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DEPRESSION OF THEOPHYLLINE METABOLISM AND ELIMINATION BY TROLEANDOMYCIN AND ERYTHROMYCIN

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Abstract—Clinical observations have suggested that elevated levels of theophylline may occur during the use of macrolide antibiotics. In the present study, the plasma clearance of theophylline was studied in rabbits treated with troleandomycin or erythromycin (400 mg·kg^{-1}·day^{-1}) over a 10-day period. The elimination of theophylline was impaired significantly after 10 days of antibiotic treatment. No change in theophylline elimination occurred when the antibiotics were given for shorter periods of time. Protein, cytochrome P-450 and cytochrome b_5 levels, and aminopyrine *N*-demethylase and benzo[*a*]pyrene hydroxylase activities were unchanged in hepatic microsomes prepared from rabbits treated with antibiotics for 10 days. Pretreatment of rabbits for 10 days with troleandomycin completely abolished production of 1-methyluric acid from theophylline in isolated hepatic microsomes, but production of 1,3-dimethyluric acid was unaffected. Troleandomycin, when added *in vitro* to microsomes, had no direct effect on theophylline metabolism. It is concluded that long-term treatment with troleandomycin selectively blocks or destroys one pathway of theophylline metabolism. This mechanism may explain the clinically observed interaction between theophylline and the macrolide antibiotics.

Recently, several clinical reports [1–3] have indicated a possible drug interaction between theophylline and macrolide antibiotics. Elevated serum theophylline levels and signs of theophylline toxicity were noted in several asthmatic patients receiving chronic theophylline therapy simultaneously with troleandomycin or erythromycin. The interaction between theophylline and erythromycin was, however, questioned by Pfeifer *et al.* [4], who reported that there was no kinetic interaction between theophylline and erythromycin in healthy volunteers given antibiotics for 48 hr. This discrepancy between reports creates concern since the wide use of macrolide antibiotics for infection and the wide use of theophylline for asthma, emphysema and bronchitis make the concomitant use of these two drugs a real possibility. In this study, we investigated whether a true drug interaction occurs between theophylline and the macrolide antibiotics.

MATERIALS AND METHODS

Male New Zealand white rabbits (2 kg) were obtained from Reiman's Fur Ranch, St. Agatha, Ontario, Canada, were housed in stainless steel mesh-floored cages, and were fed rabbit chow (Purina).

Theophylline, 8-chlorotheophylline, and 1,3-dimethyluric acid were obtained from the Sigma Chemical Co. St. Louis, MO, U.S.A.; erthyromycin (ilotycin gluceptate) was obtained from Eli Lilly & Co. Ltd., Toronto, Ontario, Canada. Troleandomycin was a gift from the Chas. Pfizer & Co., Inc., New York, NY, U.S.A.; and 1-methyluric acid was a gift from Dr. J. Williams, University of South Florida, Tampa, FL, U.S.A.

Rabbits were treated for 10 days with troleandomycin or erythromycin (200 mg/kg, i.p., twice daily). This dose is approximately 2-fold greater than the highest doses of these antibiotics used in man. In the troleandomycin experiment, animals were divided randomly into two groups of three animals. One group received the antibiotic and the second group received equivalent volumes of saline at the same times. Plasma clearance of a single dose of theophylline (5 mg/kg) was determined at various times during antibiotic treatment. After 10 days of antibiotic treatment the animals were killed, the livers were removed, and microsomes were prepared for *in vitro* metabolism studies. In the erythromycin experiment, plasma clearance of theophylline was determined pre- and post-antibiotic in the same individual animals.

The kinetics of theophylline elimination were determined after treating rabbits with heparin (1000 units/kg, i.m.) 20 min before the administration of theophylline (5 mg/kg, i.v.). Blood samples (400 μl) were obtained from a vein in the ear at 2, 15, 30 and 60 min and then at 1-hr intervals for 8 hr. The internal standard theobromine (50 μl of 20 μg/ml) was added to 100 μl of the serum that was then extracted with 3 ml chloroform–isopropanol (90:10, v/v) using a Clin Elut column (Analytichem International). The eluant was air dried and theophylline concentrations were determined by a modification of the high pressure liquid chromatographic method

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described by Sitar *et al.* [5]. Samples were reconstituted in 100 μl mobile phase (0.01 M sodium acetate, 1% acetic acid, methanol, 53/17/30, by vol.) [6] and 20 μl was injected onto a reverse phase column (RP-8, 10 μm, Brownlee Labs) in a Spectrophysics SP3500B HPLC using a flow rate of 1.6 ml/min. Separated samples were monitored at 275 nm using a Shoeffel ultraviolet variable wavelength detector. Recovery of known amounts of theophylline added to serum was 99 per cent.

