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FORMATION OF INACTIVE CYTOCHROME P-450 Fe(II)–METABOLITE COMPLEXES WITH SEVERAL ERYTHROMYCIN DERIVATIVES BUT NOT WITH JOSAMYCIN AND MIDECAMYCIN IN RATS

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Abstract—The effects of some macrolides (4 mmoles·kg^{-1} p.o. daily for 4 days *in vivo*; 0.3 mM *in vitro*) on hepatic drug-metabolizing enzymes in rats were compared. One group of macrolides including previously studied compounds (oleandomycin, erythromycin and troleandomycin), as well as several other erythromycin derivatives, showed induction of microsomal enzymes and formation of inactive cytochrome P-450–metabolite complexes *in vivo*; this formation increased in the order: oleandomycin, erythromycin ethylsuccinate, erythromycin stearate, erythromycin itself, erythromycin propionate, erythromycin estolate and troleandomycin. Troleandomycin and, to a lesser extent, erythromycin and oleandomycin formed cytochrome P-450–metabolite complexes when incubated *in vitro* with 1 mM NADPH and microsomes from rats pretreated with troleandomycin or phenobarbital, but not with microsomes from control rats or rats treated with 3-methylcholanthrene. In contrast, two other macrolides, josamycin and midecamycin, showed no induction of microsomal enzymes and no detectable formation of cytochrome P-450–metabolite complexes *in vivo*. *In vitro*, these macrolides failed to form detectable complexes even with microsomes from rats pretreated with troleandomycin or phenobarbital. Hexobarbital sleeping time was unaffected by preadministration of josamycin or midecamycin (4 mmoles·kg^{-1} p.o.) 2 hr earlier; the *in vitro* activity of hexobarbital hydroxylase was not inhibited by 0.3 mM josamycin or midecamycin. We conclude that, unlike several erythromycin derivatives, josamycin and midecamycin do not form inactive cytochrome P-450–metabolite complexes in rats.

The macrolide group of antibiotics comprises several chemically related compounds. One structural feature they have in common is the presence of a dimethylamino function, $-N(CH_3)_2$, in one of the sugar residues.

Troleandomycin has been shown to induce drug-metabolizing enzymes in rats, and to promote its own demethylation and oxidation into a metabolite, probably the nitrosoalkane, which forms an inactive complex with the Fe(II) of cytochrome P-450 [1]. Eventually, several mono-oxygenase activities are markedly decreased [2, 3].

Administration of therapeutic doses of troleandomycin to humans has been shown to induce microsomal enzymes and to result in the *in vivo* formation of an inactive cytochrome P-450–troleandomycin metabolite complex [4]; the clearance of antipyrine was decreased by 45% [4], that of theophylline by 50% [5], and that of methylprednisolone by 64% [6]. Concomitant administration of troleandomycin and other drugs has been shown to produce ischemic accidents (ergotamine derivatives [7]), cholestasis (oral contraceptives [8]) and neurologic signs of intoxication (theophylline [5] or carbamazepine [9]).

To find a macrolide devoid of such potentially harmful effects may have important clinical applications. The macrolides have similar antibiotic activities, being more or less active against the same germs, at essentially similar therapeutic doses [10]. Therefore, a macrolide not interfering with drug metabolism may be preferred in those patients that must receive other drugs metabolized by cytochrome P-450. We, accordingly, tested a series of macrolides for their effects on hepatic drug-metabolizing enzymes in rats. In this communication we report that, while erythromycin [11], oleandomycin [12], and several erythromycin derivatives induced their own transformation into metabolites forming inactive complexes with reduced cytochrome P-450, in contrast, two other macrolides, josamycin and midecamycin, did not form such complexes in rats.

MATERIALS AND METHODS

Chemicals. Erythromycin and erythromycin estolate were purchased from Sigma, St. Louis, Missouri. Erythromycin propionate was a gift from Roussel Laboratories, Paris. Erythromycin ethylsuccinate and erythromycin stearate were kindly provided by Abbott Laboratories, Saint-Rémy-sur-Avre, France. Josamycin and midecamycin were, respectively, given by Spret Mauchant, Gennevilliers, France, and Midy Laboratories, Paris. Oleandomycin and troleandomycin were generous gifts from Pfizer Laboratories, Orsay, France. [2-^{14}C]Hexobarbital (specific activity, 8.58 mCi/mmole) was purchased from New England Nuclear, Boston, Massachusetts; its

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radiochemical purity, checked by TLC, was >99%.

