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METABOLIC ACTIVATION OF THE ANTIDEPRESSANT TIANEPTINE

II. *IN VIVO* COVALENT BINDING AND TOXICOLOGICAL STUDIES AT SUBLETHAL DOSES

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Abstract—Administration of [^{14}C]tianeptine (0.5 mmol/kg i.p.) to non-pretreated hamsters resulted in the *in vivo* covalent binding of [^{14}C]tianeptine metabolites to liver, lung and kidney proteins; this very high dose (360-fold the human therapeutic dose) depleted hepatic glutathione by 60%, and increased SGPT activity 5-fold. Lower doses (0.25 and 0.125 mmol/kg) depleted hepatic glutathione to a lesser extent and did not increase SGPT activity. Pretreatment of hamsters with piperonyl butoxide decreased *in vivo* covalent binding to liver proteins, and prevented the increase in SGPT activity after administration of tianeptine (0.5 mmol/kg i.p.). In contrast, pretreatment of hamsters with dexamethasone increased *in vivo* covalent binding to liver proteins, and increased SGPT activity after administration of tianeptine (0.5 mmol/kg i.p.). Nevertheless, liver cell necrosis was histologically absent 24 hr after the administration of tianeptine (0.5 mmol/kg i.p.) to non-pretreated or dexamethasone-pretreated hamsters. *In vivo* covalent binding to liver proteins also occurred in mice and rats, being increased by 100% in dexamethasone-pretreated animals. *In vivo* covalent binding to liver proteins was similar in untreated female Dark Agouti rats and in female Sprague–Dawley rats. These results show that tianeptine is transformed *in vivo* by cytochrome P-450, including glucocorticoid-inducible isoenzymes, into chemically reactive metabolites that covalently bind to tissue proteins. The metabolites, however, exhibit no direct hepatotoxic potential in hamsters below the sublethal dose of 0.5 mmol/kg i.p. The predictive value of this study regarding possible idiosyncratic and immunoallergic reactions in humans remains unknown.

Tianeptine is a new tricyclic antidepressant which has been marketed in France since May 1988 by Ardix Laboratories. In the preceding paper [1], we have reported *in vitro* studies showing that tianeptine is activated by microsomal cytochrome P-450 into chemically reactive metabolites. We report herein *in vivo* studies on the metabolic activation of tianeptine, and its toxicological consequences in hamsters.

MATERIALS AND METHODS

Chemicals. Tianeptine sodium, i.e. 7-[(3-chloro-6,11-dihydro-6-methyl-dibenzo[c, f][1-2]thiazepin-11-yl)amino] heptanoic acid, sodium salt, was kindly provided by Institut de Recherches Internationales Servier. [^{14}C]Tianeptine sodium (30 mCi/mmol), uniformly labelled on one of the benzene rings [1], was prepared by Commissariat à l'Energie Atomique (Gif-sur-Yvette, France); its radiochemical purity was found to be 98.8% by HPLC. Dexamethasone phosphate was a generous gift from Merck, Sharp and Dohme (Rahway, NJ).

Animals and treatments. Male Golden Syrian hamsters, weighing 90–110 g, were purchased from Fichot (Ormesson, France). Male and female Crl:CD(SD)BR Sprague–Dawley rats, weighing 160–180 g, and male Crl:CD-1(ICR)BR Swiss mice, weighing 28–35 g, were purchased from Charles River France (Saint-Aubin-lès-Elbeuf, France). Female Dark Agouti rats, weighing 160–180 g, were purchased from Centre de Sélection et d'Elevage d'Animaux de Laboratoires (Orléans, France). Animals were fed a normal standard diet given *ad lib.* (M_{25} biscuits, Extralabo, Provins, France for hamsters and mice, and Autoclavé 113, Usine d'Alimentation Rationnelle, Villemoisson, France for rats).

