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DUAL EFFECTS OF MACROLIDE ANTIBIOTICS ON RAT LIVER CYTOCHROME P-450

INDUCTION AND FORMATION OF METABOLITE-COMPLEXES: A STRUCTURE–ACTIVITY RELATIONSHIP

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Abstract—Previous studies have shown that the macrolide antibiotics, troleandomycin and erythromycin, are able to induce their own transformation into a metabolite forming an inactivated complex with rat liver cytochrome P-450. This paper reports the results of a study on the effects of several macrolide antibiotics including oleandomycin, erythromycin derivatives, josamycin, methymycin, tylosin, spiramycin and rifampicin, as well as antibiotics of other series, such as tetracycline and lincomycin, on rat liver cytochromes P-450 *in vivo* and *in vitro*. Only the antibiotics containing the desosamine and mycaminose amino sugars were able to give the dual effects already found with troleandomycin: induction of cytochrome P-450 and formation of an inhibitory cytochrome P-450–iron–nitrosoalkane metabolite complex *in vivo* or *in vitro*. From these studies, it appears that two structural factors are important for a macrolide antibiotic to lead to such effects: the presence of a non-hindered readily accessible *N*-dimethylamino group and the hydrophobic character of the molecule. These data are discussed in relation to the adverse effects observed during drug associations involving some of these macrolide antibiotics.

Several recent reports indicate drug interaction between macrolide antibiotics of the oleandomycin type and other medicines [1]. For instance, in humans the steady-state plasma level of theophylline is increased in individuals who have been treated for some days with the antibiotics erythromycin and troleandomycin (TAO) [2–4]. Toxic effects have also been observed in the case of persons receiving oral progestatives [5], ergotamine derivatives [6] or cardiotonic substances [7], after some days of simultaneous administration of TAO or erythromycin. These effects are generally associated with an abnormal increase of the administered medicine plasmatic level in the presence of the antibiotic.

Decreases of the hepatic cytochrome P-450-catalysed oxidation of either xenobiotics or endogenous substrates have been observed in rats previously treated by erythromycin [8] or TAO [9, 10]. In humans, such modifications of the cytochrome P-450 activities lead to a decrease of the endogenous cholesterol metabolism [11].

Actually, these macrolide antibiotics have been shown to affect hepatic cytochromes P-450 *in vivo* in two different ways. On one hand, troleandomycin [9, 12] and, to a lesser extent, erythromycin [8] and oleandomycin [13], are inducers of cytochrome P-450. Treatment of rats by TAO leads up to a five-fold increase of hepatic cytochrome P-450 content [9, 12], the main isozyme induced being immunologically, electrophoretically and metabolically different from the main native cytochromes P-450 and from the main cytochrome P-450 forms induced by usual inducers such as phenobarbital (PB) or 3-methylcholanthrene [12]. In rabbits, TAO induction leads mainly to a cytochrome P-450 having characteristics similar to the constitutive LM_3 cytochrome P-450 form [14].

On the other hand, TAO [9] and, to a lesser extent, erythromycin [8] and oleandomycin [13], are able to lead *in vivo* to very stable cytochrome P-450–iron–metabolite complexes which are characterized by Soret peaks around 456 nm. These complexes are believed to be derived from the metabolic oxidation of the $-N(CH_3)_2$ group of the antibiotic to the corresponding —NO group and the strong binding of the resulting nitrosoalkane metabolite to cytochrome P-450–Fe(II) [9]. These complexes are so stable that up to 80% of hepatic cytochromes P-450 of rats treated by TAO are present *in vivo* under the form of an iron–metabolite complex which survives a partial purification procedure [12]. Once engaged in such a complex, cytochrome P-450 is unable to bind and activate dioxygen, explaining the inhibition of some monooxygenase activities after TAO treatment [10].

The effects of macrolide antibiotics as inducers or inhibitors seem to be greatly dependent on their structure; for instance, TAO, which is oleandomycin triacetate, is a much more potent inducer and is able to form a much more inhibitory complex *in vivo* than oleandomycin itself [13]. The present study has been undertaken to discover if other antibiotics containing an amine function have similar effects on hepatic cytochromes P-450, and to determine the structural factors which are important for the inducing effects of oleandomycin-derived antibiotics and

for the formation of inhibitory cytochrome P-450–metabolite complexes. This paper compares the effects *in vivo* of several antibiotics on rat hepatic cytochromes P-450 and the abilities of these antibiotics to form cytochrome P-450–metabolite complexes *in vitro*.

MATERIALS AND METHODS

Materials. Nicotinamide adenine dinucleotide reduced form (NADPH), bovine serum albumin, lincomycin, erythromycin base, oleandomycin, tylosin tartrate, rifampicin were purchased from Sigma Chemical Co. (St. Louis, MO). Antibiotics were kindly supplied from different sources: troleandomycin and methylenecycline from Pfizer (Orsay, France); erythromycin estolate and erythromycin glucoheptonate from Eli Lilly (Paris, France); methymycin from Squibb & Sons Inc. (Princeton, NJ); josamycin and josamycin propionate from Spret–Mauchant (Genevilliers, France); spiramycin base and forosamine from Specia (Paris, France); M 4365 A_2, G_2, platenomycin A_1, B_1 from Tanabe Seijaku Ltd. (Japan); cirramycin A_1 from Brystol–Banyu Research Institute Ltd. (Japan).