In vitro theophylline metabolism by hepatic microsomes prepared from antibiotic-treated animals was determined by measuring the production of 1,3-dimethyluric acid and 1-methyluric acid using h.p.l.c. Incubation mixtures contained 3 mg microsomal protein, 5 mM $MgCl_2$, 10 units D-glucose-6-phosphate dehydrogenase, 10 mM D-glucose-6-phosphate, 1 mM NADP, and 0.25 mM theophylline in a total volume of 3 ml of 50 mM Tris–HCl buffer, pH 7.5. Samples were incubated for 2 hr at 37° in a Dubnoff metabolic shaking water bath. An internal standard, 100 μl 8-chlorotheophylline (100 μg/ml), was added to the incubation mixture, which was then extracted with 3 ml chloroform–isopropanol (90:10, v/v) by shaking for 30 min. Aliquots (1 ml) of the aqueous phase were air dried and then reconstituted with 100 μl mobile phase (0.01 M sodium acetate, 1% acetic acid, methanol, 64/21/15, by vol). Good separation of theophylline, 1-methyluric acid and 1,3-dimethyluric acid was achieved using a flow rate of 0.4 ml/min for the mobile phase. No metabolite was detected in the organic phase; therefore, the metabolite concentration in the aqueous phase represented total metabolite formation. Maximum sensitivity for detection of 1-methyluric acid and 1,3-dimethyluric acid occurred when samples were monitored at 295 nm using the variable wavelength detector. The rate of formation of these metabolites was linear with respect to protein concentration and time.

Microsomes were prepared as described by El Defrawy El Masry *et al.* [7] and were used on the same day as they were prepared. Microsomal protein levels were determined by the method of Lowry *et al.* [8], using bovine serum albumin as a standard.

Cytochrome(s) P-450 and cytochrome b_5 were determined by the method of Omura and Sato [9]. Benzo[*a*]pyrene hydroxylase activity was determined by the method of Watenberg and Leong [10]. Aminopyrine *N*-demethylase activity was determined by the method of Sladek and Mannering [11]. Student's

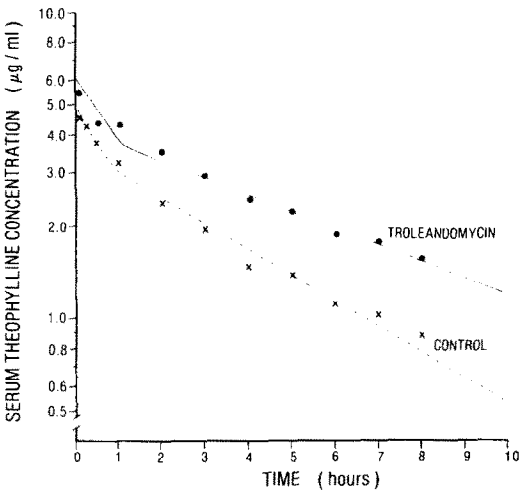

Fig. 1. Disappearance of theophylline from blood of rabbits pretreated for 10 days with troleandomycin (200 mg/kg, two times per day). Theophylline (5 mg/kg) was administered i.v. Key: (× – – – ×) control rabbit; and (●——●) troleandomycin-treated rabbit. The two curves represent the elimination of theophylline in two individual rabbits tested on the same day.

t-test for unpaired data was used to determine statistical significance between two means. Analysis of variance was used to determine statistical significance when three groups were compared. Kinetic parameters were determined using a computer fit to a two-compartment open model. The volume of distribution was calculated using the formula: $Vd = \frac{\text{dose.}}{\beta \times \text{AUC}}$ The clearance of the drug was calculated using the formula: $\text{Cl} = \frac{Vd \times 0.693}{T_{\frac{1}{2}\beta}}$.

