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Pharmacokinetics and metabolism of thioridazine during co-administration of tricyclic antidepressants

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1 Because of serious side-effects of thioridazine and tricyclic antidepressants (cardiotoxicity), a possible influence of imipramine and amitriptyline on the pharmacokinetics and metabolism of thioridazine was investigated in a steady state (2-week treatment) in rats.

2 Imipramine and amitriptyline (5 and 10 mg kg^{-1} i.p., respectively) elevated 30 and 20 fold, respectively, the concentration of thioridazine (10 mg kg^{-1} i.p.) and its metabolites (N-desmethylthioridazine, 2-sulphoxide, 2-sulphone, 5-sulphoxide) in blood plasma. Similar, yet weaker increases in the thioridazine concentration were found in the brain. Moreover, an elevation of thioridazine/metabolite ratios was observed.

3 Imipramine and amitriptyline added to control liver microsomes *in vitro* inhibited the metabolism of thioridazine *via* N-demethylation (an increase in K_m), mono-2-sulphoxidation (an increase in K_m and a decrease in V_{max}) and 5-sulphoxidation (mainly a decrease in V_{max}). Amitriptyline was a more potent inhibitor than imipramine of the thioridazine metabolism.

4 The varying concentration ratios of antidepressant/thioridazine *in vivo* appear to be more important to the final result of the pharmacokinetic interactions than are relative direct inhibitory effects of the antidepressants on thioridazine metabolism observed *in vitro*.

5 Besides direct inhibition of the thioridazine metabolism, the decreased activity of cytochrome P-450 towards 5-sulphoxidation, produced by chronic joint administration of thioridazine and the antidepressants, seems to be relevant to the observed *in vivo* interaction.

6 The obtained results may also point to inhibition of another, not yet investigated, metabolic pathway of thioridazine, which may be inferred from the simultaneous elevation of concentrations of both thioridazine and the measured metabolites.

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Keywords: Thioridazine; imipramine; amitriptyline; pharmacokinetics; metabolism; interaction

Abbreviations: AMI, amitriptyline; h.p.l.c., high performance liquid chromatography; IMI, imipramine; K_i, inhibition constant; K_m, the Michaelis constant; 2-sulphoxide of thioridazine, mesoridazine; S, substrate concentration; 2-sulphone of thioridazine, sulphoridazine; THIOR, thioridazine; V, velocity of the reaction

Introduction

Thanks to its psychotropic profile, thioridazine ('antidepressant neuroleptic') is suitable to be combined with tricyclic antidepressants in the therapy of many psychiatric disorders. However, considering serious side-effects of thioridazine and tricyclics (cardiotoxicity and anticholinergic effects in the central and autonomous nervous systems), combining these drugs may be dangerous. Heiman (1977) reported cases of life-threatening ventricular arrythmia in patients who had ingested a combination of thioridazine and imipramine or amitriptyline. The observed interaction may be of not only a pharmacodynamic, but also a pharmacokinetic character.

It has been shown that phenothiazine neuroleptics inhibit the metabolism of tricyclic antidepressants and increase their concentration in the blood plasma of man (Gram *et al*., 1974a; Vandel *et al*., 1979; Nelson & Jatlow, 1980; Bock *et al*., 1983; Brøsen *et al*., 1986) and in rats' plasma and brain (Gram *et al*., 1974b; Daniel & Melzacka, 1986; Daniel, 1991). The above effects are attributed to the inhibition of hydroxylation, and in the case of tertiary amines, also to N-demethylation. However, little is known about a possible mutual effect of tricyclic antidepressants on the metabolism of neuroleptics. The available data refer to too small a number of patients (some of who received simultaneously two neuroleptics) and to one time interval after drug administration. Moreover, they relate only to a plasma concentration of the parent drug without taking into account its main and/or active metabolites. Nevertheless, they suggest that amitriptyline can increase concentrations of phenothiazines (Jus *et al*., 1978), and also indicate that nortriptyline increases plasma concentration of chlorpromazine in the blood plasma of schizophrenic patients (Loga *et al*., 1981). Our recent studies carried out on rats showed that amitriptyline and, to a lesser extent, imipramine increased plasma and brain concentrations of promazine, a phenothiazine neuroleptic with the simplest chemical structure (Syrek *et al*., 1997). A parallel determination of concentrations of the neuroleptic metabolites *in vivo* and metabolic studies *in vitro* indicated that the investigated antidepressants inhibited main metabolic pathways of promazine. Similar interaction (an elevation of promazine concentration) was observed after joint administration of promazine and selective serotonin reuptake inhibitors to rats (Daniel *et al*., 1999c). Therefore it may be interesting to determine whether and how antidepressant drugs influence the pharmacokinetics of phenothiazines with a more complex chemical structure and serious side-effects such as, e.g. thioridazine.

Thioridazine, a prototype drug of phenothiazine neuroleptics of the piperidine-type, is a substrate and an inhibitor of polymorphic CYP2D6 (von Bahr *et al*., 1985; 1991; Meyer *et al*., 1990; Baumann *et al*., 1992; Blake *et al*., 1995), an enzyme

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which also catalyzes hydroxylation of tricyclic antidepressants (Spina *et al*., 1987; Steiner *et al*., 1988; Brøsen *et al*., 1991; Nilsen *et al*., 1994). Like other phenothiazine neuroleptics, thioridazine undergoes S-oxidation in the thiazine ring in position 5, as well as aromatic hydroxylation (mainly in position 7), N-demethylation and N-oxidation (Papadopoulos *et al*., 1985; Svendsen & Bird, 1986; Lin *et al*., 1993). However, unlike other phenothiazines – thioridazine forms a sulphoxide in position 2 of the thiomethyl substituent which is further oxidazed to a sulphone (Figure 1). The S-oxidation in position 2, catalyzed by CYP2D6, is generally recognized as a main metabolic route of thioridazine metabolism in man and animals (Lin *et al*., 1993; Daniel *et al*., 1997). Metabolites formed by S-oxidation in position 2, i.e., 2-sulphoxide (mesoridazine) and 2-sulphone (sulphoridazine), are more potent than thioridazine in blocking dopaminergic D_2 and noradrenergic α_1 receptors; moreover, N-desmethylthioridazine retains affinity for α_1 receptors (Axelsson, 1977; Bylund, 1981; Richelson & Nelson, 1984; Hyttel *et al*., 1985). Thioridazine 5-sulphoxide (a ring sulphoxide) is not pharmacologically active at dopaminergic or noradrenergic receptors, but is considered to contribute to the cardiotoxicity of the parent compound (Gottschalk *et al*., 1978; Hale & Poklis, 1986).

