DOSE-RELATED PHARMACOKINETICS AFTER ORAL ADMINISTRATION OF A NEW FORMULATION OF ERYTHROMYCIN BASE

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1 Erythromycin concentrations in serum and urine were determined in 24 healthy male, fasting subjects after oral administration of 250, 500, or 1000 mg of erythromycin base (250 mg capsules containing enteric-coated pellets). The subjects also received a film-coated erythromycin stearate tablet (equivalent to 500 mg base).

2 The mean $\pm$ s.d. maximal serum erythromycin concentrations were 1.9 ± 0.8 , 3.8 ± 1.4 , 6.5 ± 2.9 and 2.9 ± 1.7 mg/l for 250, 500, or 1000 mg base and 500 mg stearate, respectively. The serum peaks usually occurred after 2 h irrespective of dosage form given.

3 The mean $\pm$ s.d. areas under the serum concentration v time curves ($AUC_{0-\infty}$) were 4.5 ± 1.7 , 11.2 ± 4.3 , 27.2 ± 10.6 and 7.5 ± 3.4 mg l⁻¹ .h after 250, 500, or 1000 mg base, and 500 mg stearate, respectively.

4 The urinary recoveries were 5.0, 6.7, 8.6% of the base doses given and 4.4% of the stearate dose given.

5 Dose-dependent excretion of erythromycin occurred. The increase in AUC was larger than multiples of the lowest base dose.

Introduction

Despite widespread use of erythromycin, controversy exists regarding the optimal oral dosage form of the agent, due to considerable variability and unpredictability in serum concentrations (Chun & Seitz, 1977; Parsons *et al.*, 1975). Variability in disposition (distribution plus elimination) is not a major source of unpredictability in serum erythromycin concentrations, as has been shown after intravenous dosage (Austin *et al.*, 1980). A new preparation of erythromycin which consists of enteric-coated pellets of erythromycin base has been developed (McDonald *et al.*, 1977). Following its use a more predictable and quantitatively higher absorption of erythromycin seems to be reached. Therefore we designed this study to investigate the pharmacokinetic variability of erythromycin after oral administration of three clinically relevant doses of the base and one of the stearate.

Methods

The protocol for the investigation was based on the guidelines of the 1975 Tokyo revision of the Helsinki

Declaration on ethics in human experimentation. The study was approved by the Ethics committee of St Erik's Hospital, Stockholm, Sweden.

Volunteers

Twenty-four healthy male volunteers with a mean age of 26.4 (22-36) years and a mean weight of 76.7 (63-92) kg participated in the study. They were healthy according to medical history, physical examination and routine laboratory tests within 2 weeks before and after the study. Informed signed consent was obtained and the volunteers were covered by insurance against possible mishaps during the investigation. The subjects were not permitted to receive any other antibiotics, or other pharmaceutical agents for 2 weeks prior to or during the study. There was no history of drug allergy in any of the subjects.

Antibiotics

Capsules containing enteric-coated pellets of 250 mg erythromycin base (batch No. 9G563B) were supplied by Astra Läkemedel AB, Södertälje, Sweden. Film-

coated tablets of erythromycin stearate (equimolar to 500 mg erythromycin base, batch No. 05004 PD), Abbott Laboratories (Illinois, USA) were from the Swedish market.

Design of study

One subject withdrew from the study because of a sports accident, but all the other 23 volunteers took all four doses. The 23 subjects were subdivided into two groups of 11 and 12 each. The two groups were assigned to different sequences of the four erythromycin regimens, on four separate days at weekly intervals in each group (Table 1). Three doses of erythromycin base 250, 500, and 1000 mg were given as enteric-coated pellets contained in 250 mg capsules and the fourth dose was erythromycin stearate as a tablet equimolar to 500 mg base. The volunteers fasted over-night for at least 10 h before each dose. The drugs were administered with 200 ml of water. A standardized low fat breakfast and a low fat lunch were each served 1 h and 6 h after dose administration. The breakfast consisted of three slices of wholewheat bread with a little margarine, 100 ml orange juice, 200 ml low fat milk, and one banana. The lunch consisted of one slice of rye bread, a slice of lean ham, 200 ml

low fat milk, one apple, a small biscuit, and cabbage salad. The subjects were ambulant, but not permitted to engage in any strenuous physical activity during the sampling period. Caffeine containing drugs were not allowed during the study and smoking was not permitted until 2 h after dosing.