Some hamsters received piperonyl butoxide (1.2 mmol/kg i.p. in 0.5 ml of corn oil) or SKF 525-A (0.2 mmol/kg i.p. in 0.5 ml of water) 30 min before the administration of tianeptine. Other hamsters received phorone (0.7 mmol/kg i.p. in 0.5 ml of corn oil) 1 hr before the administration of tianeptine, to deplete hepatic glutathione [2, 3].

Some hamsters were given phenobarbital sodium (100 mg/kg, i.e. 0.4 mmol/kg i.p. in 0.5 ml of water), 3-methylcholanthrene (20 mg/kg, i.e. 0.07 mmol/kg in 0.5 ml of corn oil), or dexamethasone phosphate (50 mg/kg, i.e. 0.13 mmol/kg i.p. in 0.5 ml of water) daily for 3 days and were used 24 hr after the last dose of the inducer. Some male Sprague–Dawley rats and some male mice received the same dexamethasone-pretreatment.

In vivo *covalent binding studies.* Animals were killed 1, 2 or 6 hr after the administration of

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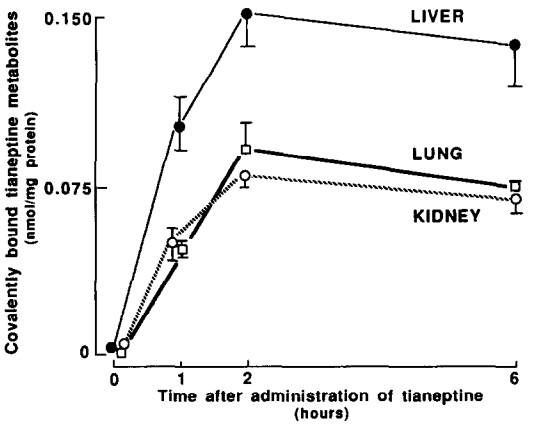

Fig. 1. Time course for the irreversible binding of [^{14}C]tianeptine metabolites to liver, lung or kidney proteins in male hamsters. Animals were killed 1, 2 or 6 hr after the administration of [^{14}C]tianeptine (0.5 mmol/kg i.p., 0.1 mCi/kg). Results are means ± SEM for 6–30 hamsters.

[^{14}C]tianeptine (0.125 or 0.5 mmol/kg, 0.1 mCi/kg) given i.p. in 0.5 ml of water. The liver, the lungs and the kidneys were removed, rinsed in ice-cold 0.154 M NaCl, and homogenized in 3 vol. of ice-cold 0.154 M NaCl. Aliquots (500 μl) of the tissue homogenates were repeatedly extracted and washed as described for *in vitro* covalent binding studies [1]. Proteins were precipitated with 2 ml of 10% perchloric acid, and extracted four times with 2 ml of ethylacetate. Proteins were then washed with, successively, 2 ml of 10% perchloric acid, acetone, ethanol, and 0.1 M sodium–potassium phosphate buffer, pH 7.4. Radioactivity could not be removed further by introducing additional extraction steps. After drying, the pellets were dissolved overnight in 2 ml of 1 N NaOH. Aliquots (500 μl) were acidified with 100 μl of 12 N H_2SO_4, and counted for ^{14}C activity. Another aliquot (20 μl) was used to determine proteins by the method of Lowry *et al.* [4].

Glutathione, SGPT and liver histology. Hepatic glutathione concentration was determined as total non-protein sulfhydryl groups by the method of Ellman [5].

Serum glutamic pyruvic transaminases (SGPT) activity was measured by the method of Reitman and Frankel [6], 24 hr after the administration of tianeptine (0.125–0.5 mmol/kg i.p.) Some hamsters were killed 24 hr after the administration of tianeptine (0.5 mmol/kg). Liver fragments were placed in Bouin's fluid, then embedded in paraffin, cut and stained with haematoxylin and eosin.

Statistical analysis. Student's *t*-test was used to assess the statistical significance of differences between means.

