Interactions between drugs and chemicals in industrial societies.
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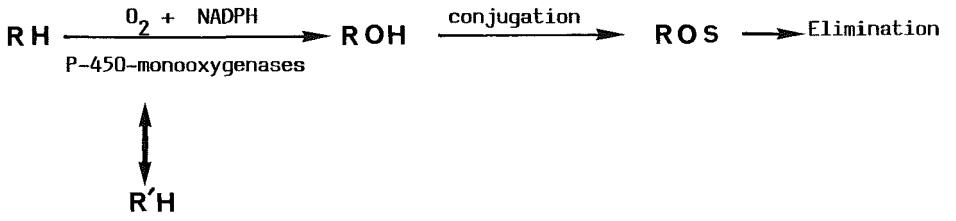
DRUG INTERACTIONS AT THE LEVEL OF DRUG-METABOLIZING ENZYMES

D. MANSUY

Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, Unité Associée au CNRS en Développement Concerté avec l'INSERM, UA 400, 45 rue des Saints-Pères, 75270 Paris Cedex 06 (France)

INTRODUCTION

Cytochrome P-450-dependent monooxygenases are ubiquitous enzymes which are in charge in most living organisms of the metabolism of exogenous as well as endogenous compounds. It is now well known that the in vivo administration of various compounds including drugs greatly modifies the activities of many monooxygenases. As shown in scheme 1, environmental compounds or drugs (R'H) may perturb in vivo the activity of some monooxygenases leading to dramatic changes of the oxidative metabolism and elimination of drugs (RH) given in association. This may result in particular in drug-drug interactions.



Scheme 1

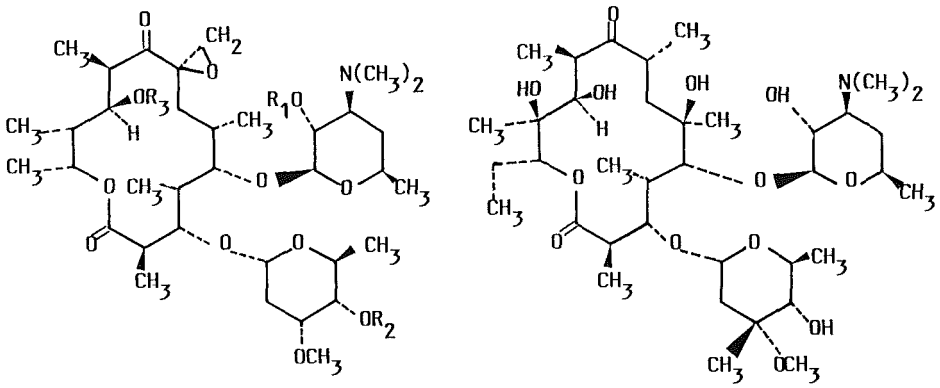
Very generally, it is admitted that such R'H exogenous compounds can act either as inducers or inhibitors of cytochrome P-450-dependent monooxygenases leading either to an increase or a decrease of their activities.

In fact, the in vivo effects of many exogenous compounds are not so well separated and the in vivo action of many xenobiotics on drug-metabolizing enzymes may be a very complex phenomenon.

The following results **illustrate the possible complexity of the effects of a given xenobiotic on cytochrome P-450-dependent monooxygenases in vivo.** They are concerned with a series of macrolactones which includes the well-known macrolide antibiotics TAO (the triacetate of oleandomycin) and erythromycin (Fig.1). These antibiotics have led to many problems of drug interactions in humans (1). Several cases of interactions between TAO and steroid derivatives such as oral contraceptives or prednisolone have been reported. In a general manner, it seems that the administration of TAO, or to a lesser extent erythromycin, leads to a decrease of the rate of metabolism and elimination of several drugs given in association with it such as carbamazepine, theophylline and dihydroergotamine. In some extreme cases the resulting increase of the plasmatic level of dihydroergotamine has led to the appearence of the secondary toxic effects of this drug and to severe clinical problems (1). The aim of the following study was to understand at the molecular level :

➤ the origin of these TAO-drug interactions, and for that purpose, the nature of the effects of TAO derivatives on drug-metabolizing enzymes.

➤ the structural factors that are important for the appearance of such effects.



