Biochemical Pharmacology, Vol. 31, No. 9, pp. 1699–1704, 1982.
Printed in Great Britain.

0006-2952/82/091699-06 $03.00/0

FORMATION OF AN INACTIVE CYTOCHROME P-450 Fe(II)-METABOLITE COMPLEX AFTER ADMINISTRATION OF TROLEANDOMYCIN IN HUMANS

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(*Received* 10 *September* 1981; *accepted* 7 *December* 1981)

Abstract—In rats, it has been shown that troleandomycin induces its own transformation into a metabolite forming an inactive complex with reduced cytochrome P-450. To determine whether similar effects occur in humans, we studied hepatic microsomes from 6 untreated patients and 6 patients treated with troleandomycin, 2 g per os daily for 7 days. In the treated patients, NADPH–cytochrome *c* reductase activity was increased by 48%; total cytochrome P-450 concentration was also increased, but 33% of total cytochrome P-450 was complexed by a troleandomycin metabolite. The cytochrome P-450 Fe(II)-metabolite complex exhibited properties identical to those of the inactive complex formed in rats: it exhibited a Soret peak at 456 nm, was unable to bind CO, and was destroyed by addition of 50 μM potassium ferricyanide.

We also measured the clearance of antipyrine in 6 other subjects. This clearance was decreased by 45% when measured again on the seventh day of the troleandomycin treatment. We conclude that repeated administration of troleandomycin induces microsomal enzymes, produces an inactive cytochrome P-450 Fe(II)-metabolite complex, and decreases the clearance of antipyrine in humans.

Troleandomycin (triacetyloleandomycin), a macrolide antibiotic, is still widely used in some countries. The drug may elicit severe drug interactions in humans. Concomitant administration of troleandomycin with oral contraceptives may produce cholestasis [1], it may also produce ischemic accidents with ergotamine [2] and neurologic signs of carbamazepine intoxication [3].

In rats, troleandomycin induces microsomal enzymes, but, in the process, promotes its own transformation into a metabolite forming an inactive complex with the iron(II) of reduced cytochrome P-450 [4, 5]; eventually, several monooxygenase activities are markedly decreased [6, 7].

Because of the high doses employed in the rat studies [4–7], and of possible species differences, it could be questioned whether similar effects would also occur after administration of therapeutic doses in humans.

In this communication, we report the effects of troleandomycin administration on hepatic drug-metabolizing enzymes and on the clearance of antipyrine in humans.

MATERIALS AND METHODS

Surgical patients. A liver specimen, removed by surgical biopsy was obtained from 14 patients. These were patients undergoing elective abdominal surgery and in whom a liver biopsy was performed for histologic examination. Primary conditions for inclusion in the protocol were: (a) abstinence from alcohol or drugs for at least two weeks before the study and no requirement for drugs until premedication (an exception was made for some patients with gastric ulcer who received aluminium phosphate), and (b) normal values for serum bilirubin and serum albumin concentrations, for the prothrombin time, and for 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 seven patients, no medication was given until premedication and anaesthesia. In a second group of seven patients, troleandomycin (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 seven days preceding surgery.

Patients were premedicated with atropine and diazepam and were anaesthetized with droperidol, fentanyl, nitrous oxide, thiopental sodium, and pancuronium bromide. A surgical liver biopsy was 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 three volumes of ice-cold 0.154 M KCl, 0.01 M sodium/potassium phosphate buffer, pH 7.4, and hepatic microsomes were prepared as previously reported [8]. The microsomal pellets were stored at −20° until the conclusions of the histologic examination were available.

As a secondary criterion for final inclusion in the study, we required that the liver be normal on histologic examination. Two patients (one in each group) had mild steatosis and their hepatic microsomes were discarded. The liver histology was normal in the 12 patients finally studied (Table 1).