The aim of the present study was to investigate a possible influence of imipramine and amitriptyline on the pharmacokinetics and metabolism of thioridazine in a steady state in rats. The observed interactions are discussed with respect to their mechanism, contribution of particular cytochrome P-450 isoenzymes to them, and their pharmacological and clinical importance.

Methods

Drugs and chemicals

Thioridazine and imipramine were provided by Polfa (Jelenia Góra, Poland). Amitriptyline was obtained from H. Lundbeck A/S (Copenhagen, Denmark). Mesoridazine and sulphoridazine (free bases) were donated by Sandoz (Basel, Switzerland). Thioridazine 5-sulphoxide was synthesized according to a previously described method (Daniel *et al*., 1997). NADP, glucose-6-phosphate and glucose-6-phosphate-dehydrogenase were purchased from Sigma (St. Louis, U.S.A.). All organic solvents with h.p.l.c. purity were supplied by Merck (Darmstadt, Germany).

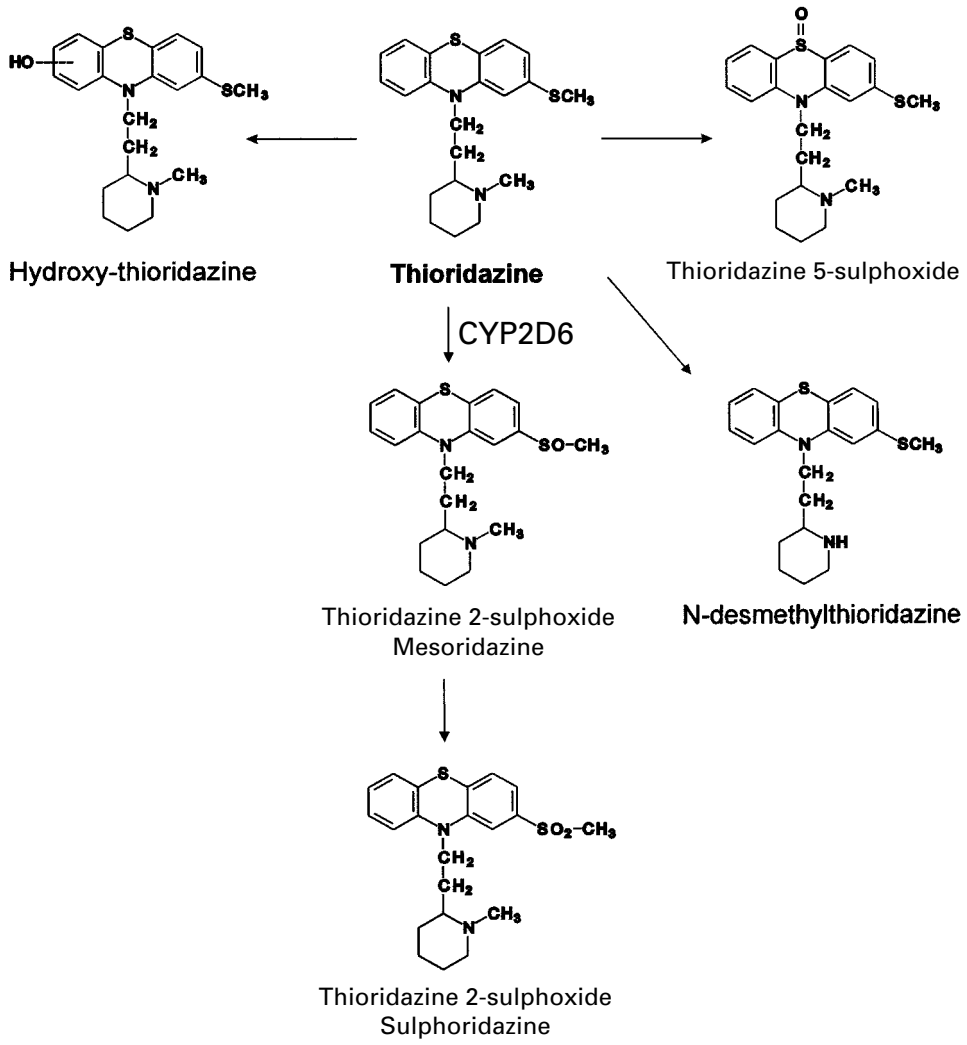

Figure 1 Metabolic pathways of thioridazine.