Erythralosamine was chemically synthesized according to the method of Flynn *et al.* [15] and recrystallized three times before use. The acidic cleavage of the osidic linkage of josamycin, leading to desmycarose-josamycin, was made by a classical procedure in this macrolide antibiotic series [16]. Desmycarose-josamycin was extracted by chloroform, purified by thin-layer chromatography on silica gel and eluted by chloroform–methanol (9:1, v/v). Its mass spectrum exhibited the expected molecular peak at *m/z* 599.

Treatment of rats. Male Sprague–Dawley rats (160–200 g) were treated by phenobarbitone (80 mg/kg per day i.p. in saline) for 3 days or by different antibiotics suspended in corn oil (500 mg/kg per day, i.p., 3 days, except for methymycin: 300 mg/kg; rifampicin: 200 mg/kg and tetracycline: 100 mg/kg). Rats were killed 24 hr after the last injection. In the case of erythralosamine, rats were treated by 200 mg/kg (i.p.), and sacrificed 48 hr after a single injection. Liver microsomes were made according to usual techniques [12] from rats either treated as indicated previously or treated by corn oil alone for controls.

Assays. Proteins [17], cytochrome P-450, cytochrome b_5 [18] and cytochrome P-450–antibiotic metabolite complexes [9] were determined as described previously. The amount of complexed cytochrome P-450 was measured as the difference between the amounts of cytochrome P-450 that were able to bind CO in microsomes before and after ferricyanide (50 μM) treatment [9, 12, 20].

Formaldehyde formation was measured according to the Nash technique [19] using 0.2 mM substrate and 1 μM microsomal cytochrome P-450 after 10 min incubation at 37°.

In vitro, cytochrome P-450–antibiotic metabolite complex formation was measured by differential spectroscopy with aerobic microsomes containing 1 μM cytochrome P-450, 0.04 mM substrate and 0.5 mM NADPH. Spectra were recorded between 400 and 500 nm every 2 min and the maximum absorbance between 456 and 490 nm was determined using 1 cm path cuvettes.

RESULTS

Effects of various antibiotics on rat hepatic cytochromes P-450 in vivo

Figure 1 gives the chemical structures of the antibiotics used in this study. Those indicated in Fig. 1d were only used in *in vitro* studies. All of them have a tertiary amino group, actually a $N(CH_3)_2$ group, except for rifampicin and lincomycin for which the amine nitrogen atom is inserted into a ring system. Most of them are macrolide antibiotics, the $N(CH_3)_2$ group of which is present on an amino sugar such as desosamine (TAO, oleandomycin, erythromycin, methymycin, M 4365 A_2 or G_2) or mycaminose (josamycin, tylosin, spiramycin, platenomycin A_1 or B_1, cirramycin A_1, desmycarose-josamycin). In the first five antibiotics of the latter group, the mycaminose sugar is inserted between the macrolide and a mycarose sugar. Tetracycline and methylenecycline, which are polycyclic antibiotics containing an $N(CH_3)_2$ function, and lincomycin, which involves a pyrrolidin ring, exhibit very different structures but have been studied for the sake of comparison.

In liver microsomes from control rats, cytochrome P-450 exists in its usual free ferric state and binds directly to CO after dithionite reduction. In liver microsomes from rats treated with TAO, it exists partly in its ferric free state and partly as a Fe(II)–nitrosoalkane 456 nm-absorbing complex, which is unaffected by CO [9]. Upon treatment by ferricyanide, its Fe(II)–RNO bond is broken, like the Fe(II)–RNO bonds of other cytochrome P-450–metabolite complexes derived from oxidation of various amines [20, 21], regenerating cytochrome P-450–Fe(III), which is then able to bind to CO after dithionite reduction (equation (1)].

$$[\text{cyt P-450} - \text{Fe(II)} \leftarrow \underset{\underset{\text{O}}{\|}}{\text{N}}\text{—R}]$$

$$\xrightarrow{K_3FeCN_6} \text{cyt P-450–Fe(III)} \xrightarrow[+\,CO]{+\,S_2O_4^{2-}}$$

$$[\text{cyt P-450–Fe(II)} \leftarrow \text{CO}] \qquad (1)$$

By taking these properties into account, it was possible to measure the amounts of free cytochrome P-450 and cytochrome P-450 engaged in stable iron–metabolite complexes able to bind to CO before and after ferricyanide treatment in microsomes from rats previously treated by the antibiotics indicated in Fig. 1.