Animals and treatments. Male Sprague–Dawley rats, Crl:CD®(SD)BR, weighing 180–220 g, were purchased from Charles River France, St-Aubin-lès-Elbeuf, France. Animals were fed a normal standard diet (Autoclavé 113, UAR, Villemoisson-sur-Orge, France) given *ad lib.*

The various macrolides (4 mmoles · kg^{-1}) dissolved in 3 ml of corn oil were administered by gastric intubation. Control rats received 3 ml of corn oil. Rats were used 2, 5 or 24 hr after a single dose, or 24 hr after the last of four daily doses. Rats treated with phenobarbital, 100 mg · kg^{-1} i.p. daily for 3 days, were used 24 hr after the last dose of the barbiturate. Other rats received 3-methylcholanthrene, 20 mg · kg^{-1}, 72 and 48 hr before being used.

Preparation of microsomes. Rats were killed by cervical dislocation and the liver was removed, minced and homogenized in three vols. of ice-cold 0.15 M KCl, 0.01 M sodium/potassium phosphate buffer, pH 7.4. The liver homogenate was centrifuged at 10,000 *g* for 30 min, and the supernatant was recentrifuged at 100,000 *g* for 60 min.

Microsomal enzymes. The cytochrome P-450 Fe(II)–metabolite complexes formed after the administration of troleandomycin, erythromycin, or oleandomycin, exhibit a Soret peak at 456 nm and are unable to bind CO [1, 11, 12]. Addition of 50 μM potassium ferricyanide oxidizes the iron to the ferric state, which disrupts the complex, and liberates equimolar amounts of uncomplexed, CO-binding, cytochrome P-450 [1, 11, 12].

Thus, uncomplexed, complexed and total cytochrome P-450 were determined as follows. A microsomal suspension containing microsomes from 62 mg of liver per ml was prepared. With a first batch of microsomes, we determined CO-binding cytochrome P-450 as described by Omura and Sato [13]. Because complexed cytochrome P-450 cannot bind CO [1, 11, 12, 14], the absorption at 456 nm measures uncomplexed cytochrome P-450 only. With a second batch of microsomes, we now looked for the presence of a cytochrome P-450–metabolite complex absorbing around 456 nm. We placed microsomes in both cuvettes and added 50 μM potassium ferricyanide to the reference cuvette to destroy the complex if any, in this cuvette. The absorption at 456 nm measured the Soret peak of complexed cytochrome P-450 in the sample cuvette. A molar extinction coefficient of 75 $mM^{-1} \cdot cm^{-1}$ [4, 14] was used to calculate the amount of complexed cytochrome P-450. To determine total cytochrome P-450, we now added 50 μM potassium ferricyanide also to the sample cuvette, which destroyed the complex in this cuvette. We ran a second spectrum to verify that the absorption at 456 nm had indeed disappeared. We then added dithionite, in excess, to both cuvettes to reduce cytochrome P-450 to the ferrous state and bubbled CO into the test cuvette. The absorption at 450 nm now measured total cytochrome P-450.

The activity of NADPH-cytochrome *c* reductase was measured as previously described [15]. Microsomal protein concentration was measured by the method of Lowry *et al.* [16].

Binding spectra. A microsomal suspension containing 2 mg of protein per ml of 0.15 M KCl, 0.01 M sodium/potassium phosphate buffer, pH 7.4, was divided between the cuvettes and the base-line was corrected. The macrolides, in 20 μl of ethanol, were added to the sample cuvette while 20 μl of ethanol was added to the reference cuvette. The binding spectra were recorded from 330 to 510 nm on an Aminco DW-2 spectrophotometer.

Demethylation. The demethylation of the macrolides was measured by formaldehyde formation as described for the demethylation of ethylmorphine [15]. The macrolide, in 20 μl of ethanol, was added to the sample flasks while 20 μl of ethanol were added to the control flasks; the incubation mixture (5 ml of 0.25 M sodium/potassium phosphate buffer, pH 7.4) contained microsomes from 250 mg of liver, 2 μmoles of NADP, 10 μmoles of glucose-6-phosphate, two enzyme units of glucose-6-phosphate dehydrogenase and 25 μmoles of $MgCl_2$; the mixture was incubated at 37° for 15 min.