Animals

The experiment was carried out on male Wistar rats (230–260 g) kept under standard laboratory conditions. To avoid a possible drug interaction at a level of absorption from the gastrointestinal tract, and to achieve a better correlation of the drug concentrations with their metabolism, the investigated psychotropics were administered intraperitoneally. Thioridazine (hydrochloride, 10 mg kg^{-1} i.p.) was injected twice a day for 2 weeks, alone or jointly with one of the tricyclic antidepressants (amitriptyline and imipramine hydrochlorides, 10 and 5 mg kg^{-1} i.p., respectively). The doses used were of pharmacological magnitude which produced 'therapeutic' plasma concentrations of the drugs (Daniel *et al*., 1981; 1997; Coudore *et al*., 1996). At 30 min, 6 and 12 h after the last dose of the drugs, the animals' trunk blood was collected in tubes moistened with a 30% solution of sodium citrate, and their brains were rapidly removed and stored frozen in solid CO_2. Blood samples were centrifuged at $2000 \times g$ for 30 min. Samples containing 1.5 ml of the plasma were stored at $-20°C$ until extraction. Liver microsomes were prepared at 24 h after the drug withdrawal by differential centrifugation in 20 mM Tris/KCl buffer (pH = 7.4), including washing with 0.15 M KCl according to a conventional method. The above procedure deprives microsomes of the presence of drugs administered *in vivo*, which was confirmed in our experiment by the h.p.l.c. method described below.

Determination of thioridazine and its metabolites

Concentrations of thioridazine and its main metabolites (2-sulphoxide, 2-sulphone, 5-sulphoxide, and N-desmethylthioridazine) were assessed in the blood plasma, brain and microsomal suspension by the h.p.l.c. method previously developed by us (Daniel *et al*., 1997). Thioridazine and its metabolites were extracted from the biological material (pH = 12) with hexane containing 1.5% of isoamyl alcohol. The residue obtained after evaporation of the plasma or brain extracts was dissolved in 100 μl of the mobile phase described below. An aliquot (20 μl) was injected into the h.p.l.c. system LaChrom (Merck-Hitachi), equipped with an UV detector, a L-7100 pump and a D-7000 System Manager. The analytical column (Econosphere C18, 5 μM, 4.6×250 mm) was purchased from Alltech (Carnforth, U.K.). The mobile phase consisted of acetate buffer of pH = 3.4 (100 mmol of ammonium acetate, 20 mmol of citric acid and 2 ml of triethylamine in 1 L of buffer, adjusted to pH = 3.4 with 85% phosphoric acid) and acetonitrile in proportion 42 : 58. The flow rate was 1.5 ml min^{-1}, the column temperature 40°C. The absorbance was measured at a wavelength of 270 nm.

In vitro *studies into thioridazine metabolism*

Thioridazine metabolism was studied in liver microsomes at linear dependence of the product formation on time and protein and substrate concentrations (Daniel *et al*., 1999a). To distinguish between a direct effect of antidepressants on the metabolism of thioridazine and changes produced by their chronic co-administration, two experimental models were used. *Model I*: pooled liver microsomes from three control rats were used. Rates of N-demethylation and 2- and 5-sulphoxidation of thioridazine (thioridazine concentration: 15–75 nmol ml^{-1}) were assessed in the absence and presence of one of the antidepressants added *in vitro* (antidepressant concentration: 50 nmol ml^{-1}). The concentrations of thioridazine and antidepressants used in *in vitro* studies were at a presumed range of concentrations in the liver after administration of pharmacological doses of the drugs (Daniel & Wójcikowski 1999). Each sample was prepared in duplicate. *Model II*: liver microsomes from thioridazine and/or antidepressant-treated rats were used. Thioridazine was added to the incubation mixture *in vitro* at a concentration of 50 nmol ml^{-1}. Thioridazine metabolism was studied in the absence of antidepressants. Incubations *(Models I and II)* were carried out in a system containing liver microsomes (0.5 mg of protein in 1 ml), Tris/KCl buffer (20 mM, pH = 7.4), $MgCl_2$ (2.5 mM), NADP (0.1 mM), glucose 6-phosphate (1.2 mM) and glucose-6-phosphate-dehydrogenase (0.3 U in 1 ml). The final incubation volume was 1 ml. After a 2-min preincubation, the reaction was started by adding thioridazine. After a 15-min incubation, the reaction was stopped by adding 200 μl of methanol and then by cooling it down to 0°C. K_m and V_{max} values in the absence or presence of the inhibitor (antidepressant) were estimated from Lineweaver-Burk's plots. K_i values were determined from secondary plots representing K_m/V_{max} ratios as a function of inhibitor concentration.

Assessment of cytochrome P-450 and cytochrome b-5

Concentrations of cytochromes P-450 and b-5 in liver microsomes were determined according to Omura & Sato (1964) and Omura & Takesue (1970), respectively, using a Beckman DU-65 spectrophotometer. Protein was assayed according to Lowry *et al*. (1951) using bovine serum albumin as a standard.

Statistics

Statistical significance was assessed using an analysis of variance followed by Dunnett's test.

Table 1 Concentrations of thioridazine and its metabolites in the blood plasma and brain at 30 min, 6 and 12 h after thioridazine withdrawal. Thioridazine was administered alone at a dose of 10 mg kg^{-1} i.p. twice daily for 2 weeks

Time after thioridazine withdrawal	*Thioridazine*	*N-desmethyl-thioridazine*	*Mesoridazine (2-sulphoxide)*	*Sulphoridazine (2-sulphone)*	*Thioridazine ring sulphoxide (5-sulphoxide)*
A. Plasma [nmol ml^{-1}]					
30 min	1.734 ± 0.330	0.076 ± 0.020	1.097 ± 0.232	0.039 ± 0.007	0.340 ± 0.050
6 h	0.086 ± 0.025	0.054 ± 0.013	0.151 ± 0.052	0.023 ± 0.007	0.180 ± 0.032
12 h	0.050 ± 0.014	0.048 ± 0.013	0.166 ± 0.063	0.020 ± 0.007	0.196 ± 0.054
B. Brain pnmol g^{-1}					
30 min	1.641 ± 0.320	0.137 ± 0.045	0.618 ± 0.094	0.038 ± 0.008	0.248 ± 0.052
6 h	1.118 ± 0.241	0.242 ± 0.046	0.425 ± 0.151	0.052 ± 0.008	0.138 ± 0.031
12h	0.586 ± 0.182	0.274 ± 0.104	1.467 ± 0.481	0.036 ± 0.012	0.128 ± 0.045

The presented values are means ± s.e.mean for $n = 5–7$

Results

In vivo *studies*

Table 1 shows steady-state concentrations of thioridazine and its metabolites in the plasma and brain of rats at 30 min and after 6 and 12 h. The obtained concentration values were similar to those observed in our previous study (Daniel *et al*., 1997), indicating that considerable amounts of mesoridazine and N-desmethylthioridazine were formed in the rat and confirming that 2-sulphoxidation of thioridazine to mesoridazine was the most important metabolic pathway of thioridazine in the species. Brain concentrations of the parent compound and N-desmethylthioridazine (and also of mesoridazine and sulphoridazine after 6 and 12 h) were higher, while those of 5-sulphoxide were lower, than in the plasma.