As shown in Table 1, treatment of rats by these antibiotics does not lead to any significant change of hepatic cytochrome b_5 content. In contrast, these antibiotics have very different effects on hepatic cytochromes P-450. In that respect, they can be divided into three groups (Table 1). Group 3 antibiotics are unable to form 456 nm-absorbing cytochrome P-450–metabolite complexes *in vivo* and do not significantly change the amount of cytochromes

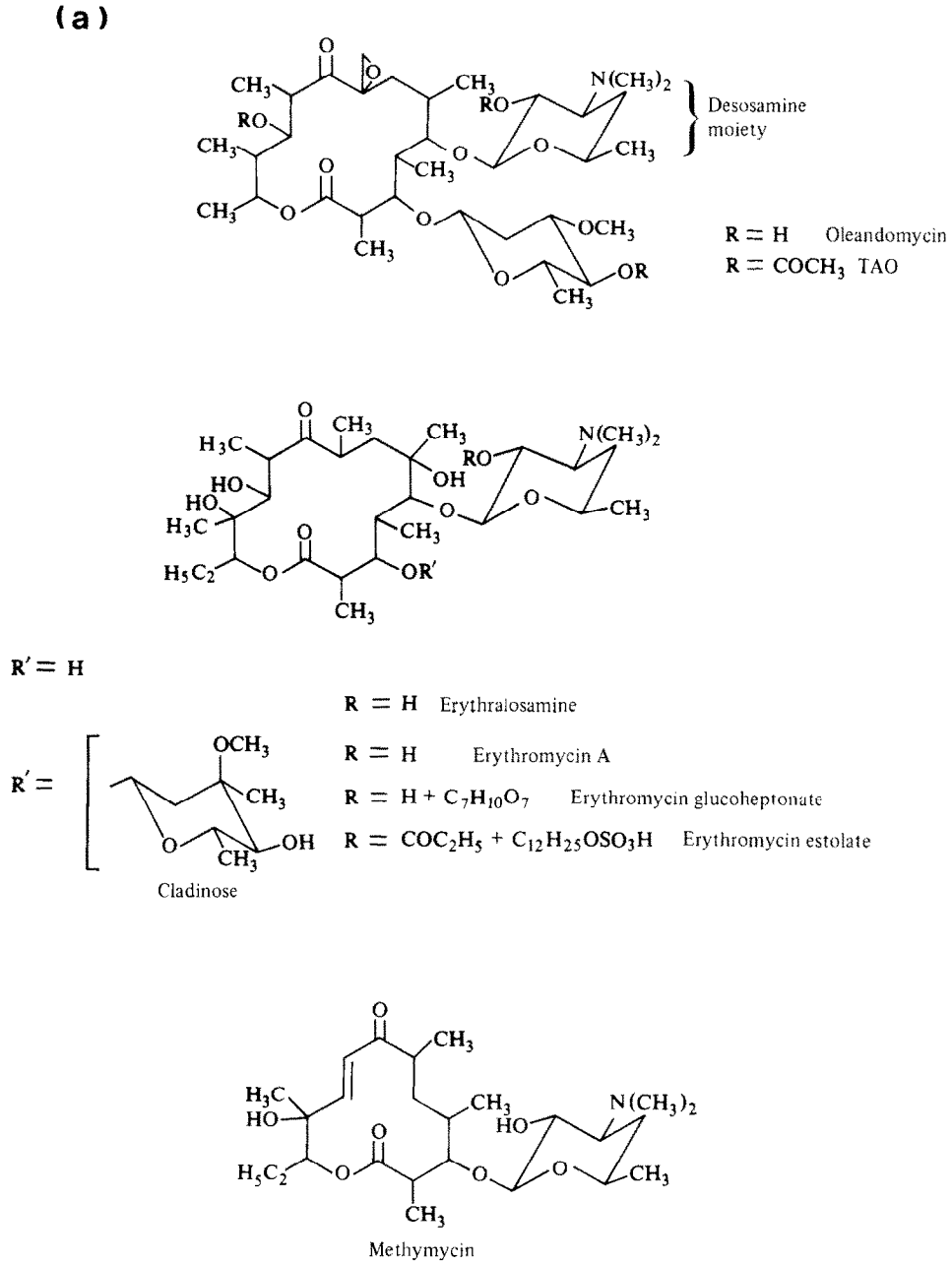
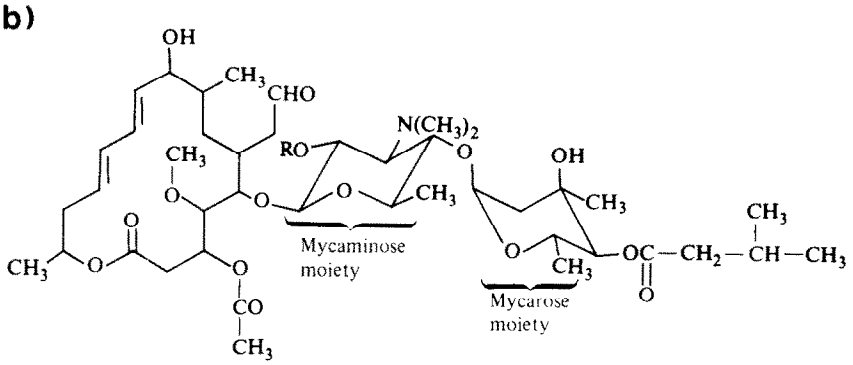
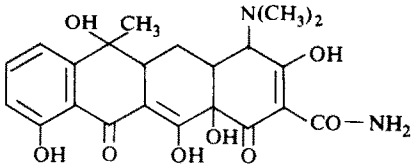


Fig. 1. Formulae of the antibiotics used in this study. $+C_7H_{10}O_7$, $+C_{12}H_{25}OSO_3H$ and $+HCO_2{-}(CH_2)_4CO_2H$ indicate glucoheptonic, laurylsulfuric and adipic acid salts of the corresponding antibiotics.