$R_1=R_2=R_3=H$ Oleandomycin

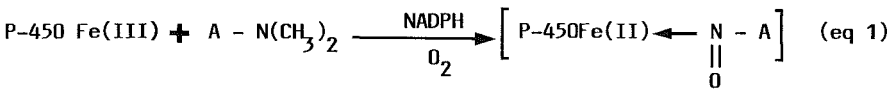
$R_1=R_2=R_3=COCH_3$ TAO

Erythromycin

Fig. 1. Formula of oleandomycin, TAO and erythromycin

SOME OLEANDOMYCIN AND ERYTHROMYCIN DERIVATIVES ARE STRONG INHIBITORS OF HEPATIC CYTOCHROME P-450 BY FORMATION OF STABLE IRON-METABOLITE COMPLEXES

Macrolide antibiotics of the oleandomycin and erythromycin series are metabolized by rat liver microsomes in the presence of NADPH at the level of their tertiary amine function. An intermediate resulting from this oxidation, presumably the corresponding nitrosoalkane, binds very strongly to the iron(II) of microsomal cytochrome P-450 (eq.1). The corresponding iron-metabolite complexes characterized by Soret peaks around 456nm are formed both in vitro and in vivo after administration of the macrolides to rats. For instance, after i.p. injection of 500 mg/Kg TAO to rats daily for 3 days, up to 70% to 80% of their liver cytochromes P-450 are blocked under the form of a P-450-Fe(II)-nitrosoalkane complex (2). The Fe(II)-RNO bond of these complexes is very stable in vitro and in vivo explaining why cytochromes P-450 engaged in such complexes loose completely their catalytic activities.



456 nm absorbing complex

$A - N(CH_3)_2$ = TAO

A study of several macrolide antibiotics including TAO, erythromycin, josamycin and spiramycin as well as many derivatives of oleandomycin and erythromycin showed us that the following structural factors are important for inhibitory metabolite complex formation (3) :

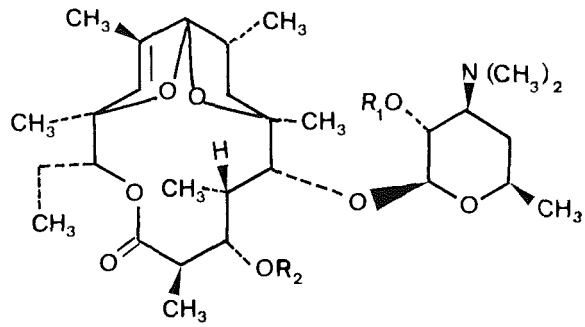
(i) ➤ the presence of a readily accessible $N(CH_3)_2$ function

(ii) ➤ the absence of a too important steric hindrance in the vicinity of the $N(CH_3)_2$ function : for instance, josamycin contrary to TAO was found unable to form such iron-metabolite complexes.

(iii) ➤ a high hydrophobicity of the molecule : for instance, TAO, the triacetate of oleandomycin is more prone to form iron-metabolite complexes than oleandomycin itself.

OLEANDOMYCIN AND ERYTHROMYCIN DERIVATIVES ARE INDUCERS OF A SPECIFIC FORM OF CYTOCHROME P-450 IN RAT LIVER.

Treatment of rats with TAO leads to a five-fold increase of their level of hepatic cytochromes P-450 (2,3). A similar strong induction of cytochrome P-450 was also observed with all the antibiotics or antibiotic derivatives previously found to form 456nm absorbing metabolite complexes in vivo. Recently, we found that several erythralosamine derivatives (MAEM or DAEM) derived from erythromycin by acidic cleavage of the bond between the macrolactone and the cladinose sugar moiety are stronger inducers of rat liver cytochrome P-450 than TAO or erythromycin themselves (Fig.2) (4).



R_1=R_2=H erythralosamine ; R_1=R_2=$COCH_3$ DAEM

R_1=H, R_2=$COCH_3$ MAEM.

Fig. 2. Formula of erythralosamine derivatives

The macrolide derivatives which were found very prone to form inhibitory cytochrome P-450-metabolite complexes in vivo, such as TAO, MAEM and DAEM, were also found as very good inducers of cytochrome P-450 in rats. However the formation of metabolite complexes is not absolutely required for the induction to take place since an erythromycin derivative which has lost the $N(CH_3)_2$ function and which is not able to form any metabolite complex was also found to induce cytochrome P-450 in rat liver (E. Sartori, M. Delaforge, D. Mansuy, in preparation).

