Biochemical Pharmacology, Vol. 32, No. 6, pp. 1063–1068, 1983.
Printed in Great Britain.

0006-2952/83/061063-06 $03.00/0

EFFECTS OF ERYTHROMYCIN ON HEPATIC DRUG-METABOLIZING ENZYMES IN HUMANS

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(*Received* 30 *July* 1982; *accepted* 21 *October* 1982)

Abstract—In rats, erythromycin has been shown to induce microsomal enzymes and to promote its own transformation into a metabolite which forms an inactive complex with reduced cytochrome P-450. To determine whether similar effects also occur in humans, we studied hepatic microsomal enzymes from six untreated patients and six patients treated with erythromycin propionate, 2 g per os daily for 7 days. In the treated patients, NADPH–cytochrome *c* reductase activity was increased; the total cytochrome P-450 concn was also increased but part of the total cytochrome P-450 was complexed by an erythromycin metabolite. The concn of uncomplexed (active) cytochrome P-450 was not significantly modified and the activity of hexobarbital hydroxylase remained unchanged. We also measured the clearance of antipyrine in six other patients; this clearance was not significantly decreased when measured again on the seventh day of the erythromycin propionate treatment. We conclude that the administration of erythromycin propionate induces microsomal enzymes and results in the formation of an inactive cytochrome P-450–metabolite complex in humans. However, the concn of uncomplexed (active) cytochrome P-450 and tests for *in vitro* and *in vivo* drug metabolism were not significantly modified.

Following several reports of severe drug interactions with troleandomycin (triacetyloleandomycin) [1–4], a few patients have been reported with possible erythromycin-induced drug interactions [5–9]. This caused considerable concern because erythromycin is widely used world-wide. It is not yet clear, however, whether erythromycin interferes or not with drug metabolism in humans. While several workers reported a decreased clearance of theophylline in patients receiving erythromycin [10–13], other workers did not confirm this finding [14–16].

Erythromycin and troleandomycin are chemically related macrolide antibiotics. Their chemical structures involve a tertiary amine function, $-N(CH_3)_2$. In rats, troleandomycin [17], erythromycin [18] and several erythromycin derivatives (Larrey *et al.*, unpublished results) have been shown to induce microsomal enzymes and to promote their own demethylation and oxidation into metabolites, probably the respective nitrosoalkanes, which form stable (inactive) complexes with the iron(II) of cytochrome P-450. Eventually, monooxygenase activities may be decreased [18, 19]. The doses of erythromycin and erythromycin derivatives that were used in these rat studies (4 mmoles.kg^{-1} daily for 4 days) were, however, considerably greater than those used therapeutically in humans. Because of these marked differences in doses, and because of possible species differences, it could be questioned whether similar effects would also occur in humans.

In this communication, we report the effects of therapeutic doses of erythromycin propionate on hepatic drug-metabolizing enzymes and on the clearance of antipyrine in humans.

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METHODS

Surgical patients. To determine the effects of erythromycin propionate on drug-metabolizing enzymes in humans, we used a protocol identical to that previously employed to assess the effects of troleandomycin in humans [20]. A liver specimen removed by surgical biopsy was obtained in patients undergoing elective abdominal surgery and in whom an histologic examination of the liver was thought advisable for various reasons (digestive carcinoma, gall-bladder stones, past intemperance, past hepatitis). We required, in addition, that these patients: (a) had abstained from alcohol and drugs for at least 2 weeks before randomization, (b) did not require any drug until premedication, and (c) had normal liver function tests at the time of randomization (serum bilirubin, serum albumin, prothrombin time, γ-glutamyl transpeptidase, alkaline phosphatase, ALAT and ASAT activities).

Eligible patients who gave their informed consent were randomly allocated into two groups. In a first group of six patients, no medication was given until premedication and anesthesia. In a second group of seven patients, erythromycin propionate (0.5 g at 8 a.m., 0.5 g at 2 p.m. and 1 g at 8 p.m.) was given per os daily for the 7 days preceding surgery. The last dose of erythromycin propionate was administered at 8 p.m. on the day preceding surgery.