Sampling procedures

Blood samples were drawn prior to dosing, which occurred at 08.00 h, and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 9 and 11 h. The samples were taken by venipuncture into evacuated tubes (Venoject^R, Terumo Corp., Tokyo, Japan) without heparin and totally 400 ml blood was taken. The blood was permitted to clot for 0.5–0.75 h before centrifugation and separation of serum. Urine was collected during the following intervals: 0–3, 3–6, and 6–9 h and 10 ml aliquots stored from each interval. All samples were then frozen at –20°C until analysis within 2 weeks.

Drug assay

The concentrations of erythromycin base were determined using the cylinder agar plate method with *Micrococcus luteus* (ATCC 9341) as test organism. The growth medium was Bacto Penassay Seed Agar (Difco Laboratories, Detroit, Michigan, USA) and the pH adjusted to 7.8 after autoclaving. The assay corresponded to the conditions described for erythromycin by Grove & Randall (1955). The lower limit of sensitivity was 0.06 mg/l.

Spiked samples were prepared by adding erythromycin base to pooled human serum. These samples were frozen together with the test samples and they were analysed in parallel with them. Adjusted theoretical concentrations of 1.23 and 0.41 mg/l had mean recoveries ($\pm$ s.d.) of 1.26 ± 0.23 mg/l ($n=9$) and 0.43 ± 0.05 mg/l ($n=8$), respectively.

Pharmacokinetic and statistical analyses

Pharmacokinetic analysis was performed with the computer program AUTOAN 2/NONLIN of Sedman and Wagner/Metzler (Wagner, 1975). This program performs curve fitting either by curve stripping and automatic model selection, or after operator assisted model selection and input of start parameters obtained from graphical extrapolations of data. The one- or two-compartment open models with first order absorption and elimination applied to most of the curves. The correlation coefficient (> 0.985) between observed and predicted values and visual inspection of the fit of the data to the computed curve, were used as criteria for acceptable curve fitting. The curve fitting was not acceptable for three subjects after 250 mg of the base, two after 500 mg base, three after 1000 mg base, and one volunteer after the 500 mg stearate

Table 1 Regimen sequences for erythromycin doses: 250 mg (A), 500 mg (B), 1000 mg base (C), and 500 mg stearate (X).

Subject number	Day			
	1	2	3	4
1	C	A	B	X
2	X	C	A	B
3	A	B	X	C
4	B	X	C	A
5	A	C	B	X
6	B	A	X	C
7	C	X	A	B
8	X	B	C	A
9	C	B	X	A
10	X	A	B	C
11	B	C	A	X
12	A	X	C	B
13*	B	C	X	A
14	X	A	C	B
15	C	B	A	X
16	A	X	B	C
17	C	A	X	B
18	X	C	B	A
19	B	X	A	C
20	A	B	C	X
21	X	B	A	C
22	A	C	X	B
23	C	X	B	A
24	B	A	C	X

*Subject No. 13 terminated the study, because of a sports accident, and he is excluded from the evaluations.

dose. Consequently, since a model allowing successful curve fitting is required for valid assessments, pharmacokinetic evaluation for these subjects were not included. The total area under the serum concentration curve (AUC) to infinity was determined both from the curve fitting data and by the trapezoidal rule. Renal clearance of erythromycin (Cl_R) was calculated by dividing the amount of erythromycin found in urine by the corresponding AUC.

Statistical analyses of the significance of differences were by Wilcoxon's rank and signed rank tests (Weber, 1972).

