Bioequivalence Study of Erythromycin Tablets in Man

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Abstract ❑ The bioavailability of some brands of film-coated erythromycin stearate tablets marketed in Egypt; brand A (250mg), brand B (250mg) and brand B̈ (500mg) and one brand of enteric-coated erythromycin tablets, was tested in order to assess their bioequivalence. The results of a single dose (500 mg) study in healthy subjects indicated wide variability in erythromycin absorption between the subjects and tablet brands with absorption failure in some cases. The relative bioavailability of erythromycin stearate tablet brands was 40, 70 and 97% for brands A, B and B̈, respectively, using brand C as a standard preparation. Judging by AUC values, calculated from mean erythromycin serum levels higher than the MEC (0.6 μg/ml), the products appear obviously inequivalent, product A being presumably inefficient and product B of marginal efficacy. The results suggested enteric-coated erythromycin base tablets as a suitable dosage form of orally administered erythromycin.

Keyphrases ❑ Film-coated erythromycin stearate tablets, enteric-coated erythromycin base tablets, bioequivalence, bioavailability.

Erythromycin, an extremely acid labile drug (1, 2), has been listed by the Academy of Pharmaceutical Sciences [APhS] (3) as having serious bioavailability and quality assurance problems. To optimize intact drug passage through the stomach, the drug is made available in a number of more stable derivatives and various oral dosage forms which include film-coated tablets of the sparingly soluble stearate salt. The salt is erratically absorbed from the GIT (4) probably because of susceptibility to gastric acid (l). This results in bioavailability variations when erythromycin stearate tablets are taken with food and fluids (5-7). For optimum absorption, the drug should be administered shortly before food (6,7). It has been reported that serum erythromycin levels vary with the chemical composition (content of stearic acid and sodium stearate) and intrinsic dissolution properties of erythromycin stearate raw material (8). Variations in serum also depend on the formulation and acid-resisting properties of the tablet coat and, hence, the degree of protection of the drug from gastric acid inactivation (9,10).

Since the ***In-vivo*** performance of erythromycin stearate tablets may depend to a great extent on pharmaceutical formulation and manufacturing coating conditions, bioavailability differences are likely to exist between preparations from different manufacturers. This has been demonstrated by several bioequivalence studies of commercial erythromycin stearate tablets (3, 10-12). The present study is concerned with the bioavailability testing of some erythromycin stearate tablet brands marketed in Egypt in order to assess potential differences in the ***In-vivo*** performance among these products. A brand of enteric-coated erythromycin tablets was used as a standard preparation.

Experimental:

Products examined: three brands of film-coated erythromycin stearate tablets manufactured locally: Brand **A**, 250mg (batch No. 109028), brand **B**, 250mg (batch No. 873693) and brand **B̈**, 500mg (batch No 870725) and brand **C**, of enteric-coated erythromycin base tablets, 250mg (batch No. 672 RD). The erythromycin content of these tablets was determined by a chemical assay method which was found to produce results comparable to those of the USP microbiological assay (13,14). The analyzed content was 262.5mg, 267mg, 519mg and 240mg as the base for the brands **A**, **B**, **B̈** and **C**, respectively.

Selection of subjects: five healthy male volunteers (age 19 to 25 years, and weight 60 to70 kg) were selected for the study. Informed consent was obtained from each subject. Volunteers were not allowed to receive antibiotics for 15 days prior to the study or to take other drugs for a week prior or during the trials.

Design of the study: The study was conducted according to a fourway crossover design with one-week washout periods. A single dose equivalent to 500mg erythromycin base (one 500mg or two 250mg tablets) was given to overnight-fasted volunteers. The tablets

were swallowed with 200ml H_2O. Fasting was continued for 4h after which a standard breakfast was allowed. Blood samples (3 ml each) were drawn from an arm vein at zero, 1/2, 1, 2, 3, 4, 5, 6 and 9 h, following drug admini-stration. Samples were collected in heparinized tubes. Plasma was separated and frozen pending analysis.

Analysis of serum samples: The erythromycin activity in serum samples was quantified microbiologically by a modified USP XXI agar diffusion technique using Sarcina lutea ATCC 9341 as the test organism and Antibiotic Assay Medium No. 1 (oxoid) adjusted to pH 8.3 as the culture medium. The antibiotic in these sera was determined by the one level assay with a standard curve technique.