The investigated antidepressants, coadministered with thioridazine, potently increased concentrations of the neuroleptic and its metabolites in the blood plasma and brain, especially after 6 and 12 h (Figures 2 and 3). Moreover, in most cases, an increase in the thioridazine/metabolite concentration ratios was observed.

Imipramine raised the plasma concentrations of thioridazine 25 fold (6 h) and 31 fold (12 h) compared to the control (Figure 2A). A simultaneous increase in N-desmethylthioridazine, mesoridazine and sulphoridazine concentrations was observed. In the brain (Figure 2B), an increase in the concentration of thioridazine was less dramatic than in the plasma and reached up to 3 fold of the control after 6 h. Concentrations of the metabolites increased or showed a tendency to increase after 6 h. At 30 min and after 12 h a significant decrease in the brain concentration of mesoridazine was observed. The thioridazine/metabolite concentration ratios were increased after 6 and 12 h in the plasma and in most cases in the brain.

Amitriptyline administrated jointly with thioridazine raised the plasma concentration of the neuroleptic 20 fold (6 h) and 8 fold (12 h) compared to the control (Figure 3A). At the same time, increases in the metabolite concentrations were observed. The exception was 5-sulphoxide whose concentration was not changed after 12 h. In the brain (Figure 3B), significant increases in thioridazine levels were observed at 30 min and after 6 h, but they were not as big as in the plasma (5 fold compared to the control after 6 h). After 12 h, a slight, non-significant increase in the level of thioridazine was observed. At the same time, increases in concentrations of N-desmethylthioridazine and sulphoridazine at 30 min, and of all metabolites after 6 h were reported. The sum of thioridazine + metabolite concentrations was raised after 6 and 12 h in the plasma, and at 30 min and after 6 h in the brain. The thioridazine/metabolite concentration ratios were increased in the plasma (especially in the case of 5-sulphoxide)

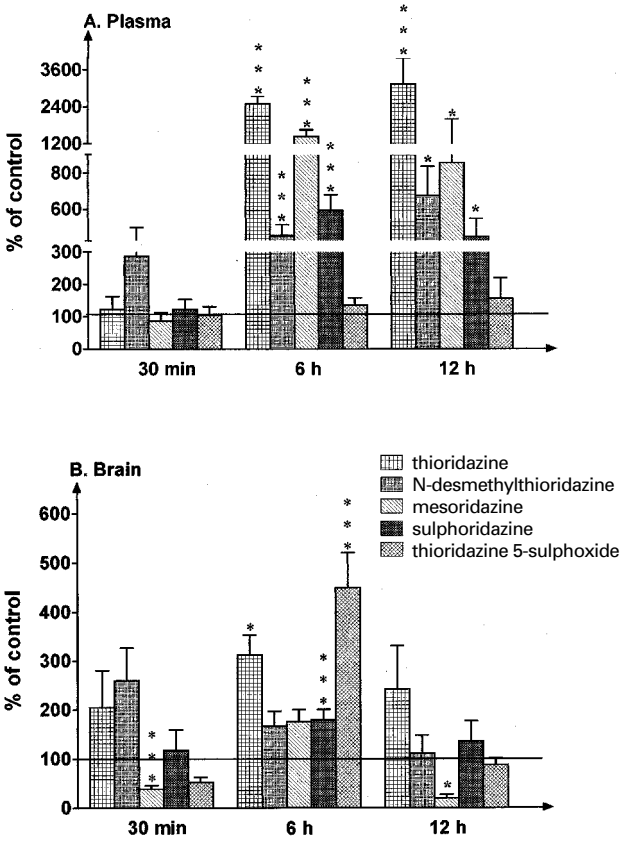

Figure 2 The influence of imipramine (5 mg kg^{-1} i.p., twice a day) on the pharmacokinetics of thioridazine (10 mg kg^{-1} i.p., twice a day) after 2-week treatment with a combination of the drugs. The plasma (A) and brain (B) levels of thioridazine and its metabolites at 30 min, 6 and 12 h after withdrawal of the drugs. $n=5-7$; $^{*}P<0.05$, $^{***}P<0.001$ (Dunnett's test). Absolute control values (animals treated with thioridazine alone) are presented in Table 1.