Results

Inter- and intrasubject variability

The mean serum concentrations after each dose of erythromycin base are shown in Figure 1. Peak concentrations after the base and the stearate dosage forms were usually observed after 1.5–2.5 h. For most subjects, the serum erythromycin peaks of the mean curve were higher after the base than these levels obtained after the stearate; these averaged 3.5 and 2.5 mg/l respectively ($P < 0.05$). The mean $\pm$ s.d. of the maximal individual serum concentration were 1.9 ± 0.8 , 3.8 ± 1.4 , and 6.5 ± 2.9 mg/l for the 250, 500, and 1000 mg doses of the base and 2.9 ± 1.7 mg/l for the stearate dose equivalent to 500 mg base.

Eight subjects showed a biphasic serum-concentration profile and one subject a late peak after one of their four doses. An example of this pattern is shown in Figure 2. The second serum peaks occurred 4–7 h after the drug intake.

Pharmacokinetics

Pharmacokinetic parameters for all doses are shown in Table 2. Similar t_{lag} values were observed after all three doses of the base. The t_{lag} for the stearate was not significantly shorter.

Figure 2 Serum level of erythromycin base and urinary excretion (% of dose) in one subject who exhibited two successive serum peaks following a single oral administration of 1000 mg erythromycin base.

Figure 1 Mean serum levels ($\pm$ s.e. mean) of erythromycin following oral administration of single doses of erythromycin base 250 mg ($\square$), 500 mg ($\circ$), 1000 mg (Δ), and stearate, 500 mg base equivalent ($\bullet$). Cross-over study in 23 healthy volunteers. Dotted line: five subjects at 11 h.

Table 2 Pharmacokinetic parameters describing erythromycin disposition in relation to dose (mean $\pm$ s.d.)

	Dose (mg base)			
	250	500	1000	500 (stearate)
<i>Serum</i>				
<i>n</i>	20	22	21	22
lag (h)	1.4 $\pm$ 0.3	1.4 $\pm$ 0.8	1.3 $\pm$ 0.4	1.1 $\pm$ 0.4
k_a (h^{-1})	3.2 $\pm$ 1.3	4.3 $\pm$ 2.2	3.2 $\pm$ 1.8	2.3 $\pm$ 0.7
k_e (h^{-1})	0.8 $\pm$ 0.3	0.5 $\pm$ 0.1	0.4 $\pm$ 0.1	0.6 $\pm$ 0.2
c_0 (mg/l)	3.8 $\pm$ 2.2	6.1 $\pm$ 2.6	10.3 $\pm$ 4.2	4.7 $\pm$ 2.6
AUC (mg l $^{-1}$ h)*	4.5 $\pm$ 1.7	11.2 $\pm$ 4.3	27.2 $\pm$ 10.6	7.5 $\pm$ 3.4
AUC (mg l $^{-1}$ h)†	5.4 $\pm$ 3.4	13.2 $\pm$ 4.1	28.6 $\pm$ 11.2	8.0 $\pm$ 3.6
$T_{1/2}$ (h)	2.0 $\pm$ 2.0	2.5 $\pm$ 1.0	3.0 $\pm$ 1.7	2.7 $\pm$ 2.3
<i>Urine</i>				
<i>n</i>	10	11	10	10
f_u	0.05 $\pm$ 0.03	0.07 $\pm$ 0.02	0.09 $\pm$ 0.05	0.04 $\pm$ 0.03
Cl _R (l/h)	3.0 $\pm$ 1.2	3.5 $\pm$ 1.6	2.9 $\pm$ 1.4	2.7 $\pm$ 1.1

lag lag time, k_a absorption constant, k_e elimination constant, c_0 sum of the hybrid intercepts A and B for each model compartment, AUC area under serum concentration ν time curve, $T_{1/2}$ half-life in serum, f_u fraction of dose excreted as microbiologically active erythromycin during 9 h, Cl_R renal clearance.