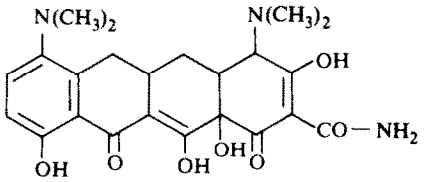
(b)



Josamycin base R = H
Josamycin propionate R = COC_2H_5



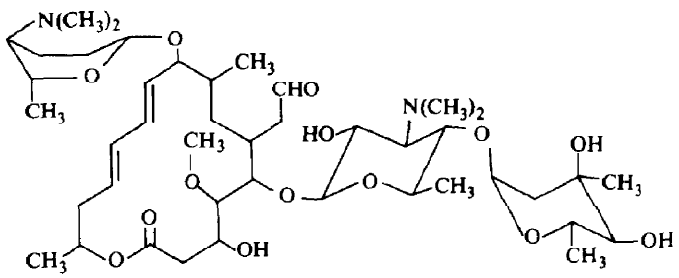
Tetracycline



Methylenecycline (minocycline)

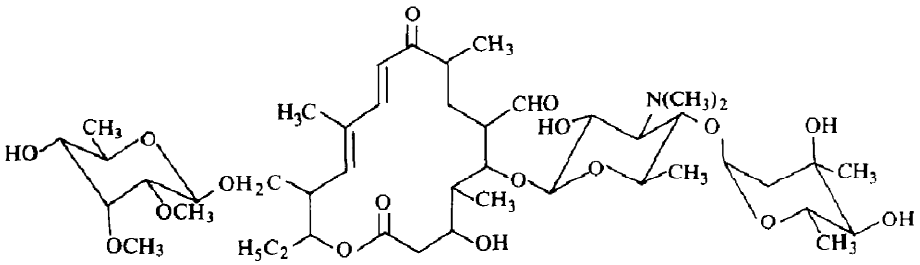
Fig. 1 (*continued*).

(c)

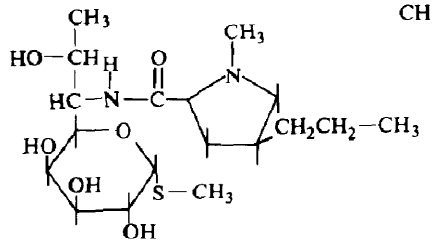


Spiramycin base

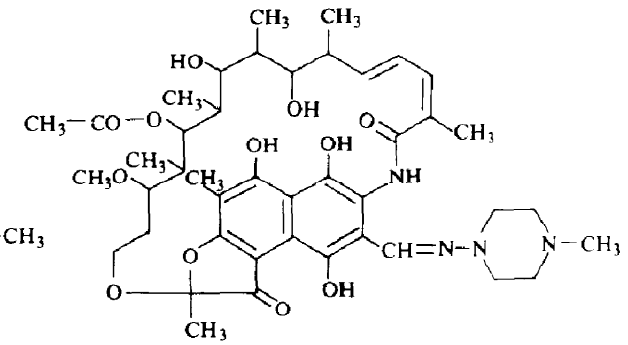
Spiramycin adipate $[+HO_2C(CH_2)_4CO_2H]$



Tylosin



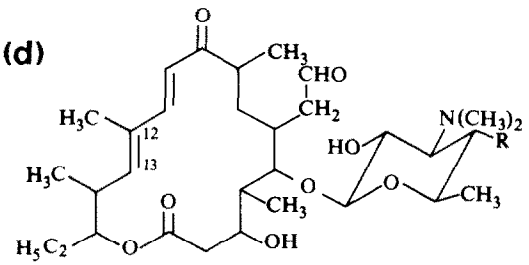
Lincomycin



Rifampicin

Fig. 1 (*continued*).

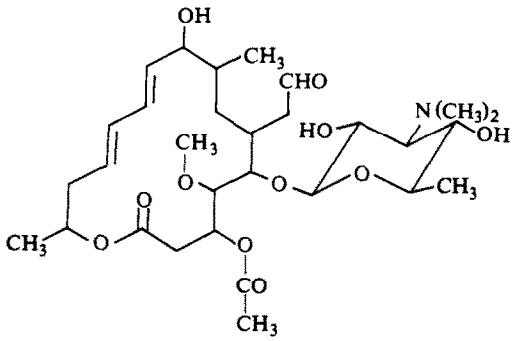
(d)



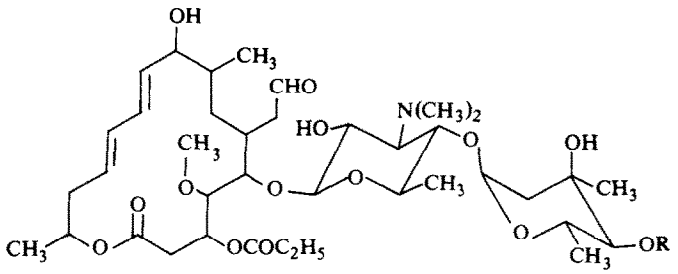
R = H M 4365 G_2

R = H, 12,13 - epoxy M 4365 A_2

R = OH, 12,13 - epoxy Cirramycin A_1



Desmycarose - josamycin



Platenomycin B_1 R = $-CO-C_2H_5$

Platenomycin A_1 R = $-CO-CH_2-CH(CH_3)_2$

Fig. 1 (*continued*).