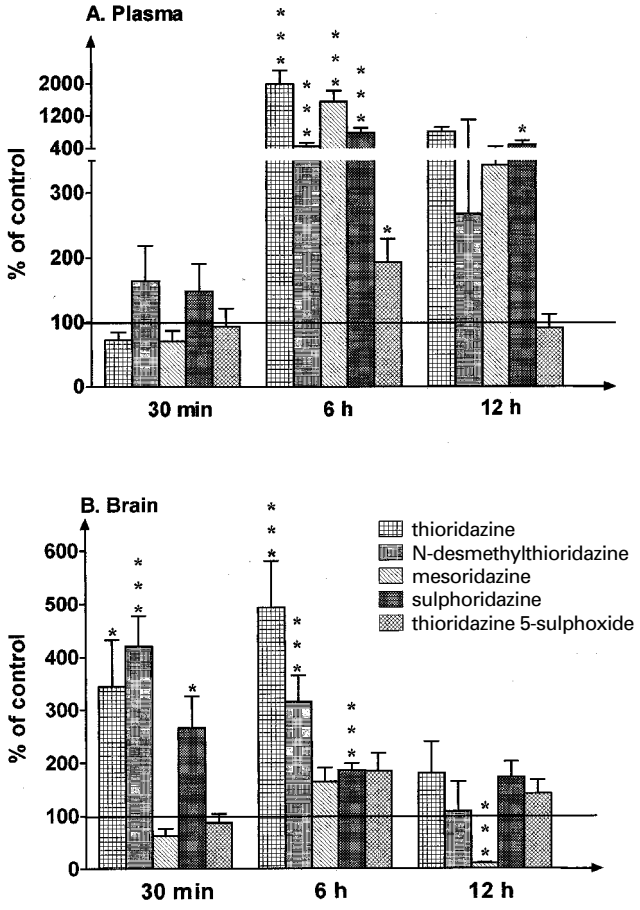

Figure 3 The influence of amitriptyline (10 mg kg^{-1} i.p., twice a day) on the pharmacokinetics of thioridazine (10 mg kg^{-1} i.p., twice a day) after 2-week treatment with a combination of the drugs. The plasma (A) and brain (B) levels of thioridazine and its metabolites at 30 min, 6 and 12 h after withdrawal of the drugs. $n=5-7$; $^{*}P<0.05$, $^{***}P<0.001$ (Dunnett's test). Absolute control values (animals treated with thioridazine alone) are presented in Table 1.

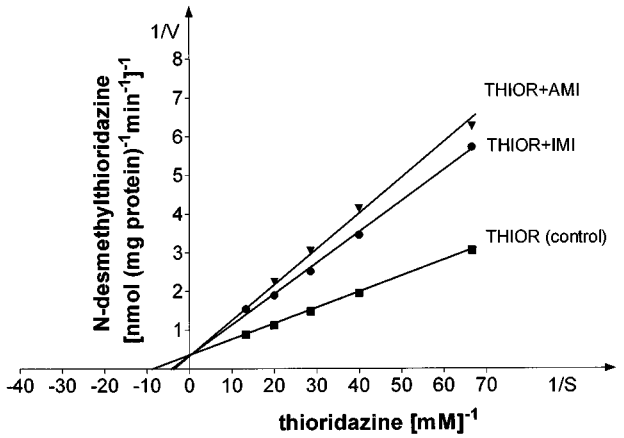

Figure 4 The kinetics of inhibition of thioridazine (THIOR) N-demethylation by imipramine (IMI) or amitriptyline (AMI) *in vitro*. V = velocity of the reaction; S = concentration of the substrate. The concentration of each inhibitor was 50 nmol ml^{-1}. The values of all kinetic parameters are presented in Table 2.

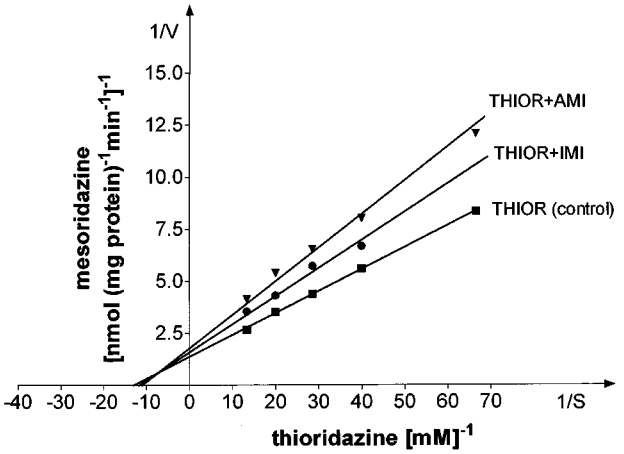

Figure 5 The kinetics of inhibition of thioridazine (THIOR) 2-sulphoxidation by imipramine (IMI) or amitriptyline (AMI) *in vitro*. V = velocity of the reaction; S = concentration of the substrate. The concentration of each inhibitor was 50 nmol ml^{-1}. The values of all kinetic parameters are presented in Table 2.

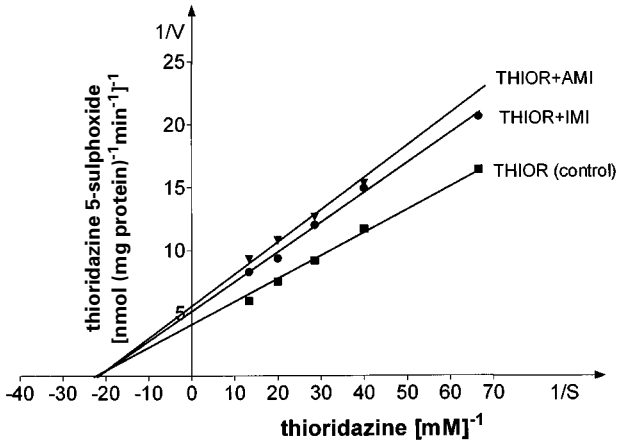

Figure 6 The kinetics of inhibition of thioridazine (THIOR) 5-sulphoxidation by imipramine (IMI) or amitriptyline (AMI) *in vitro*. V = velocity of the reaction; S = concentration of the substrate. The concentration of each inhibitor was 50 nmol ml^{-1}. The values of all kinetic parameters are presented in Table 2.

after 6 and 12 h. In the brain, increases in the parent compound/metabolite concentration ratio were observed after 6 h, and in some cases at 30 min (mesoridazine and 5-sulphoxide) and after 12 h (mesoridazine).