*AUC calculated with the trapezoidal rule; 23 subjects, 21 in the 250 mg group

†AUC calculated with the AUTOAN 2/NONLIN program

The elimination rate constant (k_e) was significantly higher after higher base doses ($P < 0.01$). The elimination half-life ($T_{1/2}$) was similar with the 500 mg base and stearate doses ($P < 0.05$), but $T_{1/2}$ was longer following higher base doses (1000 mg ν 250 mg; $P < 0.05$). There was a trend for f_u (fraction of dose excreted in urine) to increase with the dose of the base (Table 2). The renal clearance (Cl_R) was not dose dependent.

For the 500 mg doses of the two drugs, the area under the serum concentration ν time curve (AUC) was 49–65% higher after the base than after the stearate ($P < 0.01$). The AUC increased linearly with increasing doses of the base, but the increases were superproportionate for higher doses compared to the lowest dosage size (Figure 3). Thus the 500 mg dose of the base had an AUC (trapezoidal), which was 24% and the 1000 mg an AUC, which was 51% higher ($P < 0.01$) than the values which were to be expected compared to the lowest dose of 250 mg base, when the relative size of the doses are considered.

Adverse effects

No acute haematological, renal, or hepatic toxicity was observed with either dosage form as evidenced by blood chemistry. Only gastrointestinal side-effects were reported. Thus, 3.5 h after the intake of the 1000 mg dose of the base one subject vomited. After the same dose two others had epigastric discomfort with nausea. The nausea appeared soon after the breakfast (given 1 h after the dose) and lasted for 2–3 h. Loose stool occurred in one of these three subjects. The peak serum values for these three subjects were: 10.6, 10.2 and 12.2 mg/l, compared to the mean peak serum value which was 6.3 mg/l.

Discussion

The better absorption of the 500 mg base dose, with AUC being 49–65% higher than that of the stearate, compared well with findings following 250 mg doses of the same preparation of the erythromycin base (McDonald *et al.*, 1977).

The design of the protocol for this study took the points discussed by Chun & Seitz (1977) into consideration to avoid pitfalls associated with erythromycin. The doses were accordingly given 1 h before breakfast (McDonald *et al.*, 1977). Breakfast and lunch were both standardized and low in fats. This in particular followed experience derived from the study by Welling *et al.* (1978). The number of subjects was high (23) to improve the chance of discovering possible deviate patterns or findings and of improving validity, since previous studies have indicated a large variation between individuals. The doses were administered early in the morning because of several previous reports to the effect that the peak serum concentrations appear to be influenced by the time of dose ingestion (Colburn *et al.*, 1977; McDonald *et al.*, 1977). This has been discussed as a chronoavailability or circadian effect (Colburn *et al.*, 1977). The volunteers weighed within 10% of their ideal body weight compared to body length (Diem & Lentner, 1970). This was a concern, to avoid aberration due to differences in body, since a positive correlation has recently been indicated between the body weight and $T_{1/2}$ after intravenous administration of erythromycin (Austin *et al.*, 1980). Physical activity of the subjects was kept moderate because Parsons *et al.* (1977) have found longer half-lives in supine volunteers than in active persons. Frequent sampling was employed to ensure a complete monitoring of the

Figure 3 Relationship between the doses of erythromycin base and the total area under serum concentration curve ($AUC_{0-\infty}$) in 23 healthy volunteers. The bars indicate s.e. mean. Calculation of AUC with the trapezoidal rule (O) and with the AUTOAN 2/NONLIN program (Δ).

serum response and a cross-over design utilized to better permit comparisons between doses.

Considering all these precautions in the design of the investigation, it is significant that our results show a distinct dose dependency for orally administered erythromycin base. This is evidenced by superproportionate areas under the serum concentration v time curves (AUC). There was also a trend for f_u to increase with dose. This can have several explanations. A degree of capacity limited saturation of enzymes mediating biotransformation within the liver or another metabolizing system is a most probable factor. This would chiefly apply to unchanged erythromycin itself, which represents the major antibacterial component in serum, or to a metabolite with retained biological activity detected by the assay.