RESULTS

The elimination of a single dose of theophylline decreased following treatment of rabbits with troleandomycin (200 mg/kg, two times per day) for a period of 10 days (Fig. 1 and Table 1). The rate constant (β), the elimination half-life ($T_{\frac{1}{2}\beta}$), the elimination rate constant (k_{elim}) and the plasma clearance of theophylline were significantly different in animals treated with troleandomycin for 10 days compared

Table 1. Pharmacokinetic parameters of theophylline elimination in rabbits treated with troleandomycin*

Treatment	Length of treatment (days)	$T_{\frac{1}{2}\alpha}$ (min)	$T_{\frac{1}{2}\beta}$ (min)	k_{elim} (hr^{-1})	Clearance (l/hr)	V_d (l)
Saline	4	49.8 ± 6.1	167.2 ± 12.8	0.28 ± 0.03	0.39 ± 0.13	1.59 ± 0.60
Troleandomycin	4	52.8 ± 1.1	176.0 ± 19.8	0.24 ± 0.04	0.34 ± 0.08	1.42 ± 0.33
Saline	10	50.2 ± 7.1	195.5 ± 13.6	0.25 ± 0.02	0.27 ± 0.02	1.30 ± 0.20
Troleandomycin	10	49.1 ± 5.7	295.5 ± 14.3†	0.17 ± 0.01†	0.18 ± 0.01†	1.27 ± 0.11

* Each value is the mean ± S.E. of three animals. Troleandomycin (200 mg/kg) was administered i.p. twice daily. Control animals received equivalent volumes of saline.

† Significantly different from corresponding control ($P < 0.05$).

Table 2. Pharmacokinetic parameters of theophylline elimination in rabbits before and during erythromycin administration*

	$T_{\frac{1}{2}\alpha}$ (min)	$T_{\frac{1}{2}\beta}$ (min)	k_{elim} (hr^{-1})	Clearance (l/hr)	V_d (l)
0 days	57.3 ± 9.3 (3)	318.6 ± 36.6 (3)	0.19 ± 0.04 (3)	0.36 ± 0.10 (3)	2.60 ± 0.42 (3)
3 days	63.1 (1)	373.1 (1)	0.16 (1)	0.31 (1)	2.79 (1)
10 days	75.8† (2)	620.4 ± 50.3‡ (3)	0.10† (2)	0.19 ± 0.03‡ (3)	3.20† (2)

* Each value is the mean ± S.E. of the number of individual animals shown in parentheses. Erythromycin (200 mg/kg) was administered i.p. twice daily.

† Expressed as the mean of two individual animals due to insufficient data from the α-phase of one animal.

‡ Significantly different from day 0 ($P < 0.05$).

Table 3. Effect of 10-day troleandomycin treatment on rabbit liver microsomal enzymes*

	Protein (mg/ml)	Cytochrome(s) P-450 (nmoles/mg protein)	Cytochrome b_5 (nmoles/mg protein)	Aminopyrine *N*-demethylase activity [nmoles HCHO·(mg protein)$^{-1}$·hr^{-1}]	Benzo[*a*]pyrene hydroxylase activity [nmoles 3-OH-BP·(mg protein)$^{-1}$·hr^{-1}]
Control	6.31 ± 0.84	0.68 ± 0.07	0.45 ± 0.06	163.4 ± 32.6	8.18 ± 2.40
Troleandomycin	6.30 ± 0.46	0.50 ± 0.04	0.38 ± 0.04	141.5 ± 16.6	9.43 ± 0.80

* Each value is the mean ± S.E. of four animals. Troleandomycin (200 mg/kg) was administered i.p. twice daily for 10 days.

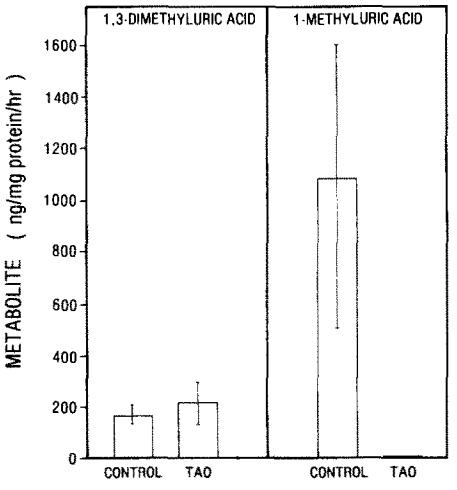

Fig. 2. Production of 1-methyluric acid and 1,3-dimethyluric acid in microsomes prepared from rabbits treated with troleandomycin (200 mg/kg, two times per day) for 10 days. Metabolite formation was determined as described in Materials and Methods. Each bar represents the mean ± S.E. obtained from microsomes prepared from four individual rabbits. Control animals received equivalent volumes of saline for 10 days.

