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Effects of thioridazine and diazepam on the pharmacokinetics of [^{14}C]imipramine in rat: acute study

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The pharmacokinetics of [^{14}C]imipramine (10 mg kg^{-1}) were tested in male Wistar rats for interaction with thioridazine (16 mg kg^{-1}) or diazepam (10 mg kg^{-1}). All drugs were administered orally with the test substances being given 40 min before [^{14}C]imipramine dosing. Bile and urine were collected for 90 min after the radioactive drug was given. The animals were then killed and the tissues removed. Thioridazine reduced the excretion of radioactivity into the bile and urine, and increased the weight of the contents within the gastrointestinal tract. These effects were interpreted as being mainly due to a reduction in gastrointestinal motility resulting in a slower stomach emptying of [^{14}C]imipramine. No effect on metabolism was detected. Diazepam pretreatment reduced the concentration ratio of radioactivity in the small intestinal contents to that of plasma, but did not alter the tissue distribution, metabolism or excretion of [^{14}C]imipramine.

The concurrent use of major and minor tranquillizers with tricyclic antidepressant compounds has been recommended by several authors (Hollister, Overall & others, 1967; Haider, 1967; Goodman & Gilman, 1970). Neuroleptics are combined with tricyclic antidepressants when both schizoaffective and depressive symptoms are present (Hollister, 1972). Minor as well as major tranquillizers are often used together with tricyclic antidepressants to help control anxiety symptoms which frequently accompany the depressive state (Splitter, 1965; Hare, 1971).

The hazards of such drug combinations have not been adequately assessed at either the pharmacokinetic or physiological levels. This study has attempted to examine the effects of thioridazine or diazepam on the pharmacodynamics of [^{14}C]imipramine in rats. Effects on biliary and urinary production as well as on stomach emptying were also measured.

MATERIALS AND METHODS

Animals and drugs. Male Wistar rats (Woodlyn Farms, Guelph, Ontario), 200–240 g, had free access to pellet-food and water for one week after purchase. Food was removed 24 h before the common bile duct and left femoral vein were cannulated under ether anaesthesia. Venous cannulation was done with a polyethylene catheter (PX011, Beckton, Dickinson and Co.) but Teflon tubing (0·013″ × 0·031″, T-21195, DuPont) was used for bile collection to avoid absorption of imipramine or its metabolites (Bickel & Minder, 1970a). The animals were then transferred to restraining cages (Bollman, 1948) where they were infused with 0·9% saline at the rate of 1 ml h^{-1} by means of a Harvard Apparatus pump. Rectal temperature was monit-

ored on a Tele-Thermometer, the probe being inserted 5 cm. Body temperature was maintained at 36–38° by means of an incandescent lamp. Two hours after anaesthetic, the rats were administered thioridazine (16 mg kg^{-1}), diazepam (10 mg kg^{-1}) or 0·25% (w/v) gum tragacanth (controls) by mouth. [^{14}C]Imipramine (2μCi ml^{-1}), prepared in distilled water, was given orally (10 mg kg^{-1}) 40 min later. Dosing volume was 5 ml kg^{-1} in all instances, administered through a stainless steel needle.

Bile and urine were collected for 90 min after the animals had received the [^{14}C]imipramine. The rats were then re-anaesthetized for the removal of 2–6 ml of blood from the abdominal aorta. This was collected in heparinized tubes which were placed on ice and later centrifuged to obtain the plasma. The animals were then decapitated and the tissues removed and stored at −20° for analysis of radioactivity.

In the non-cannulated study with thioridazine, neither surgery nor restraint was employed. The dosing schedule was as before except that the radioactivity was reduced to 0·4 μCi ml^{-1}. Nine animals per group were used; they were killed 20 min after [^{14}C]imipramine administration and the stomach contents were removed for examination.