Naturally, it was important to determine the nature of the cytochrome (s)

P-450 induced in rat by these macrolide antibiotics. In that regard, it was recently shown that TAO, DAEM or MAEM are specific inducers in rats of a cytochrome P-450 form which exhibits metabolic, electrophoretic and immunological characteristics identical to those of the major cytochrome P-450 form induced in rat by a steroid, pregnenolone-16 α-carbonitrile (PCN)(4,5). Although PCN and TAO both act as inducers of the same form of cytochrome P-450 (P-450-PCN), the apparent result of their administration to rats are different. The former leads to a maximum increase of the amounts of total rat liver cytochromes P-450 of about 80%, these cytochromes P-450 being in the usual and active ferric state. The latter as well as the other inducing macrolides leads to a much higher increase of total cytochrome P-450 of about 300% and the greatest part of this cytochrome P-450 is present under the form of an iron(II)-metabolite complex (Fig.3). Interestingly, macrolide derivatives, such as desamino-erythromycin, which are inducers but unable to form iron-metabolite complexes, gave results very similar to PCN. A possible explanation for this considerably higher amount of cytochrome P-450 induced by TAO or DAEM compared to PCN could be a prolonged half-life of the P-450-PCN form in vivo when it is engaged in a stable 456nm absorbing cytochrome P-450-metabolite complex. Such a prolongation of the half life of a cytochrome P-450 when it is engaged in a stable iron-metabolite complex, is another possible effect by which a xenobiotic can modify the activities of drug-metabolizing enzymes.

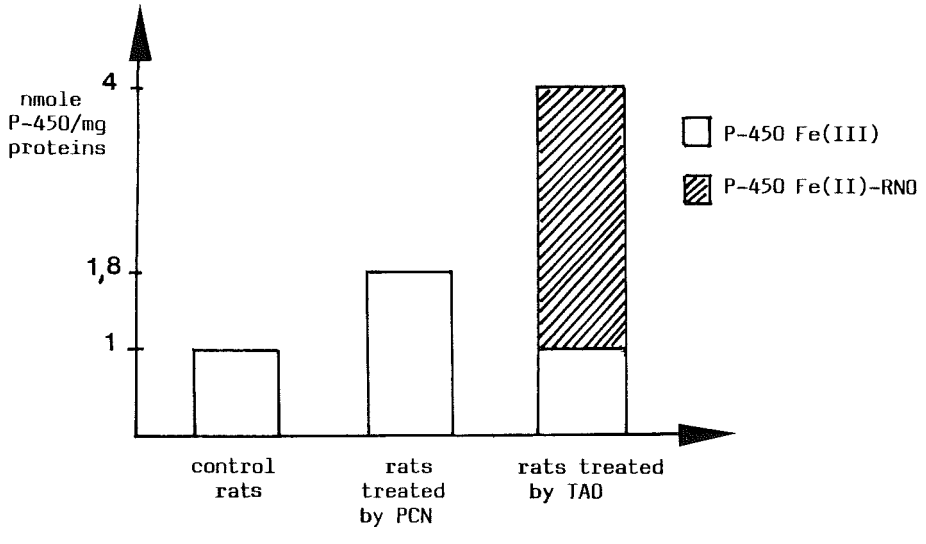


Fig. 3. Induction of liver cytochromes P-450 by PCN and TAO in rats.

EFFECTS OF TAO IN MAN

Recent results have shown that many of the effects of TAO observed in rat were also found in man. In particular, 456mn absorbing cytochrome P-450-iron-metabolite complexes are formed in the liver of patients that have received TAO (6). Moreover, studies on the liver of humans treated by TAO revealed the induction of a cytochrome P-450 form which had a particular ability to N-demethylate erythromycin and which seemed to be the equivalent of the rat liver cytochrome P-450 form (7). Very interestingly, this human liver cytochrome P-450 is not only induced by TAO but also by the corticosteroid dexamethasone. Thus, in humans as in rats, one finds this **surprising common ability of macrolide antibiotics on one side and of certain steroids on the other side to specifically induce the same cytochrome P-450 form.**

CHARACTERISTICS OF THE CYTOCHROME P-450 INDUCED BY MACROLIDE ANTIBIOTICS

In order to understand the pharmacological consequences of the different effects of TAO on the in vivo activities of cytochrome P-450-dependent monooxygenases, it is important to know the substrates that could be oxidized by the cytochrome P-450 induced by TAO. Unfortunately, very few is presently known about the substrates and activities of this cytochrome in rat or human liver. In fact, it is particularly prone to bind hydrophobic macrolide antibiotics such as TAO or DAEM and to N-demethylate them. In particular, it was shown that the amounts of cytochrome P-450-TAO derived metabolite complex formed in vitro upon oxidation of TAO by liver microsomes from rats treated by various inducers correlate well with the percentage of the cytochrome P-450-PCN form present in these microsomes (4).