to controls. The distribution rate constant (α), the distribution half-life ($T_{\frac{1}{2}\alpha}$), and the volume of distribution (V_d) were not significantly different from controls. In the same experiment, no significant change in the plasma clearance of theophylline could be determined after only 4 days of treatment with troleandomycin.

Treatment of rabbits with erythromycin (200 mg/kg, two times per day) for a period of 10 days resulted in similar decreases in theophylline elimination, as shown in Table 2. In one of the animals studied in this experiment, no apparent change in theophylline elimination had occurred after 3 days of erythromycin. Elimination half-life was increased 2.8-fold in this particular animal after 10 days of antibiotic treatment.

Troleandomycin treatment for 10 days had no effect on protein, cytochrome(s) P-450 or cytochrome b_5 content, or aminopyrine *N*-demethylase and benzo[*a*]pyrene hydroxylase activities in hepatic microsomes compared to microsomes prepared from saline-treated control animals (Table 3). Similarly, the administration of erythromycin for 10 days had no effect on protein, cytochrome(s) P-450 or cytochrome b_5 content, or aminopyrine *N*-demethylase and benzo[*a*]pyrene hydroxylase activities in hepatic microsomes.

The formation of 1,3-dimethyluric acid and 1-methyluric acid from theophylline in hepatic microsomes prepared from rabbits treated with troleandomycin for 10 days is illustrated in Fig. 2. The rate of formation of 1-methyluric acid in four control rabbits was variable, ranging from 0.12 to 2.61 $\mu g \cdot (mg\ protein)^{-1} \cdot hr^{-1}$ with a mean of $1.08 \pm 0.58\ \mu g$ 1-methyluric acid$\cdot (mg\ protein)^{-1} \cdot hr^{-1}$. In animals treated with troleandomycin, however, the formation of 1-methyluric acid was completely abolished. The lowest limit for detection of 1-methyluric acid was $0.015\ \mu g$ 1-methyluric acid$\cdot (mg\ protein)^{-1} \cdot hr^{-1}$. In contrast, the rate of formation of 1,3-dimethyluric acid in hepatic microsomes prepared from troleandomycin-treated rabbits was identical to controls.

Microsomes prepared from normal rabbits were incubated with 2.5 mM troleandomycin for 2 hr to determine if troleandomycin had a direct inhibitory effect on theophylline biotransformation. The rate of formation of 1-methyluric acid and 1,3-dimethyluric acid was identical to the rate of formation of these metabolites in microsomes incubated without the addition of troleandomycin (Table 4).

DISCUSSION

Our results indicate that the elimination of theophylline is decreased significantly in rabbits treated with troleandomycin or erythromycin for a 10-day period. These results support and substantiate the preliminary clinical reports [1–3] which described increases in serum theophylline levels when troleandomycin or erthromycin was used in patients already receiving theophylline. In the clinical reports [1–3], the antibiotics were administered to the patients to treat infections. As certain infections have been shown to increase the elimination half-life of theophylline [12] the reported increases in theophylline levels in these patients may have resulted from the infectious disease process rather than from interaction with the antibiotics themselves. This study in healthy rabbits demonstrates clearly that a true interaction occurs between theophylline and macrolide antibiotics which is independent of an infectious disease process. This does not discount the possibilty that an infection together with the use of these antibiotics may produce additive effects in decreasing the elimination of theophylline.

Table 4. *In vitro* metabolism of theophylline in microsomes incubated with troleandomycin*

	1-Methyluric acid [$\mu g \cdot (mg\ protein)^{-1} \cdot hr^{-1}$]	1,3-Dimethyluric acid [$\mu g \cdot (mg\ protein)^{-1} \cdot hr^{-1}$]
Control	3.83	0.263
Troleandomycin†	4.06	0.240

* Each value represents an individual incubation mixture using microsomes from the same control animal.