P-450 in rat liver. Group 2 antibiotics are also unable to form iron–metabolite complexes *in vivo*, but act as weak inducers of cytochrome P-450, josamycin leading for instance to a 60% increase of liver cytochrome P-450. Group 1 antibiotics lead to an increase of total liver cytochrome P-450 and to the *in vivo* formation of a 456 nm-absorbing complex whose characteristics—i.e. stability in the presence of CO and oxidative destruction of its iron–metabolite bond by ferricyanide—are similar to those of SKF 525 A-[21] or propoxyphene-[22] derived complexes which seem to be cytochrome P-450–nitrosoalkane complexes [23–29]. TAO is at the same time the most potent inducer of cytochrome P-450, leading to a 4- to 5-fold increase of its hepatic content, and the best precursor for iron–metabolite complex formation, about 2.4 nmole of complex per mg protein having been detected *in vivo*. Erythromycin estolate is almost as potent as an inducer but only 40% of total cytochrome P-450, compared to 78% for TAO, is found *in vivo* as an iron–metabolite complex. The less hydrophobic erythromycin base or glucoheptonate are much less potent inducers. A similar conclusion arises when comparing TAO to its less hydrophobic analogue oleandomycin [13]. Moreover, erythralosamine, which is derived from acid hydrolysis of erythromycin and removal of a sugar bound to the macrocycle, is, contrary to erythromycin base itself, a good inducer of cytochrome P-450 and is able to form important amounts of the 456 nm-

Table 1. Effects of various antibiotics on rat hepatic cytochromes P-450 and b_5 *in vivo*

Treatment of rats	Cytochrome b_5 (nmole/mg protein†)	Cytochrome P-450 (nmole/mg protein†)	% of cytochrome P-450–metabolite complex	Induced P-450* / Control P-450
Control	0.63 ± 0.07	0.67 ± 0.15	0	1.0
Troleandomycin	0.62 ± 0.05	3.09 ± 0.25†	78	4.60
Erythromycin base	0.58 ± 0.05	1.21 ± 0.10†	43	1.80
Erythromycin estolate	0.67 ± 0.08	2.76 ± 0.10†	40	4.12
Erythromycin glucoheptonate	0.46 ± 0.05	0.86 ± 0.05	18	1.28
Erythralosamine‡	0.50; 0.54‡	1.82; 1.90‡	31	2.78
Oleandomycin phosphate	0.56 ± 0.04	1.11 ± 0.10†	10	1.66
Methymycin	0.46 ± 0.07	1.13 ± 0.05†	17	1.69
Josamycin base	0.59 ± 0.04	1.08 ± 0.04†	n.d.§	1.61
Methylenecycline	0.62 ± 0.04	1.00 ± 0.05†	n.d	1.49
Josamycin propionate	0.61 ± 0.05	0.83 ± 0.07	n.d.	1.24
Tetracycline	0.47 ± 0.09	0.85 ± 0.14	n.d.	1.29
Tylosin tartrate	0.48 ± 0.04	0.67 ± 0.10	n.d.	1.00
Spiramycin base	0.51 ± 0.03	0.52 ± 0.05	n.d.	0.78
Spriamycin adipate	0.53 ± 0.05	0.57 ± 0.10	n.d.	0.85
Rifampicin	0.48 ± 0.06	0.69 ± 0.08	n.d.	1.03
Lincomycin	0.47 ± 0.08	0.49 ± 0.15	n.d.	0.73

Results are presented as means ± S.E.M. for 3–6 determinations.
* Ratio of cytochrome P-450 contents in treated and control rats.
† Significantly different from control; $P < 0.05$ or better.
‡ In the case of erythralosamine, only two measurements were made; individual values are given.
§ n.d.: Not detected from difference spectroscopy, i.e. < 3% metabolite–complex was formed.

absorbing complex (about 30% of total cytochrome P-450). It must be noted that, because of the low amounts of erythralosamine available for this study and its high toxicity, rats were only treated by a single dose (200 mg/kg) and killed after 48 hr in the case of this antibiotic. In identical conditions, erythromycin base failed to give any induction of cytochrome P-450 and 456 nm-absorbing complex formation [8].

Comparison of the ability of liver cytochromes P-450 induced by various antibiotics to form iron–antibiotic metabolite complexes in vitro