In vitro *formation of cytochrome P-450–metabolite complexes.* Aliquots of microsomal suspension containing 2 mg of protein per ml of 0.15 M KCl, 0.01 M sodium/potassium phosphate buffer, pH 7.4, and maintained at 37°, were divided between the cuvettes. The macrolide, in 20 μl of ethanol, was added to the sample cuvette, while 20 μl of ethanol were added to the reference cuvette. The base-line was corrected. The reaction was initiated by adding 1 mM NADPH to both cuvettes. The successive difference spectra were recorded from 380 to 500 nm on an Aminco DW-2 spectrophotometer.

Hexobarbital hydroxylase, hexobarbital sleeping time. The hydroxylation of [2-^{14}C]hexobarbital was measured by the technique of Kupfer and Rosenfeld [17]. The macrolides, dissolved in 20 μl of ethanol, were added to some flasks, while 20 μl of ethanol were added to the control flasks; the incubation mixture (4 ml of 0.05 M sodium/potassium phosphate buffer, pH 7.4) contained microsomes from 250 mg of liver, 1.2 μmoles of [2-^{14}C]hexobarbital, 2 μmoles of NADP, 10 μmoles of glucose-6-phosphate, two enzyme units of glucose-6-phosphate dehydrogenase and 25 μmoles of $MgCl_2$; the mixture was incubated at 37° for 15 min. The hexobarbital sleeping time was measured as the time interval between loss and recovery of the righting reflex after administration of hexobarbital, 150 mg · kg^{-1} i.p.

RESULTS

The effects of the administration of various macrolides (4 mmoles · kg^{-1} p.o. daily for 4 days) on hepatic drug-metabolizing enzymes are compared in Table 1. From the results, two groups of macrolides can be distinguished. With a first group (oleandomycin, troleandomycin, erythromycin and its derivatives), the concentration of microsomal protein and the activity of NADPH-cytochrome *c* reductase were increased. The concentration of CO-binding, uncomplexed, cytochrome P-450 was variously modified, being either slightly decreased, unchanged, or slightly increased. After administration of these macrolides, however, part of the total cytochrome P-450 was present in the form of cytochrome P-450–metabolite complexes that were unable to bind CO. The complexes exhibited a Soret

Table 1. Effects of various macrolides on hepatic drug-metabolizing enzymes*

	Liver weight/body weight (%)	Microsomal protein (mg·g liver^{-1})	NADPH-cytochrome *c* reductase (nmoles·min^{-1}· mg protein^{-1})	Uncomplexed cytochrome P-450	Total cytochrome P-450	Complexed cytochrome P-450
				(nmoles·mg protein^{-1})		
Control	4.2 ± 0.2	32 ± 3	83 ± 10	0.77 ± 0.05	0.76 ± 0.05	ND
Josamycin	4.1 ± 0.2	32 ± 4	97 ± 23	0.78 ± 0.09	0.78 ± 0.09	ND
Midecamycin	3.7 ± 0.3	37 ± 6	100 ± 23	0.74 ± 0.09	0.72 ± 0.09	ND
Oleandomycin	4.4 ± 0.4	40 ± 7†	124 ± 28†	0.67 ± 0.09†	0.98 ± 0.13†	0.41 ± 0.16†
Erythromycin ethylsuccinate	3.9 ± 0.3	45 ± 12†	105 ± 14†	0.91 ± 0.11†	1.41 ± 0.28†	0.59 ± 0.21†
Erythromycin stearate	4.6 ± 0.3†	46 ± 9†	130 ± 23†	0.88 ± 0.09†	1.81 ± 0.25†	0.90 ± 0.18†
Erythromycin	4.4 ± 0.4	46 ± 5†	138 ± 19†	1.11 ± 0.22†	1.99 ± 0.27†	0.98 ± 0.23†
Erythromycin propionate	4.3 ± 0.4	49 ± 4†	160 ± 31†	0.75 ± 0.09	1.87 ± 0.21†	1.07 ± 0.23†
Erythromycin estolate	4.7 ± 0.4†	45 ± 4†	153 ± 33†	0.81 ± 0.13	2.04 ± 0.38†	1.18 ± 0.24†
Troleandomycin	5.9 ± 0.6†	52 ± 4†	173 ± 21†	0.40 ± 0.07†	2.64 ± 0.43†	2.43 ± 0.35†