† Troleandomycin (2.5 mM) was added to the microsomal incubation mixture.

Pfeifer *et al.* [4], however, reported that three antibiotics—erythromycin, cephalexin and tetracycline—had no effect on theophylline elimination kinetics in humans. The present study offers an explanation for the apparent discrepancy between the clinical reports and the results of Pfeifer *et al.*, who studied subjects treated with antibiotics for 24 hr prior to theophylline administration [4]. In our study in rabbits, antibiotic treatment for 3 or 4 days produced no difference in theophylline kinetics compared to controls, but after 10 days of antibiotic treatment theophylline elimination was impaired significantly. Similarly, in the clinical studies reported by Weinberger *et al.* [3], elevated theophylline levels were observed after troleandomycin had been administered for 10 days or more. The lack of effect reported by Pfeifer *et al.* [4] may have resulted from the short period of antibiotic treatment.

A number of different factors might explain why theophylline elimination is impaired during the administration of erythromycin or troleandomycin. As biotransformation by the cytochrome P-450 system in the liver [13–15] controls to a large extent the rate of theophylline elimination, this would be a likely location for the interaction to occur. The production of 1-methyluric acid was abolished in microsomes prepared from rabbits treated for 10 days with troleandomycin, but there was no significant change in the production of 1,3-dimethyluric acid. These results indicate that the antibiotic selectively blocks or destroys the pathway that converts theophylline to 1-methylxanthine, which is then converted to 1-methyluric acid by xanthine oxidase. Although xanthine oxidase is a soluble enzyme found in liver supernatant, sufficient quantities of this enzyme must have been present in our microsomal preparations, as only 1-methyluric acid (and no 1-methylxanthine) was detected in the control incubation mixtures. If the antibiotic was acting on xanthine oxidase, it could be expected that 1-methylxanthine would appear in the incubation mixtures. As this metabolite could not be detected in the microsomes from antibiotic-treated animals, it is likely that the antibiotic had no effect on xanthine oxidase. Further evidence that the antibiotic is unlikely to act on xanthine oxidase is suggested by the finding that complete blockade of this enzyme by allopurinol has been shown to have no effect on theophylline levels or theophylline elimination rates [16, 17]. Though our evidence is indirect it is concluded that the most likely site of action of the antibiotic is on the cytochrome P-450-mediated oxidation of theophylline to 1-methylxanthine.

Using the technique we describe, we were unable to detect the formation of 3-methylxanthine in the *in vitro* incubation mixtures. It is therefore unknown if impairment of this pathway by these antibiotics is also a factor contributing to the decreased elimination of theophylline.

Several reports support the concept that multiple forms of cytochrome P-450 exist [18–20]. At least two of the metabolic pathways of theophylline in the rat—the oxidation of theophylline to 1,3-dimethyluric acid and the *N*-demethylation of theophylline to 1-methylxanthine—may be dependent on different cytochrome(s) P-450 as both pathways can be induced by 3-methylcholanthrene whereas phenobarbital induces only the pathway from theophylline to 1,3-dimethyluric acid [15]. In the rabbit, troleandomycin affects at least one cytochrome P-450-mediated pathway of theophylline metabolism, while having no effect on total cytochrome P-450 levels or on other cytochrome P-450-mediated pathways. This indicates a form of selective inhibition or destruction.

The rate of metabolite formation from theophylline was not altered after the addition of troleandomycin *in vitro* to an incubation mixture containing microsomes. This indicates that the antibiotic does not directly affect cytochrome P-450, providing an explanation for the lack of antibiotic effect after short-term treatment.

In conclusion, this study indicates that treatment of rabbits for 10 days with troleandomycin selectively blocks production of 1-methyluric acid from theophylline. As about 25 per cent of a dose of theophylline is excreted as this metabolite in man [21–24], blockade of this pathway by macrolide antibiotics is likely to contribute to the mechanism involved in the drug interaction between theophylline and these antibiotics. Blockade of other pathways in man may also contribute to this effect. Depression of theophylline metabolism by macrolide antibiotics causes an increase in the elimination half-life of theophylline, with a corresponding decrease in plasma clearance of theophylline. Continuous theophylline therapy would lead to increased serum theophylline levels, accumulation of theophylline, and theophylline toxicity.

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