It has been shown that control [9] or phenobarbital-induced (unpublished results) rat liver microsomes have a very low ability to form a 456 nm-absorbing complex *in vitro* when treated by NADPH and TAO. On the contrary, liver microsomes from rats pretreated by TAO exhibit, after treatment by ferricyanide to destroy the iron–metabolite complex formed *in vivo* and to regenerate cytochrome P-450 in its active form, a very high activity for NADPH-dependent TAO oxidation and iron–nitrosoalkane complex formation *in vitro* (Table 2). If one takes into account the ε(456–490) value of 0.080 $\mu M^{-1}cm^{-1}$ previously determined for the cytochrome P-450–TAO metabolite complex [12], one finds that 70–75% of cytochrome P-450 is thus engaged in iron–TAO metabolite complex formation *in vitro*. Since 78% of the complex was originally present *in vivo* in TAO-induced rat microsomes, one can conclude that almost all the metabolite complex can be formed again *in vitro*. Microsomes from rats treated by TAO also exhibit a remarkable ability to form iron–nitrosoalkane complexes upon *in vitro* oxidation of erythromycin base or oleandomycin. On the contrary, ferricyanide-treated microsomes from oleandomycin-induced rats are comparable to PB-induced microsomes for their very low ability to form iron–metabolite complexes upon *in vitro* oxidation of any of the three antibiotics (Table 2). Erythromycin base induction leads to an intermediate situation, the corresponding microsomes being only able to form important amounts of iron–metabolite complexes upon TAO oxidation (Table 2). It thus appears that TAO is the best substrate for metabolite complex formation with all the microsomes used. Moreover, TAO-induced cytochromes P-450 have by far the greatest ability to form 456 nm-absorbing complexes after macrolide antibiotic oxidation *in vitro*. This result led us to choose microsomes from TAO-treated rats in order to compare the ability of various antibiotics to form iron–metabolite complexes *in vitro*.

Interaction of various antibiotics with TAO-induced microsomes

Most antibiotics used in this study (Table 3) interact directly with ferricyanide-treated microsomes from TAO-pretreated rats, leading either to type I (λ_{max} = 390 nm, λ_{min} = 420 nm) or reverse type I difference spectra. As expected, the most hydrophobic antibiotics, like TAO, bind to the protein hydrophobic active site (type I interaction), the amount of cytochrome P-450–antibiotic substrate complex being considerably greater with TAO-treated (ΔOD_{max} 390–420 nm/P-450-(μM) = 14×10^{-3}) than PB-treated rat microsomes (ΔOD_{max} 390–420 nm/P-450-(μM) = 3.9×10^{-3}). Some less hydrophobic antibiotics containing several

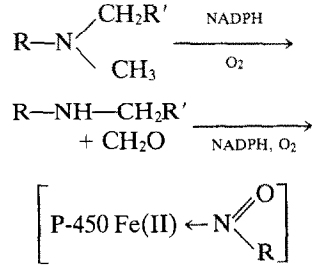
Table 2. Ability of liver cytochromes P-450 from rats treated by various antibiotics to form 456 nm-absorbing metabolite complexes *in vitro*

Substrate used *in vitro*	Rat treatment			
	$10^3 \times \Delta OD_{max}$ 456–490 nm/P-450 (μM) Using microsomes from rats pretreated by:			
	Phenobarbital	Oleandomycin	Erythromycin base	Troleandomycin
Oleandomycin	0.4 ± 0.2	2.0 ± 1.5	2.0 ± 0.8	10.0 ± 1.5
Erythromycin base	0.5 ± 0.3	2.0 ± 0.9	4.0 ± 1.7	17.0 ± 2.7
Troleandomycin	3.0 ± 0.5	3.4 ± 0.5	11.0 ± 1.0	60.0 ± 2.0

Rats were treated as indicated in Materials and Methods. Results are expressed as means ± S.E.M. of three determinations. Microsomes were pretreated with ferricyanide (5 μM) for 5 min. Complex formation was measured after 20 min incubation of microsomes (1 μM cytochrome P-450) with the antibiotics (20 μM) and NADPH (500 μM) at 30°.

hydroxy groups, such as oleandomycin, give weak reverse type I spectra which could correspond to the binding of a hydroxy group to high-spin cytochrome P-450–iron(III).

The first step in the formation of 456 nm-absorbing cytochrome P-450–Fe(II)–nitrosoalkane complexes upon microsomal oxidative metabolism of compounds containing a tertiary amine function is the *N*-dealkylation of this function [equation (2)] [28, 30].



Data concerning metabolite complex formation and formaldehyde production upon oxidation of various antibiotics by TAO-treated and PB-treated rat microsomes are compared in Table 3. Corresponding data on benzphetamine oxidation, a classical substrate of PB-treated rat microsomes [30], are also given for the sake of comparison. Using PB-treated rat microsomes, only benzphetamine appears to be a good substrate for *N*-demethylation and metabolite complex formation. PB-treated rat liver microsomes exhibit a poorer demethylation activity for group 1 (Table 1, Fig. 1a) antibiotics and for the antibiotics of group 1′ (Fig. 1d) which contain a mycaminose sugar (cirramycin A_1) or a desosamine sugar (M 4365 A_2, G_2). These PB microsomes also form only small amounts of cytochrome P-450–metabolite complex upon oxidation of these antibiotics. They have an even poorer ability to *N*-demethylate groups 2 and 3 antibiotics (< 0.5 nmole HCHO/min per nmole cytochrome P-450) and are unable to form the corresponding iron–metabolite complexes.

TAO-treated rat liver microsomes are considerably less active for benzphetamine demethylation and benzphetamine–metabolite complex formation. On the contrary, they are more active for demethylation of groups 1 and 1′ antibiotics, whereas they are almost as active or even less active for demethylation of groups 2 and 3 antibiotics and platenomycin A_1 and B_1. Moreover, they are considerably more potent than PB-treated rat liver microsomes in leading to 456 nm-absorbing complexes *in vitro* upon oxidation of groups 1 and 1′ (except platenomycins) antibiotics whereas they are unable to form such complexes upon oxidation of groups 2 and 3 antibiotics. Close relationships were always observed between demethylation activity and the extent of metabolite complex formation (Table 3). Furthermore, a good relationship was observed most generally between the extent of type I spectrum formation, *N*-demethylation and metabolite complex formation, as shown for instance in Table 3 for some structurally closely related antibiotics.