* Rats were killed 24 hr after the last dose of the macrolides, 4 mmoles·kg^{-1} p.o. daily for 4 days. Uncomplexed cytochrome P-450 was measured as the CO-difference spectrum of dithionite-reduced microsomes. Total cytochrome P-450 was similarly measured after previous addition of 50 μM potassium ferricyanide to the microsomes. Complexed cytochrome P-450 was measured by its Soret peak at 456 nm, applying a molar extinction coefficient of 75 mM^{-1}·cm^{-1}. Results are means ± S.D. for eight rats. ND, not detected.

† Significantly different from control rats, $P < 0.05$ (Student's *t*-test for independent data).

Table 2. *In vitro* formation of 456 nm absorbing cytochrome P-450–metabolite complexes*

	Formation of cytochrome P-450–metabolite complexes in the presence of various macrolides (pmoles·5 min^{-1}·nmole cytochrome P-450^{-1})				
	Josamycin	Midecamycin	Oleandomycin	Erythromycin	Troleandomycin
Control rats	ND	ND	ND	ND	ND
Josamycin-treated rats	ND	—	—	—	ND
Midecamycin-treated rats	—	ND	—	—	ND
Troleandomycin-treated rats	ND	ND	7 ± 4	16 ± 10	97 ± 5
Phenobarbital-treated rats	ND	ND	6 ± 5	10 ± 6	39 ± 19
3-Methylcholanthrene-treated rats	ND	ND	ND	ND	ND

* Rats were treated with josamycin or midecamycin (4 mmoles·kg^{-1} p.o. daily for 4 days), or received troleandomycin (0.5 mmoles·kg^{-1} p.o. daily for 4 days); isolated microsomes were then treated with 50 μM potassium ferricyanide. Other rats received phenobarbital (100 mg·kg^{-1} i.p. daily for 3 days) or 3-methylcholanthrene (20 mg·kg^{-1}, 72 and 48 hr before being killed). Microsomal suspensions containing 2 mg of microsomal protein per ml were incubated with various macrolides (0.3 mM) and 1 mM NADPH; the amount of cytochrome P-450–metabolite complexes formed after 5 min of incubation was measured from the Soret peak at 456 nm, applying a molar extinction coefficient of 75 mM^{-1}·cm^{-1}. Results are means ± S.D. for 3–6 rats. ND, not detected.

Table 3. Amplitude of binding spectra, and demethylation rates*

	Amplitude of binding spectrum (O.D. × 10^3·ml·nmole cytochrome P-450^{-1})			Formation of formaldehyde (nmoles·min^{-1}·nmole cytochrome P-450^{-1})		
	Josamycin	Midecamycin	Troleandomycin	Josamycin	Midecamycin	Troleandomycin
Microsomes from control rats	2.9 ± 1.3†	3.9 ± 0.5†	3 ± 0.6‡	0.16 ± 0.01	0.09 ± 0.09	0.81 ± 0.17
Microsomes from troleandomycin-treated rats	16.4 ± 2.9‡§	3.2 ± 1.0‡	48 ± 7‡§	0.12 ± 0.03	0.06 ± 0.02	1.36 ± 0.06§

* Hepatic microsomes from six control rats (cytochrome P-450, 0.69 nmoles·mg microsomal $protein^{-1}$), or from six rats receiving troleandomycin, 0.5 mmoles·kg^{-1} p.o. daily for 4 days, were pooled; in the latter case, microsomes were treated with 50 μM potassium ferricyanide (cytochrome P-450, 1.9 nmoles·mg microsomal $protein^{-1}$). Results are means ± S.D. for three expts. O.D., optical density.

† Reverse type I binding spectrum.

‡ Type I binding spectrum.

§ Different from that with microsomes from control rats; $P < 0.05$ (Student's *t*-test for independent data).