RESULTS

In vivo *covalent binding of tianeptine metabolites*

Administration of [^{14}C]tianeptine (0.5 mmol/kg i.p.) to male hamsters resulted in the *in vivo* covalent binding of a ^{14}C-labelled material mainly to liver proteins, but also, to lung and kidney proteins (Fig. 1); the amount of irreversibly bound material reached a maximum 2 hr after the administration of [^{14}C]tianeptine (Fig. 1). Covalent binding was highest in the liver (Fig. 1). Two hours after a lower dose of [^{14}C]tianeptine (0.125 mmol/kg i.p.), the amount of covalently bound material per mg of protein was 0.056 ± 0.010 nmol in liver, 0.024 ± 0.002 in lung and 0.029 ± 0.006 in kidney (mean ± SEM for 8 hamsters).

Table 1 shows the effects of cytochrome P-450 inhibitors, given 30 min before the administration of [^{14}C]tianeptine, on *in vivo* covalent binding measured 2 hr after the administration of [^{14}C]tianeptine (0.5 mmol/kg i.p.). Pretreatment with piperonyl butoxide significantly decreased covalent binding to liver proteins, but not to lung or kidney proteins (Table 1). In contrast, pretreatment with SKF 525-A did not significantly decrease covalent binding to liver proteins, although it significantly decreased it to lung and kidney proteins (Table 1).

Table 2 shows the effects of various cytochrome P-450 inducers on *in vivo* covalent binding to liver proteins measured 2 hr after the administration of [^{14}C]tianeptine. In hamsters, covalent binding was decreased by pretreatment with 3-methylcholanthrene; it was not significantly increased by pretreatment with phenobarbital, but was significantly increased by pretreatment with dexamethasone (Table 2). The enhancing effects of

Table 1. Effects of various inhibitors of cytochrome P-450 on the *in vivo* covalent binding of [^{14}C]tianeptine metabolites to liver, lung and kidney proteins in male hamsters

	Covalently bound [^{14}C]tianeptine metabolites		
	Liver	Lung (nmol/mg protein/2 hr)	Kidney
Non-pretreated	0.155 ± 0.010	0.093 ± 0.010	0.082 ± 0.007
Piperonyl butoxide	0.106 ± 0.011*	0.093 ± 0.031	0.095 ± 0.036
SKF 525-A	0.136 ± 0.041	0.017 ± 0.003*	0.029 ± 0.005*

The cytochrome P-450 inhibitors (piperonyl butoxide, 1.2 mmol/kg or SKF 525-A, 0.2 mmol/kg) were administered 30 min before the administration of [^{14}C]tianeptine (0.5 mmol/kg i.p., 0.1 mCi/kg). Hamsters were killed 2 hr after the administration of [^{14}C]tianeptine. Results are means ± SEM for 30 non-pretreated hamsters and 6–10 pretreated hamsters.

* Significantly different from non-pretreated hamster, $P < 0.05$.

Table 2. Effects of various cytochrome P-450 inducers on the *in vivo* covalent binding of [^{14}C]tianeptine metabolites to liver proteins in male hamsters, male mice or male rats

	Covalently bound [^{14}C]tianeptine metabolites (nmol/mg liver protein/2hr)
Hamsters	
Non-pretreated	0.155 ± 0.010
3-Methylcholanthrene	0.116 ± 0.040*
Phenobarbital	0.174 ± 0.013
Dexamethasone	0.206 ± 0.011*
Mice	
Non-pretreated	0.049 ± 0.004
Dexamethasone	0.096 ± 0.010*
Rats	
Non-pretreated	0.070 ± 0.009
Dexamethasone	0.142 ± 0.024*

Animals were pretreated for 3 days with various cytochrome P-450 inducers as described in Materials and Methods. Animals were killed 2 hr after the administration of [^{14}C]tianeptine (0.5 mmol/kg i.p., 0.1 mCi/kg). Results are means ± SEM for 4–11 animals, except for non-pretreated hamsters (30 animals).
* Significantly different from non-pretreated animals, $P < 0.05$.