In vitro *studies*

(a) Model I Studies with control liver microsomes showed that imipramine and amitriptyline added to the incubation mixture *in vitro* inhibited the metabolism of thioridazine *via* N-demethylation (an increase in K_m), mono-2-sulphoxidation (an increase in K_m and a decrease in V_{max}) and 5-sulphoxidation (mainly a decrease in V_{max}) (Figures 4–6). As shown by Lineweaver-Burk's plots, amitriptyline was a more potent inhibitor of the thioridazine metabolism than imipramine. Kinetic parameters of the thioridazine metabolism *in vitro* in the absence and presence of tricyclic antidepressants are shown in Table 2. The kinetics of di-2-sulphoxidation of thioridazine was not analysed, since sulphoridazine was not formed directly from thioridazine which was used as a substrate in our study.

(b) Model II *Model II* shows the effect of chronic treatment with pharmacological doses of the drugs, in contrast to the acute effect of the antidepressants on the rate of thioridazine metabolism (shown in *Model I*). Studies with microsomes of rats treated chronically with thioridazine and/or tricyclic antidepressants (the drugs administered *in vivo* were washed out from microsomes) indicated that imipramine given alone did not significantly change the concentrations of cytochrome P-450 or b-5, while amitriptyline significantly decreased the level of cytochrome P-450 (Table 3). Both those antidepressants inhibited the rate of sulphoridazine formation; amitriptyline also significantly decreased the sum of rates of mono-2- and di-2-sulphoxidation, i.e. the total rate of 2-sulphoxidation. Thioridazine given separately or in a combination with antidepressants decreased the concentration of cytochrome b-5, having not significantly affected that of cytochrome P-450 (Table 4). Administration of thioridazine alone significantly inhibited mono-2-sulphoxidation and sulphoridazine formation and, consequently, decreased the total rate of 2-sulphoxidation process expressed as a sum of the two rates. Joint administration of thioridazine and imipramine enhanced the rate of N-demethylation and decreased the rate of 5-sulphoxidation in comparison with those parameters in both control and thioridazine-treated animals. The above combination decreased the rate of di-2-sulphoxidation and the total 2-sulphoxidation process compared with the control; however, the rates of mono- and di-2-sulphoxidation and, consequently, the total 2-hydroxylation were enhanced compared to those in thioridazine-treated rats. Chronic treatment with thioridazine and amitriptyline did not affect the N-demethylation of thioridazine, but decreased the rates of di-2- and 5-sulphoxidation compared with the control. The rates of mono-2-sulphoxidation and total 2-sulphoxidation were elevated in comparison with thioridazine-treated rats.

Discussion

The obtained results show that the investigated tricyclic antidepressants substantially influenced the pharmacokinetics of thioridazine in rats. Imipramine and amitriptyline elevated 30 and 20 times, respectively, the concentration of thioridazine in the blood plasma. The elevation of thioridazine concentration by tricyclic antidepressants was accompanied with simultaneous increases in the levels of its metabolites. Similar,

though less potent increases in the thioridazine concentration took place in the brain. Such different degrees of elevation of the drug concentration may result from a concurrent distributive interaction between thioridazine and the investigated antidepressants, which may affect the relationship between drug concentrations in the blood plasma and tissues (Daniel & Wójcikowski, 1999).

The observed pharmacokinetic interactions were much stronger than in the case of promazine studied previously whose concentration increased three times after co-administration of amitriptyline (Syrek *et al*., 1997). Imipramine produced less distinct changes in promazine pharmacokinetics. In the blood plasma, imipramine did not produce any significant alterations in promazine concentration, but it significantly raised the level of the neuroleptic in the brain. Therefore it seems that the results of drug interactions involving the simplest phenothiazine neuroleptic promazine cannot be referred to phenothiazine neuroleptics with a more complex chemical structure which determines their affinity to different cytochrome P-450 isoenzymes, as well as their metabolism. This fact is of particular importance in the case of thioridazine which shows serious side-effects.

After chronic joint administration of thioridazine and tricyclic antidepressants, both direct *(Model I)* and indirect *(Model II)* mechanisms of their interactions with cytochrome P-450 are feasible. As shown by *in vitro* studies, these interactions have a reverse effect on N-demethylation and 2-sulphoxidation of thioridazine, i.e. they may lead to direct inhibition by the antidepressants and to elevation of their rates by chronic treatment with the drug combinations compared with rats treated with thioridazine alone. However, the final outcome of an *in vivo* interaction is a resultant of the direct effect on the enzyme and of adaptive changes (enhancement of the rates) produced by the drug combination. In fact, the findings of *in vivo* studies point to inhibition rather than enhancement of thioridazine metabolism by tricyclic antidepressants, as indicated by increases in the thioridazine concentration and in the concentration ratio of the parent compound/metabolite in the blood plasma.