Table 4. Hexobarbital hydroxylase activity and hexobarbital sleeping time in the presence of josamycin and midecamycin*

	Hexobarbital hydroxylase activity (nmoles·min^{-1}·mg microsomal protein^{-1})	Hexobarbital sleeping time (min)
Control	2.0 ± 0.6	33 ± 9
With josamycin	2.1 ± 0.5	36 ± 8
With midecamycin	2.2 ± 0.4	32 ± 12

* The hydroxylation of hexobarbital (0.3 mM) was measured in microsomes from control rats and an NADPH-generating system, in the absence or the presence of 0.3 mM josamycin or 0.3 mM midecamycin. Results are means ± S.D. for three expts. The hexobarbital sleeping time was measured as the time interval between loss and recovery of the righting reflex after administration of hexobarbital, 150 mg·kg^{-1} i.p., either in control rats or in rats that received josamycin or midecamycin (4 mmoles·kg^{-1} p.o.), 2 hr earlier. Results are means ± S.D. for eight rats.

peak at 456 nm (Table 1). Addition of potassium ferricyanide suppressed the absorption peak at 456 nm and liberated uncomplexed, CO-binding, cytochrome P-450. Total cytochrome P-450, measured by CO-binding after addition of potassium ferricyanide and subsequent reduction with dithionite, was markedly increased (Table 1). In contrast, with a second group of macrolides (josamycin and midecamycin) there was no evidence for an induction of microsomal enzymes: microsomal protein concentration and NADPH-cytochrome *c* reductase activity were not significantly modified and cytochrome P-450 concentrations remained unchanged (Table 1). Neither was there any evidence for the formation of cytochrome P-450–metabolite complexes: microsomes did not absorb at 456 nm, and their CO-binding capacity was not increased by the addition of potassium ferricyanide (Table 1).

The ability of various macrolides (0.3 mM) to form, *in vitro*, cytochrome P-450–metabolite complexes in the presence of 1 mM NADPH and microsomes from various sources is compared in Table 2. With microsomes from control rats, none of these macrolides formed detectable cytochrome P-450–metabolite complexes. Similarly, with microsomes from rats pretreated with josamycin or midecamycin, no cytochrome P-450–metabolite complexes were formed during incubation with josamycin, midecamycin, or troleandomycin (Table 2). Microsomes from rats pretreated with troleandomycin (0.5 mmoles·kg^{-1} daily for 4 days) did not form cytochrome P-450–metabolite complexes when incubated with josamycin or midecamycin, although they formed complexes during incubation with oleandomycin, erythromycin and troleandomycin (Table 2). Similarly, microsomes from rats pretreated with phenobarbital did not form cytochrome P-450–metabolite complexes from josamycin or midecamycin, although they formed complexes from oleandomycin, erythromycin and troleandomycin (Table 2). Microsomes from rats pretreated with 3-methylcholanthrene did not form complexes, even with oleandomycin, erythromycin and troleandomycin (Table 2).

Pretreatment of the animals with phenobarbital considerably increased the *in vivo* formation of a cytochrome P-450–metabolite complex, 5 hr after the administration of a single dose of troleandomycin, 4 mmoles·kg^{-1} p.o.: the amount of complexed cytochrome P-450 was 0.05 ± 0.06 nmoles·mg microsomal protein^{-1} in non-pretreated rats and 0.45 ± 0.30 in phenobarbital-pretreated rats (mean ± S.D. for four rats). In contrast, no cytochrome P-450–metabolite complex could be detected 5 or 24 hr after the administration of a single dose of josamycin or midecamycin, 4 mmoles·kg^{-1} p.o., even in phenobarbital-pretreated rats.

Josamycin and midecamycin, 0.3 mM, gave a small reverse type I binding spectrum with microsomes from control rats; troleandomycin, 0.3 mM, gave a small type I binding spectrum (Table 3). With microsomes from rats treated with troleandomycin, the three macrolides gave type I binding spectra; the amplitude, expressed per nmole of cytochrome P-450 per ml, was increased with josamycin, albeit much less than with troleandomycin; the amplitude was unchanged with midecamycin (Table 3).

Josamycin and midecamycin were demethylated by microsomes from control rats, albeit much less than troleandomycin (Table 3). With microsomes from rats treated with troleandomycin, the demethylation rate, expressed per nmole of cytochrome P-450, was increased with troleandomycin but not with josamycin and midecamycin (Table 3).

