced lower serum erythromycin concentrations than the entericoated erythromycin base preparation at all sampling times except 0, 1, 2, and 66 h. The AUCs for the film-coated stearate preparation were 48 and 59% less than the AUC for the entericoated base formulation in the 0- to 24- and 48- to 72-h periods, respectively.

![Fig. 5. Average serum erythromycin concentrations in 21 healthy subjects after 12 250-mg doses of entericoated erythromycin base, film-coated erythromycin base, or film-coated erythromycin stearate administered four times daily immediately after meals. Symbols: □, 250-mg entericoated erythromycin base tablets (lot no. 082-FM); ■, 250-mg film-coated erythromycin base tablets (lot no. 70-716-AF); ▲, 250-mg film-coated erythromycin stearate tablets (lot no. 64-984-AF).](http://aac.asm.org/)
These results, along with the data from studies 3 and 4, demonstrated that under optimal fasting conditions or under realistic conditions, an enteric-coated erythromycin base formulation produces substantially higher serum erythromycin concentrations than either a film-coated erythromycin base preparation or a film-coated erythromycin stearate preparation.

Study 6. In this study we compared erythromycin levels in blood after a commercially available enteric-coated erythromycin base formulation was administered to volunteers with meals and under fasting conditions. As Fig. 6 shows, there was a slight lag in absorption when enteric-coated erythromycin base tablets were given immediately after meals and at bedtime compared with administration under fasting conditions (every 6 h). Otherwise, the average serum concentration curves for the two dosage regimens were virtually identical. The AUCs (Table 2) were essentially identical ($P < 0.05$) for both the 0- to 24- and 48- to 72-h periods.

This study demonstrated that food did not interfere with absorption of a properly prepared enteric-coated erythromycin base formulation and that this preparation could be given in a conventional four times a day regimen with meals and still maintain optimum bioavailability.

DISCUSSION

It is known that erythromycin absorption is impaired by exposure to gastric acidity, particularly when treatment accompanies administra-

![Fig. 6. Average serum erythromycin concentrations in 18 healthy adults after 12 250-mg doses of enteric-coated erythromycin base (lot no. 081-FM) administered four times daily immediately after meals (■) or administered every 6 h with a 2-h fast before treatment and a 4-h fast after treatment (○).](image)

<table>
<thead>
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<th>Time (h)</th>
<th>Mean AUC (µg·h/ml) with treatment schedule*</th>
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<td>Four times a day after meals</td>
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<tr>
<td>0–24</td>
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<td>48–72</td>
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* Differences between treatment schedules were not significant as determined by analysis of variance.
emptying is slowed, allowing disintegration and dissolution of the tablets in the acid environment of the stomach, with subsequent greater gastric acid inactivation of the erythromycin. However, in clinical settings, prolonged fasting is not compatible with the multiple daily dosing required for erythromycin treatment or with the nutritional needs of the patients. Therefore, a protective coating is necessary for optimal erythromycin absorption.

The single-dose study (study 2) in which different coatings were compared by using the shorter fasting times that are inherent in the multiple-dose treatment necessary with clinical use suggested that the protection afforded by a simple film coating was ample and not different from the protection afforded by an enteric coating. Since absorption of the enteric-coated formulation lagged, the film-coated preparation appeared to be more desirable. However, when preparations were actually tested in multiple-dose studies (studies 3 and 4), we found that the film-coated base and stearate preparations were less protected than the enteric-coated erythromycin preparation, even under fasting conditions. About 25% less erythromycin was absorbed from the film-coated base tablets than from the enteric-coated tablets, and 37% less was absorbed from the film-coated stearate tablets than from the enteric-coated base tablets.

Even so it is difficult to impose clinically an antibiotic treatment regimen that requires fasting before and after dosing. For example, Petrick and Kleinmann (6) demonstrated that avoidance of food-drug interference was difficult at best even in a relatively controlled hospital environment. Obviously, the use of a formulation that can be administered without regard to time of meal ingestion would be ideal. Study 5 illustrated that when the enteric-coated erythromycin base product was administered with meals, it produced serum erythromycin levels that were consistently and substantially higher than the levels resulting from administration of either the film-coated base preparation or the film-coated stearate preparation. To confirm the hypothesis that an enteric-coated erythromycin base preparation could be administered without concern for time of meal ingestion, we performed a final study (study 6), which clearly documented equivalent absorption values when commercially available enteric-coated tablets were administered every 6 h under fasting conditions and four times a day immediately after regular meals and at bedtime.

These studies showed clearly that the bioavailability of erythromycin base or stearate is intimately dependent upon the degree of protection afforded the core tablets by a properly prepared enteric coating. Such a preparation can be administered irrespective of meals with the expectation of optimum bioavailability. Furthermore, single-dose tests, whether performed under prolonged fasting conditions or concomitant with food intake, were inadequate to demonstrate the differences in the absorption characteristics of erythromycin preparations that were related to the degrees of acid protection afforded by different coatings. Valid assessments of the bioavailabilities of erythromycin and erythromycin stearate clearly require multiple-dose testing.

It is apparent that in comparative bioavailability studies of a drug preparation that is acid labile multidose testing should be used under clinically relevant study conditions.

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LITERATURE CITED