Patients were premedicated with atropine and diazepam, and were anesthetized with droperidol, nitrous oxide, thiopental sodium, phenoperidine and pancuronium bromide. A surgical liver biopsy was

Table 1. Hepatic drug-metabolizing enzymes in untreated patients (U) and in patients treated with erythromycin propionate (T)*

Patient	Sex	Age (yr)	Surgical disease	Microsomal protein (mg.g liver^{-1})	NADPH–cytochrome *c* reductase (nmoles.min^{-1}. mg microsomal protein^{-1})	Uncomplexed cytochrome P-450 (nmoles.mg microsomal protein^{-1})	Total cytochrome P-450 (nmoles.mg microsomal protein^{-1})	Complexed cytochrome P-450 (nmoles.mg microsomal protein^{-1})	Hexobarbital hydroxylase (nmoles.min^{-1}. mg microsomal protein^{-1})
U_1	F	61	Gall-bladder stones	37	51	0.41	0.41	N.D.	0.34
U_2	F	59	Carcinoma of the esophagus	23	47	0.22	0.23	N.D.	0.47
U_3	M	38	Gall-bladder stones	30	45	0.16	0.16	N.D.	0.23
U_4	M	40	Carcinoma of the ampulla of Vater	30	56	0.19	0.21	N.D.	0.08
U_5	M	57	Carcinoma of the esophagus	23	44	0.34	0.36	N.D.	0.18
U_6	F	36	Colic endometriosis	45	56	0.61	0.62	N.D.	0.29
Mean ± S.D.		49 ± 12		31 ± 8	50 ± 5	0.32 ± 0.17	0.33 ± 0.17		0.27 ± 0.13
T_1	F	71	Carcinoma of the stomach	36	78	0.45	0.68	0.25	0.22
T_2	M	45	Carcinoma of the esophagus	50	86	0.54	0.70	0.15	0.38
T_3	F	55	Gall-bladder stones	34	92	0.49	0.56	0.08	0.36
T_4	M	72	Carcinoma of the esophagus	28	126	0.26	0.42	0.19	0.19
T_5	M	50	Carcinoma of the esophagus	40	118	0.61	0.71	0.10	0.37
T_6	M	49	Carcinoma of the esophagus	32	92	0.48	0.57	0.12	0.36
Mean ± S.D.		57 ± 12		37 ± 8	99 ± 19†	0.47 ± 0.12	0.61 ± 0.11†‡	0.15 ± 0.06	0.31 ± 0.08

* Uncomplexed cytochrome P-450 was measured as the CO-difference spectrum of dithionite-reduced microsomes; total cytochrome P-450 was similarly measured after first adding 50 μM potassium ferricyanide to the microsomes; complexed cytochrome P-450 was calculated from its Soret peak at 456 nm with a molar extinction coefficient of 75 mM^{-1}.cm^{-1}; N.D., not detected.

† Significantly different from that in untreated patients, $P < 0.01$ (Student's *t*-test for independent data).

‡ Significantly different from uncomplexed cytochrome P-450, $P < 0.01$ (Student's *t*-test for dependent data).

performed immediately after laparotomy. A liver fragment was placed in Bouin's fluid for histologic examination. Another liver fragment was placed in ice-cold 0.154 M NaCl and immediately brought to the near-by laboratory. Liver fragments were minced and homogenized in 3 vols of ice-cold 0.154 M KCl, 0.01 M sodium/potassium phosphate buffer, pH 7.4. The homogenate was centrifuged at 10,000 *g*, and the supernatant was centrifuged at 100,000 *g* for 60 min. The microsomal pellets were stored at −20° until the conclusion of the histologic examination was available.

As a last criterion for final inclusion in the study, we required that the liver be normal on histologic examination. One patient (in the treated group) had fibrosis of the liver, probably related to past alcoholism. His hepatic microsomes were discarded. The liver histology was normal in the 12 patients finally studied (Table 1).