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Table 1. Hepatic drug-metabolizing enzymes in untreated patients (U) and in patients treated with repeated doses of troleandomycin (RDT), 2 g per os daily for 7 days

Patients	Sex	Age (yrs)	Surgical disease	Microsomal protein concentration (mg · g liver^{-1})	NADPH-cytochrome *c* reductase (nmole · min^{-1} · mg protein^{-1})	Uncomplexed cytochrome P-450* (nmole · mg protein^{-1})	Total cytochrome P-450† (nmole · mg protein^{-1})	Complexed cytochrome P-450‡ (O.D. × 10^3)
U_1	M	56	Carcinoma of the esophagus	22	33	0.33	0.32	3
U_2	M	55	Carcinoma of the esophagus	25	50	0.46	0.46	0
U_3	M	53	Gastric ulcer	27	59	0.51	0.50	2
U_4	M	38	Gastric ulcer	33	51	0.50	0.47	2
U_5	M	40	Diverticulosis of the sigmoid	37	37	0.38	0.38	0
U_6	F	75	Gallstones	30	70	0.21	0.22	3
Mean ± S.D.		53 ± 13		29 ± 5	50 ± 14	0.40 ± 0.12	0.39 ± 0.11	2 ± 1
RDT_1	F	44	Gastric ulcer	28	79	0.39	0.70	43
RDT_2	F	73	Carcinoma of the rectum	26	64	0.32	0.49	32
RDT_3	M	69	Gallstones	30	68	0.64	0.80	36
RDT_4	M	54	Carcinoma of the esophagus	30	95	0.43	0.71	56
RDT_5	M	59	Carcinoma of the sigmoid	20	85	0.56	0.90	29
RDT_6	M	34	Gastric ulcer	27	54	0.42	0.55	20
Mean ± S.D.		55 ± 15		27 ± 4	74 ± 15§	0.46 ± 0.12	0.69 ± 0.15§	36 ± 12§

* Measured as the CO-difference spectrum of dithionite-reduced microsomes.
† Measured, after addition of 50 μM potassium ferricyanide, as the CO-difference spectrum of dithionite-reduced microsomes.
‡ Measured by its Soret peak at 456 nm (see Materials and Methods) with a microsomal suspension containing microsomes from 62 mg of liver per ml.
§ Significantly different from that in untreated patients, $P < 0.02$ (Student's *t*-test for independent data).

Microsomal enzymes. The cytochrome P-450 Fe(II)-troleandomycin metabolite complex which is formed in rats has several properties 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 [4]. The cytochrome P-450 Fe(II)-troleandomycin metabolite complex is unstable in the ferric state; treatment of the microsomes with 50 μM potassium ferricyanide oxidizes the iron to the ferric state, destroys the complex, and regenerates native cytochrome P-450 [4].

Accordingly, uncomplexed, complexed, and total cytochrome P-450 were determined as follows. (a) With a first batch of microsomes, the concentration of CO-binding cytochrome P-450 was measured as described by Omura and Sato [9]: microsomes were placed in both cuvettes, dithionite added in both cuvettes and CO bubbled in the test cuvette. Because complexed cytochrome P-450 cannot bind CO, the difference spectrum at 450 nm (Fig. 1) measures uncomplexed cytochrome P-450 only [4]. (b) With a second batch of microsomes, the presence of a 456 nm-absorbing complex was looked for. Microsomes were placed in both cuvettes. 50 μM potassium ferricyanide was added to the reference cuvette to destroy the complex, if any, in this cuvette. The difference spectrum at 456 nm was then recorded (Fig. 1). (c) Using the same batch of microsomes, 50 μM potassium ferricyanide was also added to the test cuvette, so that the complex should now be destroyed in both cuvettes. A second difference spectrum was run at 456 nm (Fig. 1) to verify that this was indeed the case. Dithionite (in excess) was then added to both cuvettes to reduce cytochrome P-450 Fe(III) to the ferrous state, and CO was bubbled into the test cuvette. Because the complex, when present, had been destroyed, the difference spectrum at 450 nm (Fig. 1) now measured total cytochrome P-450.