The activity of hexobarbital hydroxylase was not inhibited in the presence of 0.3 mM josamycin or 0.3 mM midecamycin, nor was the hexobarbital sleeping time prolonged, 2 hr after the administration of josamycin or midecamycin, 4 mmoles·kg^{-1} p.o. (Table 4).

DISCUSSION

Oleandomycin, erythromycin and troleandomycin have been shown to induce microsomal enzymes and to promote their own transformation into metabolites forming inactive complexes with the Fe(II) of cytochrome P-450 [1, 2, 11, 12]. The results of the present investigation indicate that several other erythromycin derivatives produce similar effects in rats (Table 1). The results also give a comparison of the respective potency of these various macrolides in forming cytochrome P-450–metabolite complexes *in vivo*. A less active macrolide was oleandomycin,

followed by erythromycin ethylsuccinate, erythromycin stearate, erythromycin itself, erythromycin propionate and erythromycin estolate; by far the most active was troleandomycin, the amount of complexed cytochrome P-450 formed with this macrolide being twice that formed with erythromycin estolate and six times that formed with oleandomycin (Table 1).

Interestingly, two other macrolides, namely josamycin and midecamycin, failed to form cytochrome P-450–metabolite complexes *in vivo* (Table 1). Comparison of the effects of these two macrolides with those of the others may help delineate at which step they differed in their interaction with cytochrome P-450. Oleandomycin, erythromycin and troleandomycin do not form cytochrome P-450–metabolite complexes when incubated with 1 mM NADPH and cytochrome P-450 from control rats [1, 11, 12]. Similarly, josamycin and midecamycin did not form any complexes when incubated with 1 mM NADPH and cytochrome P-450 from control rats (Table 2).

Oleandomycin, erythromycin and troleandomycin, however, induce microsomal enzymes resulting in the appearance of cytochrome P-450 species with a high affinity and activity for the respective macrolides. These induced species actively transform oleandomycin, erythromycin, or troleandomycin into metabolites which form stable complexes with the Fe(II) of cytochrome P-450 [1, 11, 12]. In contrast, josamycin and midecamycin failed to significantly increase the concentration of microsomal enzymes (Table 1). More specifically, they failed to induce species of cytochrome P-450 active with the macrolides. This is shown by the observation that microsomes from rats pretreated with josamycin or midecamycin did not form cytochrome P-450–metabolite complexes in the presence of troleandomycin (Table 2).

Not only were josamycin and midecamycin unable to induce active species of cytochrome P-450, but they were also unable to form cytochrome P-450–metabolite complexes even in the presence of species of cytochrome P-450 induced by other compounds. This is shown by the following observations. A first way of inducing cytochrome P-450 species active with the macrolides is to administer troleandomycin. This treatment increased the *in vitro* formation of cytochrome P-450–metabolite complexes not only with troleandomycin [1] but also with oleandomycin and erythromycin (Table 2). However, when josamycin and midecamycin were incubated with 1 mM NADPH and cytochrome P-450 from troleandomycin-treated rats, no *in vitro* formation of cytochrome P-450–metabolite complexes could be detected (Table 2). A second way of inducing species of cytochrome P-450 active with the macrolides is to administer phenobarbital. Species of cytochrome P-450 induced by phenobarbital, albeit apparently different from those induced by troleandomycin [18], are also active in forming cytochrome P-450–metabolite complexes with oleandomycin, erythromycin and troleandomycin (Table 2). However, when microsomes from rats pretreated with phenobarbital were incubated with 1 mM NADPH, and josamycin or midecamycin, again no *in vitro* formation of cytochrome P-450–metabolite complexes could be detected (Table 2). This observation *in vitro* was further corroborated by *in vivo* studies. When a single dose of troleandomycin is administered to control rats, complex-generating species of cytochrome P-450 are absent (or present in very low concentrations) at the time of administration; as a consequence, the *in vivo* formation of cytochrome P-450–metabolite complexes is delayed and, then, slowly increases as the active species progressively appear (present results and unpublished observations). In contrast, when a single dose of troleandomycin is administered to rats pretreated with phenobarbital, complex-generating species of cytochrome P-450 are present in the liver at the time of administration; as a consequence, there is an immediate and extensive formation of the cytochrome P-450–troleandomycin metabolite complex *in vivo*. In sharp contrast, when a single dose of josamycin or midecamycin was administered to rats pretreated with phenobarbital, no cytochrome P-450–metabolite complex was formed.