Microsomal enzymes. The cytochrome P-450 Fe(II)–erythromycin metabolite complex which is formed in rats has several properties [18] which should allow its easy characterization if it is also formed in humans. The complex exhibits a Soret peak at 456 nm and is unable to bind CO [18]. The complex, however, is unstable in the ferric state: addition of 50 μM potassium ferricyanide to the microsomes oxidizes the iron to the ferric state, destroys the complex, and regenerates uncomplexed (CO-binding) cytochrome P-450 [18].

We, therefore, measured uncomplexed, complexed and total cytochrome P-450 as follows. Microsomal suspensions containing microsomes from 62 mg of liver.ml^{-1} were prepared. With a first batch of microsomes, we measured CO-binding cytochrome P-450 as described by Omura and Sato [21]: we added dithionite in both cuvettes and bubbled CO in the test cuvette. Because complexed cytochrome P-450 cannot bind CO, the difference spectrum at 450 nm (Fig. 1) measured uncomplexed cytochrome P-450 only. With a second batch of microsomes, we now looked for the presence of a 456-nm-absorbing complex. We placed microsomes in both cuvettes and added 50 μM potassium ferricyanide in the reference cuvette to destroy the complex, if any, in this cuvette. The difference spectrum at 456 nm (Fig. 1) measured the Soret peak of complexed cytochrome P-450 in the test cuvette. With the same batch of microsomes, we now added 50 μM potassium ferricyanide in the test cuvette also, so that the complex was now destroyed in both cuvettes. We ran a second spectrum, to show that the 456-nm-absorbing complex had indeed disappeared (Fig. 1). We now added dithionite, in excess, in both cuvettes to reduce cytochrome P-450 Fe(III) to the ferrous state, and we bubbled CO in the test cuvette as described by Omura and Sato [21]. Because the complex, when present, had been destroyed, the absorption at 450 nm (Fig. 1) now measured total cytochrome P-450.

NADPH–cytochrome *c* reductase activity was measured as previously reported [22]. Microsomal protein concn was measured by the method of Lowry *et al.* [23]. The *in vitro* activity of hexobarbital (0.25 mM) hydroxylase was measured as described by Kupfer and Rosenfeld [24].

Medical patients. The clearance of antipyrine was determined on two occasions in six patients admitted for mild respiratory tract infections (Table 2). Temp. was less than 38°. Other conditions for inclusion were abstinence from alcohol or drugs for at least 2 weeks before the study and absence of other disease; in particular, no clinical or biochemical evidence of liver disease, as judged by normal values for serum bilirubin, serum albumin, prothrombin time, γ-glutamyl transpeptidase, alkaline phosphatase, ALAT and ASAT activities.

In eligible patients who gave their informed consent, the first determination of the antipyrine clearance was made 2–4 days after admission. Starting on the following day, the patients were then treated with erythromycin propionate (0.5 g at 8 a.m., 0.5 g at 2 p.m. and 1 g at 8 p.m., daily for 7 days). A second determination of the antipyrine clearance was made on the seventh day of this treatment.

We had previously observed that, in patients admitted for mild respiratory tract infections and receiving no treatment, the clearance of antipyrine measured 2–4 days after admission was not significantly modified when measured again 7 days later [20].

Antipyrine clearance. The clearance of antipyrine was determined as follows: antipyrine (15 mg.kg body wt^{-1}) was administered intravenously at 8 a.m., and blood samples were drawn at 11 a.m., 2 p.m., 5 p.m. and 8 p.m. The concn of antipyrine in plasma was measured as reported by Brodie *et al.* [25]. We verified that erythromycin propionate, in concns previously reported in humans [26], does not interfere with this assay. Both before, and during, the administration of erythromycin propionate, the natural logarithm of the antipyrine concn (C) decreased linearly with time (t) according to the equation: $\ln C = \ln C_0 - \beta t$. $\ln C_0$ and β were calculated as the intercept and the slope, respectively, of the linear regression line. The apparent volume of distribution (V_d) was calculated as the dose/C_0 ratio; the clearance of antipyrine was determined as the $\beta.V_d$ product.

RESULTS

Microsomal enzymes

The administration of erythromycin propionate, 2 g per os daily for seven days, did not significantly modify hepatic microsomal protein concn but increased by 98% the activity of NADPH–cytochrome *c* reductase (Table 1).