The elimination rate constant, k_e , was higher and serum half-life values consequently longer, with higher doses of the base ($P < 0.05$). A dose-related urinary excretion of erythromycin has also been found after intravenously administered erythromycin (Austin *et al.*, 1980). They also found that the distribution volume at steady-state (V_{dss}) and $T_{1/2}$ were subject to dose dependency. In contrast to our findings, they also observed AUC not to be superproportionate to dose in only 3–5 subjects. We would put

more emphasis on the conclusions from the larger number of subjects involved in our study.

A recent observation shows that erythromycin is accumulated inside macrophages and that this loading is dependent on oxidative metabolic processes (Johnson *et al.*, 1980). Erythromycin base may be actively secreted into the intestinal lumen (Holland & Quay, 1980). It is not known whether these processes are influenced by dose variations. Dose-dependent pharmacokinetics, though, has been described for a few other antibiotics e.g. the ureidopenicillins azlocillin (Bergan *et al.*, submitted for publication; Leroy *et al.*, 1980), mezlocillin (Bergan, 1978), and piperacillin (Bergan & Williams, in preparation).

The well established fact that low serum concentrations of erythromycin are sometimes experienced was verified by this study. The absorption of early doses may occur late or not at all, as also pointed out by other investigators (Mather *et al.*, 1981). Higher serum concentrations than the mean serum levels, however, may follow subsequent doses, which we have observed in a multiple dose study with the microencapsulated base (Josefsson *et al.*, 1982). These observations raise the question whether the dose dependency noted may be more related to the first dose and would disappear during establishment

of steady-state conditions. The serum concentrations and the AUC-values were compatible with other published data on either the new formulation or that of the stearate (McDonald *et al.*, 1977; Berend *et al.*, 1979). The mean AUC values ($\pm$ s.d.) after 500 mg stearate was 7.5 ± 3.4 mg l⁻¹ h in this study which is comparable with the 7.5 ± 3.9 mg l⁻¹ h reported by Berend *et al.* (1979), but higher than the values reported for fasting subjects by Malmberg (1979).

If it is in the acid stomach for a longer period of time, the filmcoating on the stearate tablet will become dissolved and the drug partially destroyed before absorption in the duodenum (Welling *et al.*, 1978). This process may be influenced by the volume of fluid used to swallow the drug. Fasting individuals absorb the stearate 43% less when the volume of the co-administered water is reduced from 250 to 20 ml (Welling *et al.*, 1978). The capsules of erythromycin base are dissolved in the gastric fluid releasing the enteric-coated pellets but the coating protects the antibiotic until the pellets pass through the pyloric sphincter. The enteric coat is readily released inside the duodenal contents.

A biphasic serum concentration curve was observed once after one of their four doses in nine subjects. The reason for this phenomenon is not fully known, but it does not appear to be subject related, since it only occurred once in each subject. Consequently, in spite of the fairly large number of subjects involved in

this cross-over study, it is difficult to predict who will show this serum curve pattern. After the 500 mg doses, a second serum peak occurred in only one subject after the stearate compared with two subjects after the base but this might be a coincidence rather than indicating a true difference between the compound since the stearate liberates base as such in the body. Our observations of two successive serum peaks in the same individuals may indicate an entero-hepatic pathway as proposed for another macrolide, josamycin (Bergan & Øydvinn, 1972). Bimodal serum curves have also been observed after food consumption following intravenous doses of erythromycin (Austin *et al.*, 1980). Secretions of high erythromycin concentrations into the gastrointestinal tract and subsequent reabsorption were suggested as a possible explanation (Holland & Quay, 1976). Another possibility mentioned was displacement by lipids or other food components.

In conclusion this study indicates that the encapsulated enteric-coated formulation of erythromycin base gave significantly higher maximal concentrations than the stearate formulation ($P < 0.05$). With increasing doses, erythromycin base demonstrated a dose-dependent pharmacokinetics, which was reflected by AUC and the urinary excretion, which were superproportionate compared to dosage size.

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