DISCUSSION

The formation of stable inhibitory cytochrome P-450–metabolite complexes characterized by a 456 nm peak upon metabolite oxidation of macrolide antibiotics is thus a general phenomenon *in vivo* and *in vitro*. At least it is not limited to the previously described cases with oleandomycin [13], TAO [9] or erythromycin base [8]. So far, we have observed the formation of such complexes with antibiotics containing a desosamine (TAO, oleandomycin, erythromycin derivatives, methymycin, M 4365 A_2, M 4365 G_2, erythralosamine) or a mycaminose (cirramycin A_1, desmycarose-josamycin) sugar moiety. It is worth noting that, so far, all the antibiotics that were shown to form a 456 nm-absorbing complex *in vivo* also form this complex *in vitro* with TAO-treated rat liver microsomes. Moreover, the ability of an antibiotic to form a nitrosoalkane–cytochrome P-450 complex *in vivo* seems to be linked to its inducing effect on hepatic cytochrome P-450. The antibiotics causing the greatest increase of cytochrome P-450 levels, TAO and erythromycin estolate, are at the same time those which give the highest level of metabolite complexes *in vivo* and *in vitro*. This increase of hepatic cytochrome P-450 level upon TAO treatment is not simply due to a protection of cytochrome P-450 when it is engaged in a 456 nm-absorbing complex which could delay its biodegradation, since a new form of cytochrome P-450 appears after TAO treatment [12] and since control or PB-treated rat liver microsomes are almost unable

Table 3. Interactions of various antibiotics with microsomes from PB- or TAO-induced rats

Substrate	Difference spectra on TAO-microsomes	Nitrosoalkyl-cytochrome P-450 complex formation ΔOD (456–490 nm) × $10^3/\mu$M cytochrome P-450		Formaldehyde formation nmole/min per nmole cytochrome P-450	
	Type ΔOD (390–420 nm)/ μM cytochrome P-450	PB microsomes	TAO microsomes	PB microsomes	TAO microsomes
Benzphetamine	I (0.015)	13	1.0 ± 0.4	8.4 ± 0.1	1.5 ± 0.3
TAO	I (0.056)	3 ± 0.5	60 ± 2.0	2.0 ± 0.2	5.8 ± 0.3
Oleandomycin	RI (0.006)	0.4 ± 0.3	10 ± 1.5	0.87 ± 0.15	0.95 ± 0.10
Erythromycin base	RI (0.015)	0.5 ± 0.3	17 ± 2.7	1.30 ± 0.10	2.7 ± 0.1
Erythromycin estolate	I (0.031)	2.0 ± 0.8	23 ± 1.4	0.53 ± 0.05	2.4 ± 0.1
Erythralosamine	I (0.022)	n.m.	41 ± 2.0	0.84 ± 0.12	1.9 ± 0.1
Methymycin	I (0.015)	tr	3 ± 0.7	0.63 ± 0.05	1.0 ± 0.05
M 4365 A_2	I (0.039)	tr	17 ± 2.0	0.67 ± 0.10	0.85 ± 0.15
M 4365 G_2	I (0.058)	tr	22 ± 2.0	0.93 ± 0.08	1.33 ± 0.13
Cirramycin A_1	I (0.020)	n.m.	8 ± 2.0	n.m.	0.42 ± 0.15
Desmycarose-josamycin	I (0.009)	n.m.	3 ± 1.0	n.m.	n.m.
Josamycin	I (0.012)	n.d.	n.d.*	0.43 ± 0.10	0.29 ± 0.12
Spiramycin base	RI (0.010)	n.d.	n.d.	0.20 ± 0.12	0.26 ± 0.08
Tylosin	RI (0.014)	n.d.	n.d.	0.10 ± 0.05	0.23 ± 0.10
Platenomycin A_1	I (0.021)	n.m.	n.d.	0.37 ± 0.05	0.23 ± 0.05
Platenomycin B_1	I (0.023)	n.m.	n.d.	0.43 ± 0.06	0.19 ± 0.07

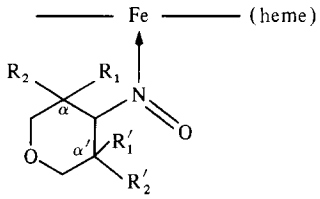
Results are expressed as means ± S.E.M. for three determinations on phenobarbital (PB)- or troleandomycin (TAO)-treated rat liver microsomes previously treated with ferricyanide. Metabolite–complex formations were performed as in Table 2. Formaldehyde formation was measured according to the Nash method, using 0.2 mM substrate and an NADPH-generating system (NADP 1 mM, G6P 10 mM, G6PDH1 UI) and incubation at 37° for 10 min. I and RI indicate type I and reverse type I difference spectra.