The *in vitro* metabolic studies carried out in control liver microsomes *(Model I)* show that both imipramine and amitriptyline inhibit directly the metabolism of thioridazine. Inhibition of N-demethylation proceeds *via* an increase in the K_m value, without a distinct change in V_{max}, which may suggest its competitive character (Table 2). Inhibition of mono-2-sulphoxidation involves an increase in K_m and a decrease in V_{max}. In the case of 5-sulphoxidation, inhibition of the process is caused mainly by a decrease in V_{max}, which may implicate a non-competitive mechanism. However, since only one concentration of the inhibitor was used, and since Lineweaver-Burke's analysis is not very accurate, precise estimation of the mechanisms of inhibition requires further (more detailed) studies. As reflected by the K_i values, the inhibitory effect of amitriptyline on thioridazine metabolism was more pronounced than that of imipramine in that experimental *in vitro* model; hence surprisingly, the effect of imipramine on thioridazine pharmacokinetics was stronger than that of amitriptyline. This finding may implicate that the concentration of imipramine in the vicinity of cytochrome P-450 in our *in vivo* experiment was higher than that of amitriptyline. The observed interactions are particularly pronounced after longer time intervals, which may be due to different pharmacokinetics of thioridazine and the antidepressants. Thioridazine is rapidly absorbed from the intraperitoneal cavity (Daniel *et al*., 1997), while the antidepressants need more time to reach a maximum concentration (Daniel *et al*., 1981; Coudore *et al*., 1996; Melzacka *et al*., 1986). Thus the antidepressants exert their direct inhibitory effect on thioridazine metabolism after a longer time, i.e. when their concentrations are higher than that of thioridazine. The stronger inhibitory effect of imipramine than that of amitriptyline *in vivo* (at its weaker direct effect *in vitro*) probably results from a higher imipramine/thioridazine than amitriptyline/thioridazine concentration ratio reached *in vivo*. It may therefore be concluded that the varying concentration ratios of antidepressant/thioridazine *in vivo* appear to be more important to the final result of the pharmacokinetic interactions than are relative direct inhibitory effects of the antidepressants on thioridazine metabolism, observed in *Model I in vitro*. Moreover, the decreased activity of cytochrome P-450 towards 5-sulphoxidation, produced by chronic joint administration of thioridazine and the antidepressants, seems to be relevant to the observed *in vivo* interaction, since it is logically related to the increased concentration of the parent compound thioridazine, and is shown as a spectacular increase in the thioridazine/5-sulphoxide concentration ratio in the blood plasma.

Enhancement of the rates of thioridazine N-demethylation by imipramine (vs both the control and the thioridazine group) or of 2-sulphoxidation by imipramine and amitriptyline (vs thioridazine group), observed after chronic administration of the drug combinations *(Model II)*, does not seem to be of basic significance for the final result of the pharmacokinetic interactions *in vivo* in the presence of the inhibitor (antidepressant). Although the level of N-desmethylthioridazine and mesoridazine was raised, the concentration of thioridazine and the thioridazine/metabolite concentration ratios also rose. It seems that the direct inhibitory action of tricyclic antidepressants on cytochrome P-450 isoenzymes, observed in *Model I*, surpasses the effect of chronic treatment on thioridazine N-demethylation and 2-sulphoxidation found in

Table 2 The effect of imipramine and amitriptyline on the kinetics of thioridazine metabolism *in vitro (Model I)*

Type of reaction	*Drug* (inhibitor)	K_i [μM]	K_m [μM]	V_{max} [nmol of product mg protein^{-1} min^{-1}]	$V_{max}\ K_m^{-1}$
N-demethylation	Control		113	2.873	0.025
	Imipramine	48	229	2.873	0.012
	Amitriptyline	32	287	2.873	0.010
2-sulphoxidation	Control		75	0.763	0.010
	Imipramine	72	85	0.622	0.007
	Amitriptyline	39	89	0.542	0.006
5-sulphoxidation	Control		44	0.250	0.005
	Imipramine	145	45	0.190	0.004
	Amitriptyline	104	45	0.180	0.004

The presented parameters were calculated on the basis of Lineweaver-Burk's plots, shown in Figures 4–6. V_{max} the maximum velocity of the reaction, K_m the Michaelis constant, K_i, the inhibition constant.

Table 3 Hepatic metabolic parameters after different pretreatments; concentrations of cytochrome P-450 and b-5, and rates of thioridazine demethylation and sulphoxidation in liver microsomes of rats trated with tricyclic antidepressants for 2 weeks

Treatment	*Cytochrome P-450* [nmol (mg protein)$^{-1}$]	*Cytochrome b-5* [nmol (mg protein)$^{-1}$]	*N-demethylation Specific activity N-desmethyl-thioridazine* [nmol (mg protein)$^{-1}$ min^{-1}]	*mono-2-sulphoxidation Specific activity mesoridazine* [nmol (mg protein)$^{-1}$ min^{-1}]	*di-2-sulphoxidation Specific activity sulphoridazine* [nmol (mg protein^{-1}) min^{-1}]	*2-sulphoxidation (total) Specific activity mesoridazine + sulphoridazine* [nmol (mg protein)$^{-1}$ min^{-1}]	*5-sulphoxidation Specific activity thioridazine 5-sulphoxide* [nmol (mg protein)$^{-1}$ min^{-1}]
Control	0.850±0.070	0.626±0.062	1.040±0.234	0.368±0.058	0.044±0.007	0.412±0.065	0.155±0.020
Imipramine	0.999±0.060	0.625±0.034	1.118±0.085	0.316±0.018	0.022±0.001***[a]	0.338±0.019	0.125±0.008
Amitriptyline	0.624±0.042*[a]	0.539±0.023	0.858±0.142	0.248±0.018	0.013±0.002***[a]	0.261±0.019*[a]	0.117±0.004

[a]Values are means±s.e.mean, $n=6$; *$P<0.005$, ***$P<0.001$, in comparison with control (Dunnett's test).

Table 4 Hepatic metabolic parameters after different pretreatmeants; concentrations of cytochrome P-450 and b-5, and rates of thioridazine demethylation and sulphoxidation in liver microsomes of rats treated with thioridazine or thioridazine + antidepressant for 2 weeks