Very recent results showed that cytochrome P-450-PCN has a particular ability to bind theophylline and ergotamine. Although liver microsomes from control rats led to a very weak type I binding spectrum in the presence of ergotamine, liver microsomes from rats either treated by PCN or by TAO led to a very intense binding spectrum (E. Sartori, M. Delaforge, D. Mansuy, in preparation). This shows clearly that P-450-PCN binds selectively ergotamine and suggests that it could be involved in the oxidative metabolism of this drug.

Moreover, since P-450-PCN is induced by certain steroids (PCN in rat and dexamethasone in man) it is tempting to speculate that this cytochrome which is preexisting in control livers is involved in the metabolism of some endogenous or exogenous steroids.

PHARMACOLOGICAL CONSEQUENCES OF THE IN VIVO EFFECTS OF TAO AND RELATED MACROLIDE ANTIBIOTICS ON CYTOCHROMES P-450.

The in vivo effects of TAO on liver cytochromes P-450 can be summarized as follows :

► TAO highly induces a cytochrome P-450 form that preexists in control liver and that is also induced by some steroids. This cytochrome has a particular ability to oxidize macrolide antibiotics such as TAO but could also be involved in the oxidative metabolism of other drugs such as ergotamine

► After TAO administration, this cytochrome P-450 is inactivated by formation of an iron-metabolite complex that is very stable in vivo.

These data allow one to explain at least in part the consequences observed after TAO association with drugs. When the drug given in association is metabolized by cytochrome P-450-PCN (or its equivalent in human liver), one should expect that it would be less rapidly eliminated when associated with TAO since this cytochrome is inactivated by metabolite complex formation. This could be the case for dihydroergotamine which has a particular affinity for cytochrome P-450-PCN.

As for the more general problem of possible interactions of drugs metabolized by cytochrome P-450-PCN with macrolide antibiotics, there are three possible situations :

1 ► the macrolide antibiotic is at the same time an inducer of the cytochrome P-450-PCN form and an inhibitor of that form by iron-metabolite complex formation. This is the case of TAO, of some hydrophobic derivatives of erythromycin and of the erythralosamin derivatives. With such compounds, one should expect a decrease of the elimination rate of drugs which are given in association with them and which are metabolized by P-450-PCN.

2 ► the macrolide antibiotic is an inducer of cytochrome P-450-PCN but is not able to form an inhibitory iron-metabolite complex. This seems to be the case of the antibiotic rifampicin in man. In that case, one should expect the opposite result : an increase of the elimination rate of drugs given in association.

3 ► the macrolide antibiotic is neither an inducer nor an inhibitor. This is the case of josamycin and one should expect only very weak changes of the metabolism of drugs given in association with it.

Accordingly, presently available clinical data from the literature indicate that TAO administration leads to a decrease of the elimination rate of several drugs given in association with it whereas rifampicin has the opposite effect.

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Discussion - Drug interactions at the level of drug-metabolizing enzymes.

G.L. Plaa

I am intrigued with TAO as a tool. How quickly does the inhibition occur in the rat and how long does it last?

Mansuy

It is difficult to say how quickly it occurs because we have not measured it in vivo. The only thing that we did was to measure the amount of the inhibiting complex as a function of time. It reaches a maximun after two days when TAO is given as one injection daily for three days, and baseline values in rat liver are obtained in about ten days.

G.L. Plaa

Can you even get an inhibitory phase separated from the induction phase?

D. Mansuy

We have not tried to differentiate these two effects as a function of time. However, I can say that the complex formation is very fast.

P. du Souich

Do you know which one of the metabolic pathways of theophylline in man is inhibited by TAO?

D. Mansuy

We have not studied that specifically. The only experiment we did in that direction was to measure the binding affinity of theophylline to liver microsomes from treated animals. These microsomes contained different proportions of P 450 PCN and we found that the binding constants were higher after induction of P 450 PCN.

H. Vainio

Does the induction by macrolide antibiotics extend to extrahepatic drug metabolizing tissues?

D. Mansuy

I do not know. We have studied only the liver.

M.M. Reidenberg

As you give the inducer that forms the inhibitory complex and get more enzyme protein formed, what happens to enzyme activity? You get a very prompt inhibition and then over time a lot of enzyme protein is synthesized? Is there any change in enzyme activity or is all of this new protein that is synthsized inhibited?

D. Mansuy

We have not done the necessary studies to answer this question. In our three day or eight day studies we have observed both a inhibition of the N-demethylation of TAO, for instance, and this increase in protein.

R. Lauwerys

Your results imply that the half life of TAO in the liver is quite long.

D. Mansuy

The inhibitory complex is very strong and what we observe is a prolongation of the half life of the particular form of cytochrome P 450 that has bound the metabolite.