Drugs used: imipramine hydrochloride was purchased from Empire Laboratories, Canada. Thioridazine hydrochloride was donated by Sandoz Ltd., Canada, and diazepam by Hoffman-La Roche Ltd., Canada. Desipramine hydrochloride was donated by Ciba-Geigy Canada Ltd. Iminodibenzyl, 2-hydroxyimipramine hydrochloride, 2-hydroxydesmethylimipramine oxalate and 10-hydroxyimipramine hydrochloride were gifts from Ciba-Geigy Ltd., Basle. [^{14}C]imipramine labelled in the 10, 11 position with a specific activity of 7·24 mCi mmol^{-1} was purchased from Amersham/Searle Corporation.

Preparation of biological materials. Brain, spinal cord, heart, liver, kidneys, lungs, spleen, testes, and contents of the stomach and small intestine were homogenized in glass containers with 3 parts triethanolamine–HCl buffer 0·15 M, pH 7·4 (Bickel & Weder, 1968) using a tissumizer (Model SDT, Tekmar Co., Ohio.). Duplicate 400 μl samples of homogenate were placed in scintillation vials and evaporated to dryness overnight. The residue was then dissolved in 1 ml of Soluene 100 (Packard). Duplicate 50–100 mg samples of oesophagus, skeletal muscle and perirenal fat were added directly to 1 ml of Soluene 100, as were 100 μl plasma and 10 μl biliary samples. Whole stomach and small intestine were dissolved in 5 to 10 ml respectively of Soluene 100, of which a 400 μl aliquot was used for counting. After the materials had been completely dissolved, 10 ml of scintillation fluid (42 parts Liquifluor, New England Nuclear Canada Ltd., to 1000 parts toluene) was added to each vial. Urine from the bladder at the time of death was combined with the 90 min collection and duplicate 100 μl samples were mixed in 10 ml Aquasol (New England Nuclear Canada Ltd.).

Chromatography. The extraction procedure and the thin-layer chromatography (t.l.c.) system used were similar to the methods of Bickel & Weder (1968). A 2 ml portion of liver homogenate was titrated with 1 N NaOH to pH 10·0 and then extracted three times by shaking for 15 min with 5 ml aliquots of 1,2-dichloroethane. Portions of the extracts were dried sequentially under nitrogen in the same 1 ml centrifuge tube. The residue was then re-constituted in 100 μl of methanol of which 20 μl was used for spotting.

100 μl samples of bile were incubated at 37° for 24 h with 100 μl of 0·2 M Na acetate buffer, pH 5·0 and 7 μl of Glusulase (Endo Laboratories Inc.) containing 158 700 units

of β-glucuronidase and 52 400 units of sulfatase per ml. 10 μl of the incubation mixture was used directly for chromatography without further extraction.

Chromatograms were developed on 250 μm thick silica gel plates (60F–254, EM Laboratories Inc.) with chloroform–n-propanol–saturated ammonia (100: 100: 2). All biliary samples were applied on the thin-layer plates together with approximately 250–500 ng each of imipramine and its metabolites. Visualization of the spots was by the procedure of Bickel & Baggiolini (1966). The desired spots were then scraped individually into scintillation vials and suspended in a mixture of 15 ml Aquasol to 5 ml distilled water radioactivity measured in a scintillation counter. Quenching was corrected by the external standard channel ratio method.

Statistics. Student's *t*-values were calculated and evaluated for significance using Dunnet's method of comparing several treatments with a control (Dunnet, 1955). Logarithmic transformations were applied to ratios, and arcsin transformations to percentages before testing for significance. For readability, however, mean values and "standard error" are always presented in tables and in text from untransformed data.

RESULTS

Radioactivity in bile. Biliary concentrations of radioactivity after an oral dose of [^{14}C]imipramine (10 mg kg^{-1}) are shown in Fig. 1. Diazepam pretreatment (10 mg kg^{-1}) 40 min earlier caused no significant change in the excretion of radioactivity into the bile. Thioridazine (16 mg kg^{-1}), however, produced a delay in the appearance of the peak and significantly reduced the magnitude of [^{14}C] concentrations at the 20 and 30 min time intervals.