Bioavailability parameters were calculated from individual serum erythromycin levels at different times using STRIPE computer program (College of Pharmacy, University of Illinois, Chicago, USA). The data obtained were subjected to statistical analysis using the t-test and analysis of variance.

RESULTS and DISCUSSION

The bioavailability of film-coated erythromycin ste-

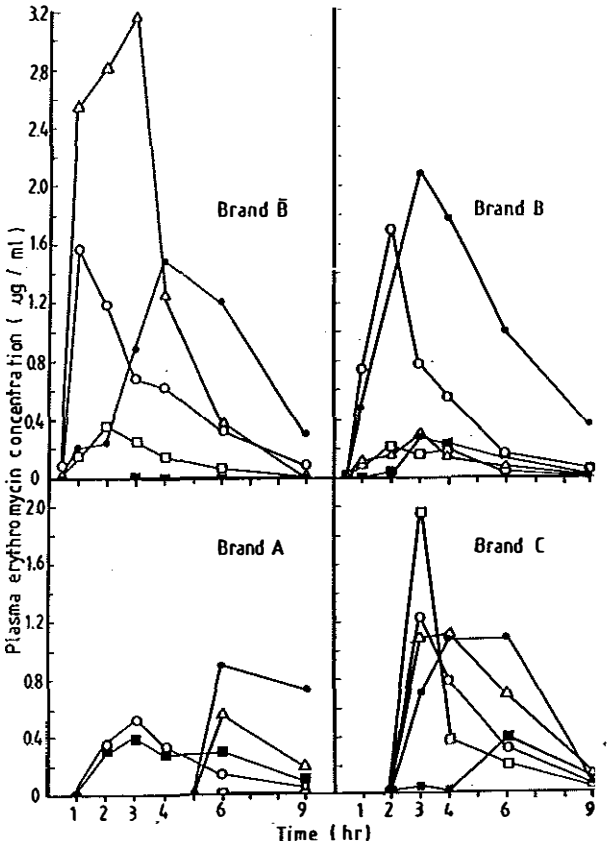

Fig.1: **Erythromycin plasma concentrations-time profiles following the administration of 500mg single doses of erythromycin tablet brands A, B, and B̄ and C to volunteers No. 1 (●), No 2 (○), No 3 (■), No 4 (□) and No 5 (Δ).**

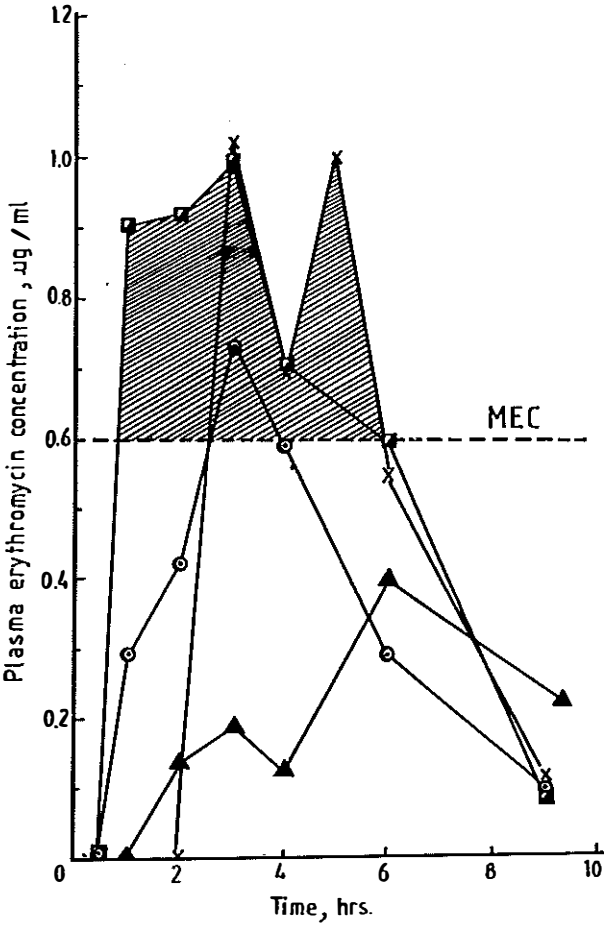

Fig. 2: **Mean erythromycin plasma concentrations-time profiles following the administration of 500mg single doses of tablet brands A (▲), B (○), B̄ (◪) and C (X) to five volunteers.**