NADPH-cytochrome *c* reductase activity was measured as previously reported [8]. Microsomal protein concentration was determined by the technique of Lowry *et al.* [10].

Medical patients. The clearance of antipyrine was determined on two occasions in 18 patients admitted for mild respiratory tract infections (Table 2); rectal temperature was less than, or equal to, 38°. Additional conditions for inclusion in the study were: (a) absence of other disease and, in particular, no clinical or biochemical evidence of liver disease, as judged by normal values for serum bilirubin and serum albumin concentrations, for the prothrombin time, and for γ-glutamyl transpeptidase, alkaline phosphatase, ALAT and ASAT activities, and (b) abstinence from alcohol or drugs for at least two weeks before the study. Eligible patients who gave their informed consent were randomly allocated into three groups; the clearance of antipyrine was determined one to three days after randomization and then again seven days after the first determination. (a) In six patients, serving as controls, no medication was given. (b) In six other patients, a single dose of troleandomycin (1 g per os) was given concomitantly with the intravenous administration of antipyrine for the second determination of the antipyrine clearance. (c) In six other patients, troleandomycin (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 seven days, starting on the day after the first determination of the antipyrine clearance and ending after the second determination of the antipyrine clearance.

Antipyrine clearance. The clearance of antipyrine was determined as follows: antipyrine, 15 mg per kg body weight, 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 concentration of antipyrine in plasma was determined as reported by Brodie *et al.* [11]. We verified that troleandomycin,

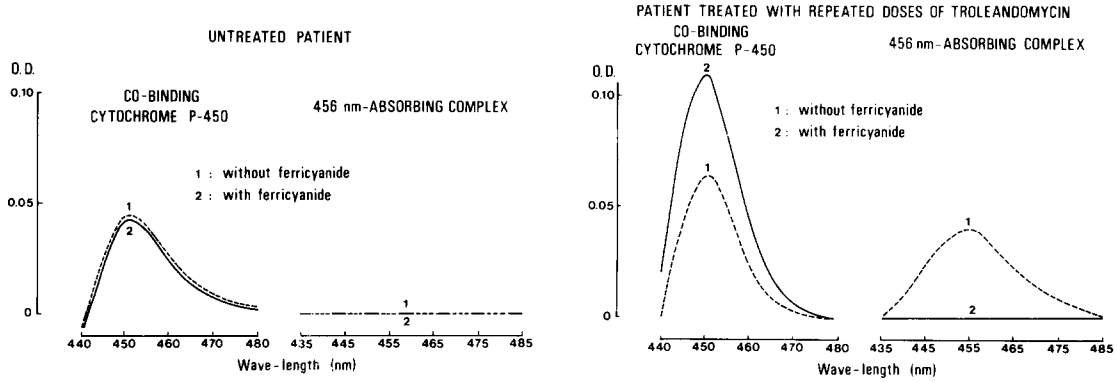

Fig. 1. Difference spectra obtained with microsomes from an untreated patient (patient U_1 of Table 1) and a patient treated with repeated doses of troleandomycin (patient RDT_1 of Table 1). Microsomes from the troleandomycin-treated patient contain a cytochrome P-450 Fe(II)-troleandomycin metabolite complex which exhibits a Soret peak at 456 nm, is unable to bind CO, but is destroyed by addition of potassium ferricyanide: microsomes absorb light at 456 nm (see Materials and Methods); addition of 50 μM potassium ferricyanide suppresses the absorption peak at 456 nm and concomitantly increases the CO-binding capacity of dithionite-reduced microsomes. In contrast, no absorption at 456 nm is observed in microsomes from the control patient and the addition of potassium ferricyanide does not modify the CO-difference spectrum in this patient. Note also that total cytochrome P-450 (measured as the CO-difference spectrum of microsomes first treated with potassium ferricyanide and then reduced with dithionite) is much higher in the troleandomycin-treated (induced) patient than in the control patient.

