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# ROLE OF THE P-450 DEPENDENT AND FAD-CONTAINING MONOOXYGENASES IN THE BIOACTIVATION OF THIOACETAMIDE, THIOBENZAMIDE AND THEIR SULPHOXIDES

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Several thioamides, among which thioacetamide (TA) and thiobenzamide (TB), as well as their sulphoxides, are known to induce liver damage [1, 2, 3] . Many evidences also suggest that one or more oxidation steps are required for the toxic action of such compounds [1, 3, 4]. Bioactivation is believed to occur within the liver endoplasmic reticulum by the mixed-function oxidase system, according to the following steps: TA and TB are oxidized at sulfur atom to give rise to the corresponding S-oxides (TASO, TBSO). In turn, the S-oxides undergo a further oxidation which possibly leads to the highly reactive S-dioxygenated products. Finally,the S-dioxides may undergo hydrolysis, from products of which acetamide or benzamide can be recovered. From the breakdown of S-dioxides also covalent binding to cell macromolecules may result, which could be responsible for the observed liver damage [6, 7] . At least two microsomal systems, i. e. the cytochrome P-450 dependent monooxygenase and the flavin-containing one (FADM),could be involved in bioactivation, since both are known to efficiently oxidize the sulfur atom of several organic substrates [1, 5] .

To investigate their relative importance as far as the thioamide-induced liver necrosis is concerned, TA, TB or their sulphoxides were given in a necrogenic amount to rats pretreated with i) SKF 525-A or metyrapone, specific inhibitors of P-450 ii) methimazole or cysteamine, substrates/inhibitors of FADM

The thioamides were also added to liver microsomes engaged in the transformation of model P-450 or FADM substrates, to evaluate what kind of change, if any, can be induced by thioamides in the kinetic pattern of such reactions.

## RESULTS AND DISCUSSION

In vivo : methimazole and cysteamine effectively reduced the thioamide-induced increase in serum ALAT* activity, while SKF 525-A or metyrapone did not modify it (Fig. 1).Accordingly, the extent of liver necrosis was significantly reduced by methimazole or cysteamine pretreatments, but not by SKF 525-A or metyrapone administration (Fig. 1).

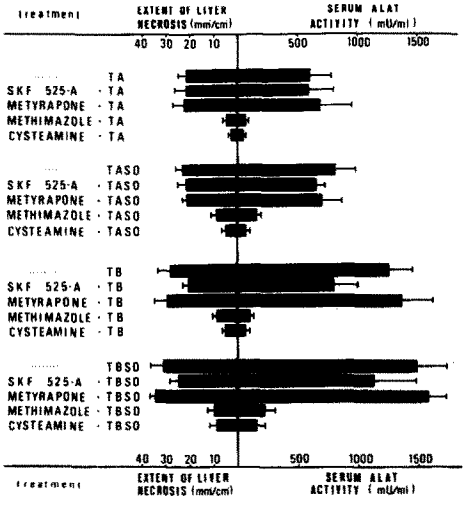


Fig. 1 Effects of various pretreatments on liver necrosis induced by thioacetamide, thioacetamide S-oxide thiobenzamide and thiobenzamide S-oxide.
Male Sprague-Dawley rats (200–250 g) were fasted 14 h before any treatment and during the experimental period. All the chemicals were purchased from commercial sources, except for the sulphoxides (TASO, TBSO) , which were synthetized in our laboratory.
TA (200 mg/kg) and TASO (220 mg/kg) were given i.p.; TB (200 mg/kg) and TBSO (220 mg/kg) were given p.o.; SKF 525-A (75 mg/kg), metyrapone (60 mg/kg) and methimazole (200 mg/kg) were given i.p., whereas cysteamine (500 mg/kg) was administered p.o.
All the pretreatments were performed 30 min before hepatotoxin administration. Rats were killed 24 h following the administration of thioamides.
Serum ALAT activity was measured by a commercial U V method. The quantitation of tissue damage was performed by a planimeter on drawings of H&E stained histological sections and expressed in $mm^2/cm^2$.
Data represent the means ± S.E. (5 rats/group). Statistical analysis was done by the Student's t-test.

* alanine aminotransferase

In vitro : all the thioamides, when added to active rat liver microsomes performing the N-oxidation of N,N-dimethylaniline, significantly inhibited the kinetic of the reaction (Fig. 2) . Conversely, the kinetic of O-deethylation of 7-ethoxycoumarin was scarcely affected by TA, TB and TASO; only TBSO had a slight effect (Fig. 3).

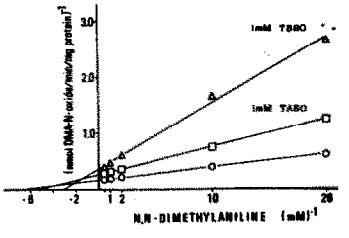


Fig. 2 Double reciprocal plot of rate of DMA N-oxide production from N,N-dimethylaniline by rat liver microsomes in absence and in the presence of 1mM TASO or 1mM TBSO. Each data point represents the mean of 2 determinations performed on separate pools of microsomes obtained from 3 rats each. The apparent Km and Vmax values are 140 μM, 6.25 nmol/min/mg protein, 192 μM, 3.85 nmol/min/mg protein and 329 μM, 2.86 nmol/min/mg protein, respectively. The enzymatic activity was measured by the method of Gold and Ziegler [8] .

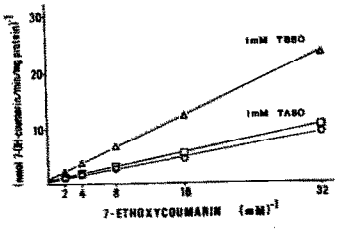


Fig. 3 Double reciprocal plot of the rate of O-deethylation of 7-ethoxycoumarin by rat liver microsomes in absence and in the presence of 1mM TASO or 1mM TBSO. Each data point represents the mean of 2 determinations performed on separate pools of microsomes obtained from 3 rats each. The apparent Km and Vmax values are 704 μM 2.5 nmol/min/mg protein for the unhinibited reaction and 909 μM, 1.25 nmol/min/mg protein in the presence of 1mM TBSO. The enzymatic activity was determined by the method of Greenlee and Poland [9] .

Our observations show that two good substrates of the liver microsomal FADM,methimazole and cysteamine, are very effective in preventing liver necrosis induced by TA, TB and their sulphoxides. On the contrary, the known inhibitors of P-450 mediated biotransformations, SKF 525-A and metyrapone lack preventive action. Furthermore, the microsomal reactions believed to be mediated by the P-450 system, are not at all, or scarcely affected by the addition of thioamides to incubation medium, whereas the oxidation of a model FADM substrate, such as N,N-dimethylaniline, is significantly inhibited by all the thioamides.

Since i) thioamides, as well as their sulphoxides have been reported to be good substrates for the purified FADM [5] ii) the microsomal metabolism of such compounds appear largely mediated by FADM and at a minor extent by P-450, at least in rat liver microsomes [6, 7, 10] , it can be hypothesized that the in vivo observed protection by FADM substrates on the liver damage induced by thioamides administration could be ascribed to a competition between the chemicals on the same enzymatic system, i.e. the FADM one. This hypothesis is also confirmed by the kinetic patterns of FADM or P-450 mediated microsomal reactions in presence of the thioamides.

Thus, it is concluded that FADM might critically control the process of activation of thioamides to their ultimate necrogenic forms, to which also the P-450 system could contribute.

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