The reason why josamycin and midecamycin failed to form cytochrome P-450–metabolite complexes, even with induced species of cytochrome P-450, is incompletely understood. Both macrolides interacted with oxidized cytochrome P-450, as shown by the appearance of binding spectra (Table 3). Both macrolides were substrates for cytochrome P-450, as shown by their demethylation (Table 3). Pretreatment of the animals with troleandomycin changed the binding spectra of josamycin and midecamycin from a ligand-type of interaction to a type I binding spectrum (Table 3), showing that the hydrophobic binding site of the induced species of cytochrome P-450 can indeed bind josamycin and midecamycin. It is noteworthy, however, that both the amplitudes of the binding spectra and the demethylation rates were considerably smaller with midecamycin or josamycin than with troleandomycin (Table 3); it is also noteworthy that the demethylation observed may have been that of the methoxy function, rather than that of the tertiary amine function, of josamycin and

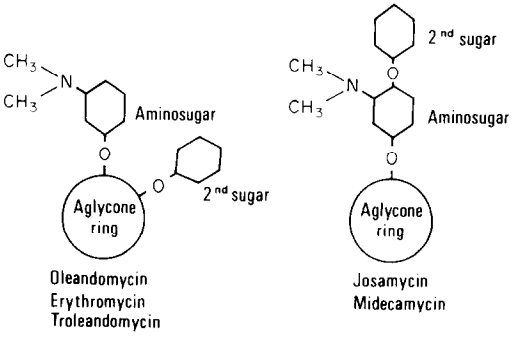

Fig. 1. Schematic structure of various macrolides. The chemical structure of the aglycone ring and that of the two sugars differ in the various macrolides. It is noteworthy, however, that in macrolides forming cytochrome P-450–metabolite complexes (oleandomycin, troleandomycin, erythromycin and its various salts and esters), the two sugars are independently attached to the aglycone ring. In macrolides not forming complexes (josamycin and midecamycin), the second sugar is attached to the aminosugar.

midecamycin. The chemical structure of josamycin and midecamycin resembles that of oleandomycin, erythromycin and troleandomycin, in that these various macrolides are made up of an aglycone ring and of two sugars, one of which bears the tertiary amine function (Fig. 1). Their chemical structures, however, differ in the following, possibly important, aspect. In oleandomycin, erythromycin and troleandomycin, the two sugars are independently attached to the aglycone ring. In josamycin and midecamycin, however, the second sugar is glycosidically attached to the aminosugar, which is itself attached to the aglycone ring (Fig. 1). Conceivably, the intimate presence of this bulky sugar may impair the proper positioning of the tertiary amine function (and its demethylated derivatives) in close proximity to the heme iron of cytochrome P-450. Structure–activity relationships suggesting steric hindrance have been reported for several amphetamines and phenylalkylamines [19, 20].

Our observations that josamycin and midecamycin did not induce microsomal enzymes (Table 1), did not form cytochrome P-450–metabolite complexes (Tables 1 and 2) and did not inhibit cytochrome P-450 activity, *in vitro* or *in vivo* (Table 4), may have important clinical applications. Until now, the concordance has been good between the effects of troleandomycin or erythromycin in rats and their effects on hepatic drug-metabolizing enzymes in humans ([1, 4, 11] and unpublished observations). Furthermore, clinical observations suggest that josamycin and midecamycin may have little effect on drug metabolism in humans. Indeed, whereas several drug interactions have been reported with troleandomycin [5, 7–9], and a few with erythromycin [21–23], no drug interaction has been reported with midecamycin in humans. A single case of ergotamine intolerance has been reported in a patient receiving josamycin [24]. In this single patient, however, the role of josamycin may be questioned since ischemic complications may occur with ergotamine given alone [25]. Indeed, josamycin or midecamycin did not decrease the clearance of theophylline [26, 27], whereas this clearance was markedly decreased by the concomitant administration of troleandomycin [5].

These observations may indicate that josamycin and midecamycin might be preferred to other macrolides in those patients who must receive other drugs metabolized by cytochrome P-450.

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