* n.m. = not measured; n.d. = not detected, tr = trace.

to form cytochrome P-450–metabolite complexes contrary to TAO-treated rat liver microsomes (Table 3). The sequence of events explaining the effects of some macrolide antibiotics on hepatic cytochromes P-450 could be: (i) the induction by the antibiotic of a cytochrome P-450 form especially able to bind and metabolize it; (ii) the oxidation of the antibiotic by this new form leading to a stable iron–metabolite complex; and (iii) the accumulation of this complex where cytochrome P-450 is completely inactivated.

The structural factors which appear important for an antibiotic to lead to a cytochrome P-450–nitrosoalkane complex are the following:

(1) *The presence of an accessible* $N(CH_3)_2$ *group in the molecule*. Antibiotics involving a tertiary amine function with the nitrogen atom inserted in a cyclic structure, such as rifampicin or lincomycin, are unable to lead to 456 nm-absorbing complexes. Antibiotics involving a $N(CH_3)_2$ group located on a compact polycyclic structure, such as tetracycline, fail to form cytochrome P-450–metabolite complexes. This may be understood if one takes into account the structure of the final possible iron–nitrosoalkane complex. As shown in model porphyrin–iron(II)–nitrosoalkane complexes, the RNO ligand is bound to the iron by its nitrogen atom (Scheme 1) [29]. It is thus likely that the stability of these complexes dramatically decreases when bulky substituents are present on the carbon bearing the NO group. Regarding this, it has been shown that tertiary nitrosoalkanes such as 2-methyl-2-nitrosopropane do not bind to the Fe(II)(porphyrins) [29] or to haemoproteins such as haemoglobin [26] and cytochrome P-450 [23–25]. In tetracycline, the $N(CH_3)_2$ group is located on a very hindered secondary carbon atom with bulky rigid substituents on carbons in α- and α'-positions (Scheme 1). Such steric factors are also very important in explaining the observed differences between the tested antibiotics.



Scheme 1.

(2) *The steric hindrance around the* $N(CH_3)_2$ *group*. Antibiotics involving a $N(CH_3)_2$ group on a desosamine (TAO, erythromycin derivatives, methymycin and M 4365 A_2 and G_2) or a mycaminose (cirramycin A_1, desmycarose-josamycin) are good precursors for metabolite complex formation. Desosamine has an unhindered α-position ($R_1 = R_2 = H$) and a relatively more hindered α'-position ($R'_1 = H$, $R'_2 = OH$ or $OCOCH_3$) (Scheme 1 and Fig. 1). Mycaminose is relatively more hindered around its nitrogen atom since it has two equivalent α- and α'-positions ($R_1 = R'_1 = OH$; $R_2 = R'_2 = H$). This could explain why M 4365 A_2, which differs only from cirramycin A_1 by the replacement of the desosamine moiety by a mycaminose moiety, gives 3–4

times more 456 nm-absorbing complex than cirramycin A_1 (Table 3). Moreover, antibiotics such as josamycin, spiramycin, tylosin and platenomycin A_1 and B_1, where one OH group of the mycaminose moiety is replaced by a considerably more bulky O-sugar group, are completely unable to form cytochrome P-450–nitrosoalkane complexes (Tables 1 and 3). Accordingly, desmycarose-josamycin, which is derived from josamycin by replacement of this O-sugar group (mycarose) (Fig. 1d) by an OH group, becomes able to form a 456 nm-absorbing complex (Table 3). Such a critical influence of the steric hindrance around the amino group on cytochrome P-450–metabolite complex formation has been previously noticed in the amphetamine series [21, 24, 31, 32].

(3) *The hydrophobicity of the molecule.* Several examples show that an increase of the hydrophobicity of a given antibiotic leads to an increase of its ability to form a 456 nm-absorbing complex. For example, oleandomycin, which involves three OH groups, gives 6–8 times less metabolite complex than TAO, where the three OH groups have been esterified. The potency of erythromycins as metabolite–complex precursors parallels their hydrophobicity: glucoheptonate <base <estolate. Moreover, erythralosamine, which has lost the cladinose moiety of erythromycin, forms at least twice as much 456 nm-absorbing complex than erythromycin (Table 3). The same conclusion arises when one compares M 4365 G_2, M 4365 A_2 and cirramycin A_1 (Fig. 1d, Table 3). Their potency as cytochrome P-450–metabolite complex precursors decreases in the order : M 4365 G_2 > M 4365 A_2 > cirramycin A_1, as their number of polar oxygen atoms increases (M 4365 A_2 contains one more epoxide oxygen atom than M 4365 G_2, and cirramycin A_1 one OH group more than M 4365 A_2).

Several factors could be at the origin of the ability of some macrolide antibiotics to modify the plasma levels and therapeutic response of drugs given in association with them [33]. In that respect, it seems that there is a relationship between the ability of macrolide antibiotics to form inhibitory cytochrome P-450–metabolite complexes in rat liver, as indicated in this paper, and their ability to modify drug metabolism in man, as indicated by data presently available in the literature. For instance, TAO, which is a very good precursor of an inhibitory- 456 nm-absorbing complex in rat [9] and human [34] livers, was shown to increase the half-life of theophylline in man [4], whereas josamycin, which fails to give such a complex in rat liver (this paper), was found unable to affect the theophylline half-life [4]. Moreover, several reports exist concerning therapeutic problems occurring after association of drugs with TAO [1], whereas few reports exist in the case of oleandomycin [13] and even less in the case of josamycin [35].

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