Table 3. *In vivo* covalent binding of [^{14}C]tianeptine metabolites to hepatic proteins in female Sprague–Dawley and female Dark Agouti rats

	Covalently bound [^{14}C]tianeptine metabolites (nmol/mg liver protein/2 hr)
Sprague–Dawley	0.058 ± 0.007
Dark Agouti	0.079 ± 0.019

Rats were killed 2 hr after the administration of [^{14}C]tianeptine (0.5 mmol/kg i.p., 0.1 mCi/kg). Results are means ± SEM for 5 rats.

dexamethasone-pretreatment on *in vivo* covalent binding were moderate in hamsters (33%), but marked in mice (97%) and rats (102%).

In vivo covalent binding to liver proteins was similar in female Sprague–Dawley rats and female Dark Agouti rats (Table 3).

Glutathione

Administration of tianeptine slightly decreased the liver weight/body weight ratio, from 4.4 ± 0.1% in control hamsters to 3.7 ± 0.2% 4 hr after the administration of tianeptine, 0.5 mmol/kg i.p. (mean ± SEM for 17 and 22 hamsters, respectively). Glutathione contents were therefore expressed per whole liver (Fig. 2, Table 4).

Administration of tianeptine resulted in a dose-related decrease in hepatic glutathione (Fig. 2), which reached a minimum between 4 and 6 hr after the drug administration (Fig. 2). Hepatic glutathione 4 hr after administration of tianeptine was not significantly higher in hamsters pretreated with piperonyl butoxide than in non-pretreated hamsters (Table 4).

Pretreatment with phorone further decreased hepatic glutathione concentration in tianeptine-treated hamsters (Table 5). This pretreatment, however, did not increase significantly *in vivo* covalent binding of [^{14}C]tianeptine metabolites to hamster liver proteins (Table 5).

SGPT activity and liver histology after tianeptine

SGPT activity was increased 4.6-fold 24 hr after the administration of tianeptine (0.5 mmol/kg i.p.) in non-pretreated hamsters (Fig. 3), but was not significantly increased after lower doses of tianeptine (0.125 or 0.25 mmol/kg).

Twenty-four hours after the administration of tianeptine (0.5 mmol/kg i.p.), SGPT activity was significantly lower in piperonyl butoxide-pretreated hamsters, and significantly higher in dexamethasone-pretreated hamsters, than in non-pretreated hamsters (Fig. 4).

Despite increased SGPT activity, there was no histologically detectable liver cell necrosis in any of 5 non-pretreated hamsters or any of 10 dexamethasone-pretreated hamsters killed 24 hr after the administration of tianeptine (0.5 mmol/kg i.p.).

DISCUSSION

Present results show that the reactive tianeptine metabolite(s) demonstrated in the preceding communication [1], is also formed *in vivo* in hamsters, mice and rats, and binds covalently to liver, lung and kidney proteins (Fig. 1, Tables 1, 2). Our results with Dark Agouti rats also confirm the view, detailed in the preceding paper [1], that the metabolic activation of tianeptine is not mediated by the cytochrome P-