Treatment	*Cytochrome P-450* [nmol (mg protein)$^{-1}$]	*Cytochrome b-5* [nmol (mg protein)$^{-1}$]	*N-demethylation Specific activity N-desmethyl-thioridazine* [nmol (mg protein)$^{-1}$ min^{-1}]	*mono-2-sulphoxidation Specific activity mesoridazine* [nmol (mg protein)$^{-1}$ min^{-1}]	*di-2-sulphoxidation Specific activity sulphoridazine* [nmol (mg protein^{-1}) min^{-1}]	*2-sulphoxidation (total) Specific activity mesoridazine + sulphoridazine* [nmol (mg protein)$^{-1}$ min^{-1}]	*5-sulphoxidation Specific activity thioridazine 5-sulphoxide* [nmol (mg protein)$^{-1}$ min^{-1}]
control	0.685±0.043	0.682±0.029	0.424±0.040	0.399±0.022	0.029±0.004	0.428±0.025	0.159±0.009
Thioridazine	0.584±0.027	0.524±0.037*[a]	0.337±0.063	0.254±0.014***[a]	0.010±0.001***[a]	0.264±0.014**[a]	0.143±0.005
Thior+IMI	0.638±0.024	0.529±0.029***[a]	0.807±0.068***[a,b]	0.345±0.013***[b]	0.019±0.001***[a],*[b]	0.363±0.014*[a], **[b]	0.112±0.009***[a],*[b]
Thior+AMI	0.608±0.033	0.559±0.023*[a]	0.374±0.029	0.358±0.008***[b]	0.011±0.000***[b]	0.369±0.009**[b]	0.127±0.007*[a]

[a]Values are means±s.e.mean, $n=6$; *$P<0.05$ **$P<0.01$, ***$P<0.001$, in comparison with control (Dunnett's test). [b]Values are means±s.e.mean, $n=6$; *$P<0.05$, **$P<0.01$,, ***$P<0.001$, in comparison with thioradazine-treated rats (Dunnett's test). Thior, thioradazine; IMI, imipramine; AMI, amitriptyline.

Model II (enhancement), which resembles of a pharmacological situation in which no functional hypersensitivity of the D_2 receptor, produced by chronic neuroleptic treatment, is observed in the presence of a receptor blocker (neuroleptic).

The observed parallel increases in concentrations of both thioridazine and its metabolites may result from inhibition of another, not yet investigated by us, metabolic pathway of thioridazine. In the case of inhibition of drug metabolism, an increase in the parent compound, a decrease in the metabolite concentration and, consequently, an increase in the parent compound/metabolite concentration ratio in blood plasma may be expected. A reverse situation is observed in the case of induction, i.e., a decrease in the concentration of the parent compound, an increase in the concentration of a metabolite and, consequently, a decrease in the parent compound/metabolite concentration ratio can be seen. In our experiment increases in the concentrations of thioridazine and in the thioridazine/metabolite concentration ratios (characteristic of the inhibition) were found at a simultaneous increase in the metabolite concentration (at variance with inhibition). A possible explanation of the observed discrepancy may be inhibition of the metabolic pathway of thioridazine, not yet investigated by us (e.g. aromatic hydroxylation), by the antidepressants under study, which would increase not only the concentration of substrate thioridazine, but also indirectly the amount of the formed N-demethylated and 2-hydroxylated metabolites. Studies by Zehnder *et al.* (1962) indicate that aromatic hydroxylation is an important metabolic pathway of thioridazine in the rat and tricyclic antidepressants are known to inhibit CYP2D1, an enzyme which can catalyze aromatic hydroxylation in the rat (Daniel & Netter, 1990; Masubuchi *et al.* 1995). This is only an example of a possible explanation, since it cannot be excluded that the observed increases in concentrations of the thioridazine metabolites are due to inhibition of their successive metabolism by the antidepressants. Unfortunately, due to some problems with the synthesis of hydroxy-thioridazine (and its lack on the market), it was not possible to investigate thioridazine hydroxylation in the present study.

Our earlier *in vitro* studies into thioridazine metabolism, carried out in the presence of specific cytochrome P-450 inhibitors, indicated that the isoenzymes CYP2D1, CYP2B (N-demethylation and mono-2-sulphoxidation) and CYP1A2 (N-demethylation, mono-2- and 5-sulphoxidation) contributed to the biotransformation of the neuroleptic in male Wistar rats (Daniel *et al.*, 1999a). Therefore it is likely that direct inhibition of the above-mentioned isoenzymes by imipramine and amitriptyline, as well as the decreased activity of CYP1A2 (which catalyzes 5-sulphoxidation) produced by chronic joint administration of thioridazine and the antidepressants, play an essential role in the observed metabolic interactions between these drugs *in vivo*. On the other hand, the observed decreases in the brain concentration of mesoridazine and the concurrent elevation of its concentration in the blood plasma may suggest either induction of brain sulphoxidase which catalyzes oxidation of 2-sulphoxide to 2-sulphone (Bhamre *et al.*, 1995), or alterations in the distribution of mesoridazine.

It is quite possible that the metabolic interactions between thioridazine and tricyclic antidepressants observed in rats also take place in humans, since the metabolism of these psychotropics in the two species is similar and their doses used in our experiment produce 'therapeutic' concentrations of the investigated drugs (Daniel *et al.*, 1981; 1997; Coudore *et al.*, 1996). Besides, the K_i values obtained in our study reflect both the order of magnitude of the respective K_m values and the presumed concentration range of the antidepressants in a lipophilic phase of the liver endoplasmatic reticulum *in vivo* (Bickel & Steele, 1974; Bickel *et al.*, 1983; Daniel & Wójcikowski, 1999). Similar metabolic interaction (an inhibition of thioridazine metabolism) was observed after joint administration of thioridazine and fluoxetine to rats (Daniel *et al.*, 1999b).

Considering serious side-effects of thioridazine and tricyclic antidepressants (cardiotoxicity, anticholinergic effects), as well as the D_2 and α_1 receptor blocking activity of the thioridazine metabolites, the above-described metabolic interactions may be of clinical importance. Besides pharmacodynamic interactions, the metabolic interactions observed in the present study may also contribute to a dangerous cardiotoxicity in patients who received combinations of thioridazine and imipramine or amitriptyline, reported by Heiman (1977). It is also noteworthy that a metabolic interaction between phenothiazine neuroleptics and tricyclic antidepressants proceeds in both directions, i.e. antidepressants inhibit the metabolism of phenothiazines and *vice versa*.

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