The concn of uncomplexed cytochrome P-450 (measured by its CO-binding in microsomes not treated with potassium ferricyanide) was not significantly increased in the treated patients (Table 1).

In the treated patients, however, part of total cytochrome P-450 was present as a cytochrome P-450–metabolite complex, unable to bind CO. Like the inactive complex formed in rats [18], this complex exhibited a Soret peak at 456 nm (Fig. 1). Quantitation of complexed cytochrome P-450 from the Soret peak at 456 nm and the molar extinction coefficient (75 mM^{-1}.cm^{-1}) previously reported for similar complexes [20, 27], indicated that, on average, 0.15 nmoles of cytochrome P-450 was complexed with the metabolite (Table 1). Like the inactive

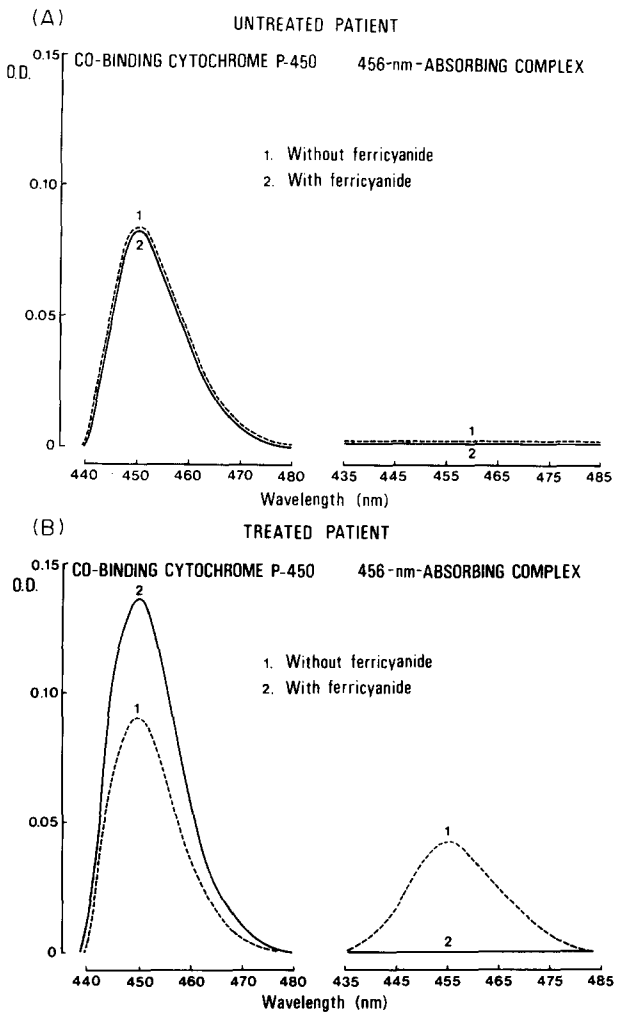

Fig. 1. Determination of uncomplexed, complexed and total cytochrome P-450. The figure shows recordings obtained with microsomes from: (A) an untreated patient (patient U_1 of Table 1), and (B) a patient treated with erythromycin propionate (patient T_1 of Table 1). The microsomal suspensions contained microsomes from 62 mg of liver.ml^{-1}. In the untreated patient, the CO-binding spectrum of dithionite-reduced microsomes was not modified by previous addition of potassium ferricyanide. There was no absorption peak at 456 nm. In microsomes from the treated patient, there were two forms of cytochrome P-450: uncomplexed cytochrome P-450, which bound CO and did not absorb at 456 nm, and complexed cytochrome P-450, which absorbed at 456 nm but did not bind CO. Addition of potassium ferricyanide to the microsomes disrupted the cytochrome P-450 Fe(II)–metabolite complex, as shown by the disappearance of its Soret peak at 456 nm. This liberated uncomplexed cytochrome P-450, and the CO-binding spectrum of dithionite-reduced microsomes increased. Note also that total cytochrome P-450 (CO-binding after the addition of potassium ferricyanide) is higher in the treated patient than in the untreated patient. O.D., optical density.