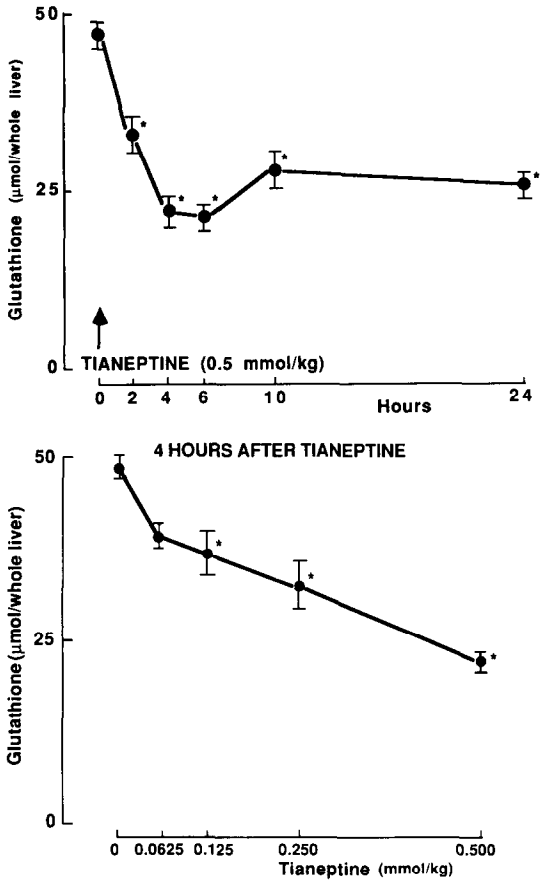

Fig. 2. Depletion of hepatic glutathione after administration of tianeptine in male hamsters. The upper part of the figure shows the time course of hepatic glutathione concentration 0–24 hr after the administration of tianeptine (0.5 mmol/kg i.p.). The lower part of the figure shows hepatic glutathione concentration 4 hr after the administration of various doses of tianeptine (0.062, 0.125, 0.25, or 0.5 mmol/kg i.p.). Results are means ± SEM for 4–34 hamsters. * Significantly different from control hamsters, $P < 0.01$.

450 isoenzyme which metabolizes debrisoquine (Table 3), but is mediated, at least in part, by glucocorticoid-inducible isoenzymes. Indeed, *in vivo* covalent binding to liver proteins was increased in hamsters, mice and rats pretreated with dexamethasone (Table 2).

Our results also show that the administration of tianeptine at the doses studied (45 to 360 times the therapeutic dose) decreases hepatic glutathione content (Fig. 2). This observation, together with the protective effect of glutathione observed in the *in vitro* studies [1], suggest conjugation of a reactive tianeptine metabolite with glutathione. Definite proof must await, however, the actual isolation of this presumed conjugate. It is noteworthy that *in vitro* covalent binding to microsomal proteins was not modified when the concentration of glutathione added to the incubation mixture was decreased from 4 to 1 mM, although covalent binding then increased as the concentration of glutathione was decreased to 0.5 mM or less [1]. This may perhaps explain that decreasing hepatic glutathione contents *in vivo* from 8 to 0.8 mM by pretreatment with phorone (Table 5) did not significantly increase the *in vivo* covalent binding of [^{14}C]tianeptine metabolites to liver proteins (Table 5).

Our results finally show that tianeptine at a very high, sublethal, dose (0.5 mmol/kg i.p.) exerted some hepatotoxic effects in hamsters. The increase in SGPT activity observed after this very high dose was prevented by pretreatment with piperonyl butoxide (Fig. 4), and was, in contrast, augmented by pretreatment with dexamethasone (Fig. 4), suggesting that it was related to the metabolic activation of tianeptine.

It is difficult to speculate on the predictive value of the present study for human therapeutics. It is thought that the formation of reactive metabolites may lead to 3 types of hepatitis in humans: predictable toxic hepatitis, idiosyncratic toxic hepatitis and and immunoallergic hepatitis [7]. It is clear that therapeutic doses of tianeptine are too low to exert predictable hepatotoxicity in humans. This is shown by 3 pieces of evidence. Firstly, only a sublethal dose of tianeptine (0.5 mmol/kg, i.e. 230 mg/kg i.p.) increased SGPT activity in hamsters (Fig. 3). This intraperitoneal dose, however, is 360 times the usual oral therapeutic dose in humans (1.4 μmol/kg daily). Secondly, the concentration of tianeptine required to produce some toxicity in isolated human hepatocytes was 200 μg/ml, i.e. 500-fold the peak plasma tianeptine concentration in humans [8]. Lastly, clinical trials have not detected hepatitis in humans [9]. It

Table 4. Effects of pretreatment with piperonyl butoxide on the loss of hepatic glutathione 4 hr after the administration of tianeptine

	Hepatic glutathione at 4 hr		
	Without tianeptine	After tianeptine (μmol/whole liver)	Per cent decrease
Non-pretreated	48 ± 2	21 ± 1	57
Piperonyl butoxide-pretreated	44 ± 2	25 ± 2	42

Some hamsters were pretreated with piperonyl butoxide (1.2 mmol/kg i.p.) 30 min before the administration of tianeptine (0.5 mmol/kg i.p.). Hamsters were killed 4 hr after the administration of tianeptine. Results are means ± SEM for 9–22 hamsters.