Table 2. Metabolism of antipyrine in untreated patients (U), in patients receiving a single dose of troleandomycin (SDT)*, and in patients treated with repeated doses of troleandomycin (RDT)†

Patients	Sex	Age (yrs)	Medical diagnosis	Apparent volume of distribution of antipyrine		Antipyrine clearance	
				First determination ($ml \cdot kg^{-1}$)	Second determination‡ ($ml \cdot kg^{-1}$)	First determination ($ml \cdot min^{-1} \cdot kg^{-1}$)	Second determination‡ ($ml \cdot min^{-1} \cdot kg^{-1}$)
U_1	M	52	Bronchitis	646	630	0.780	0.728
U_2	F	79	Cold	534	542	0.426	0.384
U_3	F	60	Bronchitis	440	494	0.246	0.309
U_4	M	74	Coryza	630	648	0.373	0.465
U_5	M	45	Coryza	751	820	0.900	0.937
U_6	M	35	Cold	602	661	0.889	0.878
Mean ± S.D.		57 ± 17		600 ± 106	632 ± 113	0.60 ± 0.29	0.62 ± 0.27
SDT_1	M	61	Cold	691	704	0.635	0.581
SDT_2	M	30	Coryza	773	883	1.140	1.082
SDT_3	F	75	Bronchitis	735	743	0.512	0.640
SDT_4	F	44	Cold	634	664	0.624	0.433
SDT_5	M	51	Cold	779	780	0.787	0.764
SDT_6	M	62	Bronchitis	771	748	0.821	0.791
Mean ± S.D.		54 ± 16		730 ± 58	754 ± 75	0.75 ± 0.22	0.71 ± 0.22
RDT_1	M	75	Bronchitis	563	580	0.631	0.174
RDT_2	F	70	Bronchitis	559	589	0.661	0.438
RDT_3	M	50	Cold	712	597	0.843	0.249
RDT_4	M	35	Coryza	760	769	1.027	0.605
RDT_5	M	69	Bronchitis	522	562	0.486	0.418
RDT_6	M	36	Cold	780	599	0.747	0.492
Mean ± S.D.		56 ± 18		649 ± 114	616 ± 76	0.73 ± 0.19	0.40 ± 0.16§

* Troleandomycin, 1 g per os, was given concomitantly with the intravenous administration of antipyrine for the second determination of the antipyrine clearance.

† Troleandomycin, 2 g os was given daily for 7 days, including that of the second determination of the antipyrine clearance.

‡ The second determination was made 7 days after the first determination.

§ Significantly different from the first determination, $P < 0.01$ (Student's t-test for dependent data).

in concentrations previously reported in humans [12], does not interfere with this assay. Both in untreated patients and in patients treated with single or repeated doses of troleandomycin, the natural logarithm of the antipyrine concentration (A) decreased linearly with time (t) according to the equation: $\ln A = \ln A_0 - \beta t$. $\ln A_0$ and β were calculated as the intercept and the slope, respectively, of the linear regression line. The apparent volume of distribution was calculated as the dose/A_0 ratio, and the clearance of antipyrine was determined as the $\beta.Vd$ product.

RESULTS

Microsomal enzymes. Administration of troleandomycin, 2 g per os daily for seven days, did not significantly modify hepatic microsomal protein concentration but increased NADPH–cytochrome *c* reductase activity by 48% (Table 1).

The concentration of uncomplexed cytochrome P-450 (measured as the CO-difference spectrum of microsomes not treated with potassium ferricyanide) was similar in untreated patients and in troleandomycin-treated patients (Table 1).