Table 2. Metabolism of antipyrine before and during the administration of erythromycin propionate*

				Volume of distribution		Clearance	
Patient	Sex	Age (yr)	Medical diagnosis	First determination (ml.kg^{-1})	Second determination (ml.kg^{-1})	First determination (ml.min^{-1}.kg^{-1})	Second determination (ml.min^{-1}.kg^{-1})
1	F	44	Coryza	759	785	0.70	0.43
2	M	46	Bronchitis	683	937	1.14	1.34
3	M	68	Coryza	765	826	0.37	0.41
4	M	78	Coryza	809	735	0.74	0.61
5	M	58	Bronchitis	836	661	1.12	0.42
6	M	31	Bronchitis	715	690	0.74	0.55
Mean ± S.D.		54 ± 17		761 ± 57	772 ± 101	0.80 ± 0.29	0.63 ± 0.36

* The metabolism of antipyrine was determined before, and on the seventh day of, an erythromycin propionate treatment (2 g per os daily).

complex formed in rats [18], the cytochrome P-450 Fe(II)–metabolite complex present in human microsomes was unstable in the ferric state. Treatment of the microsomes with 50 μM potassium ferricyanide oxidized the iron to the ferric state and disrupted the complex, as shown by the disappearance of its Soret peak at 456 nm (Fig. 1). The disruption of the complex liberated equimolar amounts of uncomplexed cytochrome P-450, as shown by the increased binding spectrum of dithionite-reduced microsomes (Fig. 1, Table 1) after the addition of potassium ferricyanide.

Total cytochrome P-450 (measured as the CO-binding of microsomes treated with potassium ferricyanide) was 85% higher in the treated patients than in the untreated patients (Table 1).

The activity of hexobarbital hydroxylase was similar in treated and untreated patients (Table 1).

Antipyrine clearance

The clearance of antipyrine was not significantly decreased when measured again on the seventh day of the erythromycin propionate treatment (Table 2). The V_d of antipyrine was similar before, and during, the administration of erythromycin propionate (Table 2).

DISCUSSION

In rats, erythromycin has been shown to induce microsomal enzymes and to promote its own transformation into a metabolite which forms an inactive complex with reduced cytochrome P-450 [18]; several erythromycin derivatives produced grossly similar effects, the estolate and the propionate appearing, however, as the most active (Larrey *et al.*, unpublished results).

Our results show that similar effects occur after the administration of therapeutic doses of erythromycin propionate in humans.

(a) In rats, the administration of erythromycin increases the activity of NADPH–cytochrome *c* reductase and the concn of total cytochrome P-450 in the liver [18]. Similarly, in humans, NADPH–cytochrome *c* reductase activity was increased by 98% and the total cytochrome P-450 concn was increased by 85% in patients treated with erythromycin propionate (Table 1).

(b) In rats, the induced species of cytochrome P-450 have a high affinity with erythromycin and actively demethylate and oxidize this macrolide into a metabolite, probably the nitrosoalkane, which forms an inactive complex with the iron(II) of cytochrome P-450 [18]. The cytochrome P-450 Fe(II)–metabolite complex exhibits a Soret peak at 456 nm and is unable to bind CO [18]. Addition of potassium ferricyanide, however, oxidizes the iron to the ferric state, destroys the complex and liberates uncomplexed (CO-binding) cytochrome P-450 [18]. Our results show that a similar complex is formed after the administration of erythromycin propionate in humans: microsomes from the treated patients exhibited a Soret peak at 456 nm (Fig. 1, Table 1); treatment of the microsomes with potassium ferricyanide suppressed the absorption peak at 456 nm and increased the CO-binding capacity of dithionite-reduced microsomes (Fig. 1, Table 1). Quantitation of complexed cytochrome P-450 from its Soret peak at 456 nm (Table 1) indicated that, on average, 0.15 nmoles.mg microsomal protein^{-1} of cytochrome P-450 was complexed with the metabolite. A similar value was obtained from the difference between total and uncomplexed cytochrome P-450 in the treated patients (Table 1). This amount of complexed cytochrome P-450 represents about half the amount of cytochrome P-450 present in the untreated patients (0.32 nmoles.mg microsomal protein^{-1}).