arate tablet brands (**A**, **B** and **B̄**) was detennined relative to that of an enteric-coated tablet brand (**C**), as this product has been accepted as a standard preparation for erythromycin tablets (3). Serum concentrations-time profiles following the administration of 500mg-doses of erythromycin tablets of the brands under study to 5 subjects are shown in ***Fig. 1.*** Mean serum levels-time curves are shown in ***Fig. 2.*** Mean values of the bioavailability parameters computed using data in ***Figs. 1*** and ***2*** are listed in ***Table I.*** The results indicate wide inter brand and intersubject variability as indicated by the generally high SD and CV values (***Table I***). This observation has been reported previously for various erythromycin derivatives and oral dosage forms (5, 12,15).

Although absorption rate-indicating parameters such as lag time and T max are probably of little significance after multiple dosing (3,16), yet they offer means of evaluating and comparing formulations in single dose studies. Erythromycin absorption from enteric-coated tablets (brand **C**) was de-

layed as expected, with a lag time of 2 hours during which no drug could be detected in serum of all subjects (***Fig. 1***). The mean T max value was 4 hours (***Table I***). Drug absorption was faster from erythromycin stearate tablet brands **B** and **B̄** with detectable erythromycin activity in blood as early as half an hour after dosing, in eight out of ten subjects taking tablets of both brands (***Fig. 1,*** brands **B** and **B̄**). Mean T max values were almost identical for these two brands (***Table I***). Absorption of brand **A** tablets was markedly slow with lag times extending beyond 4 hours in some subjects (***Fig. 1***). The mean T max was 4.5 hours (***Table I***). The difference between T max values for brands **A** and **B** and brands **A** and **B̄** was significant at $P<0.1$ and $P<0.025$, respectively, using t-test for paired data.

Differences between individual erythromycin availability data can be described by two arbitrary qualitative parameters denoting the failure of a given formulation to be absorbed, namely C max < MEC (0.6-1 µg/ml) and AUC < 3 µg.h /ml (10 ,17). According to the criterion Cmax, absorption failure was observed in four, three, two and one subjects out of five in testing the bioavailability of brands **A**, **B**, **B̄** and **C**, respectively, (***Fig. 1***) . Absorption failure has also been reported in bioequivalence studies involving erythromycin tablets with some subjects exhibiting trace levels of erythromycin activity in plasma (10,12). Moreover, wide intersubject and intrasubject variability was observed between individual Cmax values for erythromycin stearate tablets, particularly in the case of brands **B** and **B̄**, the coefficient of variation of Cmax being 36.7, 97.8 and 70.1% for brands **A**, **B** and **B̄** respectively, (***Table I***). A mean Cmax of 1.64 µg/ml was achieved 2.5 hours, after administration of brand **B̄** tablets which is in accordance with reported data (10). The mean Cmax value for brand **B** tablets was much lower (0.92 µg/ml) although both products are from the same manufacturer. The standard brand **C** tablets exhibited a mean Cmax of 1.50 µg/ml which is consistent with literature data (l8). This value is less than that of brand **B̄** tablets, nonetheless, individual data for brand **C** tablets were more consistent (lower CV%, ***Table I***).

The least mean Cmax (0.60µg/ml) was observed with brand **A** tablets which were clearly erratically absorbed (***Fig. 1***). However, because of the wide intersubject and intrasubject variability, differences between Cmax values for brand **A** and **C** and brands **A** and **B̄** were significant at $p<0.1$, while the difference between Cmax values of brands **B** and **B̄** was statistically insignificant.