A 456 nm-absorbing complex was not detected in microsomes from untreated patients (Fig. 1, Table 1). In contrast, an absorption peak at 456 nm was observed in microsomes from troleandomycin-treated patients (Fig. 1, Table 1); the absorption peak at 456 nm disappeared after addition of 50 μM potassium ferricyanide to the microsomes (Fig. 1).

Treatment of the microsomes with potassium ferricyanide did not modify the CO-binding capacity of microsomes from untreated patients but increased it by 50% in microsomes from troleandomycin-treated patients (Fig. 1, Table 1). The concentration of total cytochrome P-450 (measured after addition of potassium ferricyanide) was higher in the troleandomycin-treated patients than in the untreated patients (Table 1).

Antipyrine clearance. The apparent volume of distribution of antipyrine remained unchanged in the three groups of patients (Table 2).

The mean value for the second determination of the antipyrine clearance was not significantly different from its first value in untreated patients and in patients receiving a single dose of troleandomycin, but was decreased by 45% in patients receiving repeated doses of troleandomycin (Table 2).

DISCUSSION

The results indicate that the several effects observed after the administration of high doses of troleandomycin in rats [4, 6], also occur, albeit to a smaller extent, after administration of therapeutic doses in humans.

(a) Cytochrome P-450 from control rats is poorly able to transform troleandomycin into the complex-forming metabolite [4]; two hours after a single dose of troleandomycin, no complexed cytochrome P-450 was detected, monooxygenase activities remained unchanged, and the hexobarbital sleeping time was unaffected [4, 6]. Similarly, administration of a single dose of troleandomycin did not significantly modify the clearance of antipyrine in humans (Table 2).

(b) In rats, troleandomycin induces microsomal enzymes and, in the process, promotes its own transformation into a metabolite which forms an inactive complex with reduced cytochrome P-450: NADPH–cytochrome *c* reductase and total cytochrome P-450 concentration are increased; part of total cytochrome P-450 is complexed with the troleandomycin metabolite; the concentration of uncomplexed cytochrome P-450 remains about normal [4, 5]. Similar observations were made after administration of repeated doses of troleandomycin in humans (Table 1). Quantitation of complexed cytochrome P-450 as the difference between total, and uncomplexed, cytochrome P-450 indicated that 33% of total cytochrome P-450 was complexed by the troleandomycin metabolite in humans. A similar value was obtained when complexed cytochrome P-450 was calculated from the absorption at 456 nm and from the extinction coefficient (75 $mM^{-1} \cdot cm^{-1}$ previously reported for similar complexes [13]. This [6] and similar [13] complexes cannot bind molecular oxygen and are totally inactive.

(c) After repeated doses of troleandomycin in rats, despite normal concentrations of uncomplexed (active) cytochrome P-450, several monooxygenase activities are decreased and the hexobarbital sleeping time is considerably prolonged [6, 7]. The mechanism for this reduced drug clearance is incompletely understood [6]; it may result from several added phenomena [6]: partial replacement of normal species of cytochrome P-450 by induced species, hypoactivity of these induced species with substrates other than troleandomycin itself, competition between troleandomycin and other substrates for the hydrophobic (type I) binding site of induced cytochrome P-450, and possibly, higher *in vivo* amount of complexed cytochrome P-450 than what is actually seen in isolated microsomes. Similarly, repeated doses of troleandomycin markedly reduced the clearance of antipyrine (Table 2) and that of theophylline [14] in humans.

These observations provide a likely explanation for the several drug interactions previously reported with this antibiotic in humans [1–3]. Similar effects might occur with erythromycin, which elicits similar drug interations in humans [15–17] and which, in rats, also induces its own transformation into a metabolite forming an inactive complex with reduced cytochrome P-450 [18].

We conclude that repeated administration of therapeutic doses of troleandomycin (a) induces microsomal enzymes, (b) results in the *in vivo* formation of a metabolite forming an inactive complex with reduced cytochrome P-450, and (c) decreases the clearance of antipyrine in humans.

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