(c) In rats, the induction of cytochrome P-450 and its concomitant inactivation by the erythromycin metabolite leaves normal (or slightly increased) concns of uncomplexed (active) cytochrome P-450 [18]. Similarly, in humans, the concn of uncomplexed cytochrome P-450 was not significantly increased by the administration of erythromycin propionate (Table 1).

(d) In rats, despite normal concns of uncomplexed (active) cytochrome P-450, the activity of hexobarbital hydroxylase was found to be decreased in microsomes from rats killed 24 hr after the last dose of erythromycin (4 mmoles.kg^{-1} daily for 4 days) [18]. The mechanism for this effect is incompletely delineated and several mechanisms have been proposed [18]. Conceivably, after the administration of these very high doses, erythromycin may persist in the liver at the time of death, stick to the microsomes during their preparation, and compete with hexobarbital for binding to uncomplexed cytochrome P-450 [18]. After the administration of lower doses of erythromycin (1 mmole.kg^{-1} daily for 4 days), less of the macrolide may remain in isolated microsomes and the activity of hexobarbital hydroxylase remained unchanged [18]. Similarly, in humans, where the doses are even smaller, the activity of hexobarbital hydroxylase was not significantly modified (Table 1). Shortly after the administration of erythromycin, drug concns in the liver may be higher than those remaining at later times in isolated microsomes. We, therefore, also measured the clearance of antipyrine in subjects concomitantly receiving erythromycin propionate (Table 2). Although there was a small trend for a decreased clearance of antipyrine, this effect was not statistically significant (Table 2). Similarly, the conflicting results reported in the literature may indicate that erythromycin has little, if any, effect on the mean value for the clearance of theophylline in humans [10–16].

A few patients have been reported with possible erythromycin-induced drug interactions [5–9]. Some of these observations may have been fortuitous and mainly supported by possible analogies with the known effects of troleandomycin [1–4]. It is conceivable, however, that drug interactions may indeed occur in a few patients. Individual variability is to be expected in a system where drug-metabolizing enzymes are both induced and inactivated. Because of individual variability, drug clearances, albeit barely modified for the whole group, may nevertheless be notably decreased in an individual patient. Indeed, the clearance of antipyrine decreased by 63% in one of our patients (Table 2). It is conceivable that drug interactions may occur in those few patients

in whom drug clearances are most severely depressed.

The effects of erythromycin on hepatic drug-metabolizing enzymes resemble those of troleandomycin in that both macrolides induce microsomal enzymes and form inactive cytochrome P-450–metabolite complexes in humans [20]. Unlike erythromycin, however, troleandomycin appears as a potent inhibitor of drug metabolism in rats [17, 19] and humans [20]. In rats, several monooxygenase activities were markedly depressed after the administration of troleandomycin (1 mmole.kg^{-1} daily for 4 days) whereas only the hexobarbital hydroxylase activity was decreased after the administration of higher doses of erythromycin (4 mmoles.kg^{-1} daily for 4 days) [18]. The latter effect disappeared after administration of lower doses of erythromycin (1 mmole.kg^{-1} daily), whereas the hexobarbital hydroxylase activity was still decreased after very low doses of troleandomycin (0.06 mmoles.kg^{-1} daily) [28]. In humans, the concomitant administration of troleandomycin decreased by 45, 50 and 60%, respectively, the clearance of antipyrine [20], theophylline [2] and methylprednisolone [29]. In contrast, erythromycin has little, if any, effect on the mean value for the clearance of antipyrine (Table 2) and that of theophylline [10–16].

We conclude that erythromycin propionate, given in therapeutic doses, induces microsomal enzymes and forms an inactive cytochrome P-450–metabolite complex in humans. However, the concn of uncomplexed (active) cytochrome P-450 was not significantly modified, the activity of hexobarbital hydroxylase remained unchanged, and the mean value for the clearance of antipyrine was not significantly decreased.

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