Table 5. Effect of pretreatment with phorone on hepatic glutathione and the *in vivo* covalent binding of [^{14}C]tianeptine metabolites to hepatic proteins in male hamsters

	Glutathione (μmol/g liver)	Covalently bound [^{14}C]tianeptine metabolites (nmol/mg liver proteins)
Non-pretreated	8.3 ± 0.4	0.15 ± 0.01
Phorone-pretreated	0.8 ± 0.1*	0.19 ± 0.03

Some hamsters were pretreated with phorone (3.6 mmol/kg i.p.) 1 hr before the administration of tianeptine. Non-protein sulfhydryls ("glutathione") were determined 1 hr after the administration of tianeptine (0.5 mmol/kg i.p.); results are means ± SEM for 4 hamsters. *In vivo* covalent binding was measured 2 hr after administration of [^{14}C]tianeptine (0.5 mmol/kg i.p., 0.1 mCi/kg). Results are means ± SEM for 30 non-pretreated hamsters and 6 phorone-pretreated hamsters.

* Significantly different from that in non-pretreated hamsters, $P < 0.001$.

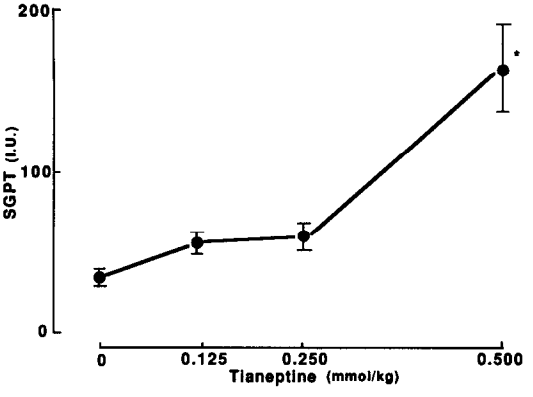

Fig. 3. SGPT activity 24 hr after the administration of various i.p. doses of tianeptine to non-pretreated male hamsters. Results are means ± SEM for 10–20 hamsters. * Significantly different from control hamsters, $P < 0.01$.

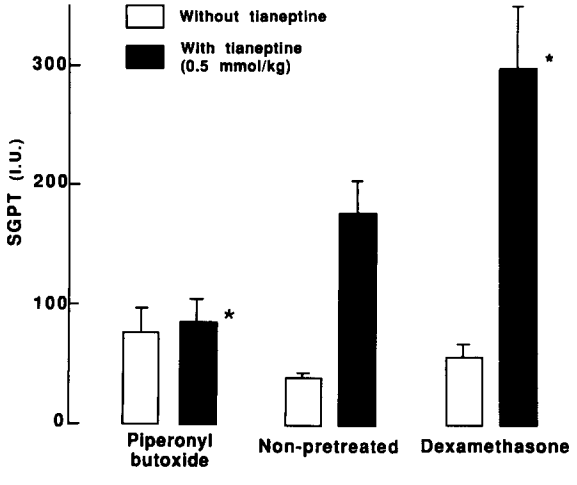

Fig. 4. SGPT activity 24 hr after administration of tianeptine (0.5 mmol/kg i.p.) to variously pretreated hamsters. Results are means ± SEM for 9–26 hamsters. * Significantly different from non-pretreated hamsters receiving tianeptine.

cannot be excluded, however, that hepatitis may some day develop, in an exceptional subject, as a result of metabolic idiosyncrasy or, more likely, of immunoallergy. As yet, metabolic activation studies have been reported for drugs already known to produce hepatitis, so that their predictive value (if any) remains unknown. Early publication of the covalent binding studies of several drugs will be necessary to assess their value (if any) for the prediction of uncommon idiosyncratic or immunoallergic drug reactions in humans.

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