Table I **: Bioavailability Parameters Computed from Erythromycin Plasma Concentrations-time Data Following Administration of 500mg-Doses of Erythromycin Tablets Brands A, B, B̄ and C to Five Volunteers.**

Parameter	Brand A	Brand B	Brand B̄	Brand C
Lag time, h	2.8±1.6*	0.5±0.3	0.4±0.3	2.0±0.0
CV%	57.1	60.0	75.0	0.0
Tmax, h	4.5±1.7	2.6±0.6	2.5±1.3	4.0±1.0
CV%	37.8	23.1	52.0	25.0
Cmax, µG/ml	0.60±0.22	0.92±0.90	1.64±1.15	1.50±0.70
CV%	36.7	97.8	70.1	46.7
$AUC_{0-\infty}$, µg.h/ml	2.09±1.46	3.64±4.23	5.02±4.49	5.19±3.34
CV%	69.9	116.2	89.4	64.4
%bioav. relative to C	40	70	97	-
AUC, µg.h/ml **	0.00	0.09	1.02	0.76

* Standard deviation. **AUC values calculated from mean plasma concentrations higher than the MEC (0.6 µg/ml).

Table II **: One way Analysis of Variance Applied to Area under the Erythromycin Plasma Concentration-time Data ($AUC_{0-\infty}$, µg.h/ml).**

Source	sum of squares	Degrees of freedom	Mean squares	F
Between brands	31.20	3	10.40	0.81 NS
Between subjects	205.32	16	12.83	
Total	236.52	19		

The mean t 1/2 value computed was 1.9 h ± 1.1 (n =16) which is very close to the value of 2 hours quoted for erythromycin after oral administration (19).

Individual $AUC_{0-\infty}$ values were wide-ranged because of large intersubject variability, CV ranged from 64.6 to 116.2% ***(Table I).*** According to the absorption failure criterion, AUC< 3µg. h/ml, brand **A** tablets failed to be absorbed in four out of five subjects (mean $AUC_{0-\infty}$ = 2.09 µg. h/ml ± 1.46). The bioavailability of brands **A**, **B** and **B̄** relative to the standard preparation (brand **C**) was 40, 70 and 97%, respectively. However, interbrand and intersubjects differences in $AUC_{0-\infty}$ were insignificant at the 5% level when the data were subjected to the analysis of variance ***(Table II)*** . This is due to the limited number of subjects participating in the study and the wide intersubject variability. Nevertheless, the difference between $AUC_{0-\infty}$ of brand **A** and brand **C** tablets was significant at $P < 0.1$ when the t-test for paired data was applied.

Bearing in mind that erythromycin must achieve a plasma concentration of 0.6 µg/ml to be effective (17), AUC values were calculated from mean erythromycin plasma levels higher than 0.6 µg/ml (shaded areas in ***Fig. 2***). The magnitude of these areas is a function of the length of time erythromycin plasma levels remained above the MEC. Judging by these AUC values ***(Table I),*** the products under study appear obviously inequivalent. According to this parameter, the products can be arranged in order of efficacy as follows: brand $\bar{B}$ > brand C> brand B > brand A. The latter brand being presumably ineffective in the treatment of infections requiring a relatively high MIC.

The present study has revealed bioavailability differences among film-coated erythromycin stearate tablets. Although bioavailability is a complex parameter, the results point to a formulation problem probably associated with tablet coating. The poor or delayed erythromycin absorption exhibited by several serum concentrations-time profiles ***(Fig. 1),*** may be a consequence of failure of drug release from tablets. The problem appears to be particularly acute in case of brand **A**. The considerable differences between brands **B** and $\bar{B}$ tablets regarding Cmax (0.92 vrs 1.64 µg/ml) and AUC calculated from plasma levels> MEC (0.09 vrs 1.02 µg.h/ml) suggests that separate bioavailability studies should be conducted for different strength tablets from the same manufacturer, as recommended by the FDA for 125mg and 250mg erythromycin capsules (19). To assess the potential implication of formulation and manufacturing factors in the bioavailability variations observed, the ***In vitro*** performance of the tablet products under study has to be tested.

Further, the results of the study raise questions concerning the overall ***in-vivo*** performance of erythromycin stearate film-coated tablets In comparison with enteric-coated erythromycin base tablets. The latter showed the least proportion of absorption failure, the highest consistency of plasma concentration data and proved efficient according to the efficacy criterion AUC, µg.h/ml calculated from mean plasma levels above the MEC ***(Table I)***. Moreover, in comparative bioavailability studies using different erythromycin base and stearate tablet formulations, the entric-coated base actually gave greater serum levels and AUC values than the stearate salt products after a multiple dose regimen (3). Accordingly, optimization of erythromycin therapy may be achieved by proper formulation of erythromycin base tablets.

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