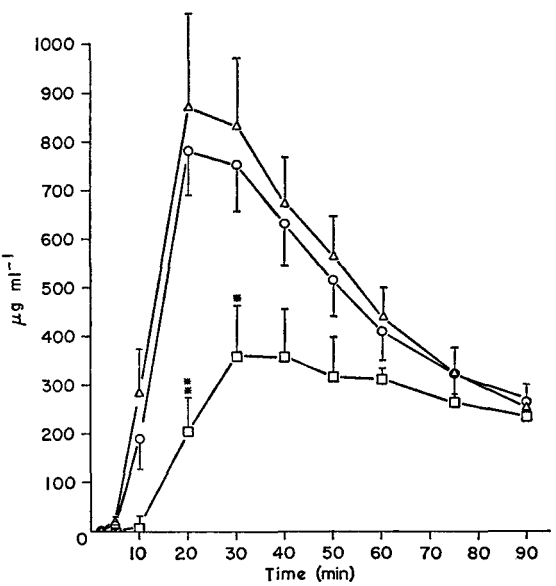

FIG. 1. Elimination of radioactivity into the bile as a function of time after oral administration of [^{14}C]imipramine (10 mg kg^{-1}). Ordinate: biliary radioactivity expressed as imipramine hydrochloride in μg ml^{-1}; Abscissa: time after [^{14}C]imipramine dosing in min. Animals were orally dosed 40 min earlier with diazepam (△, 10 mg kg^{-1}), thioridazine (□, 16 mg kg^{-1}) or gum tragacanth solution (○). Vertical bars indicate s.e. Each group contained 5 rats.

Liver metabolism. Thin-layer separation of biliary metabolites from the 90 min collection did not reveal any significant differences between control and tranquillizer pretreated groups in the proportions of the major hydroxylated metabolites (Table 1). The only significant difference obtained was an elevation in the thioridazine group of the percentage of the metabolites in the band of colour (R_F approximately 0·73 to 0·86) which immediately precedes the solvent front. Iminodibenzyl, 2-hydroxy-iminodibenzyl and perhaps other unidentified metabolites lie in this region and can be separated properly only on another thin-layer system (Bickel & Weder, 1968).

Table 1. *Metabolites (% of total radioactivity) in 90 min bile collection.*

Metabolite	Control	Pretreatment Diazepam	Thioridazine
Origin	21·2 ± 1·1	19·7 ± 0·9	20·4 ± 2·2
2-Hydroxydesmethylimipramine	23·3 ± 1·1	22·5 ± 1·3	24·3 ± 1·3
Desipramine	5·7 ± 0·6	6·1 ± 0·1	5·2 ± 0·3
10-Hydroxyimipramine	6·8 ± 0·6	5·9 ± 0·5	5·4 ± 0·5
2-Hydroxyimipramine	16·6 ± 1·3	17·3 ± 2·0	14·6 ± 1·0
Imipramine	1·0 ± 0·2	1·1 ± 0·1	1·0 ± 0·4
$R_F \sim$ 0·73–0·86	2·7 ± 0·4	3·7 ± 0·6	4·8 ± 0·6*
Other	22·7 ± 1·4	23·7 ± 3·0	24·2 ± 0·8

See Fig. 1 for dosing schedule.
Values are presented as mean ± "s.e." of untransformed percentages. The arcsin transformation was applied before statistical significance was determined (see Methods).
* Differs from control at $P < 0·05$.

Bile to liver and bile to plasma ratios were determined as a further test of blockage of liver metabolism by thioridazine or diazepam. A reduction in these ratios would be expected to result from an impaired metabolism (e.g. inhibition of glucuronide conjugation). No significant differences were found between treatment groups for either the bile to liver ratios (untransformed mean ± "s.e." for control = 14·3 ± 1·6, diazepam = 13·6 ± 0·63 and thioridazine = 12·2 ± 1·5) or bile to plasma ratios (untransformed mean ± "s.e." for control = 529 ± 71, diazepam = 424 ± 28 and thioridazine = 600 ± 52). Biliary radioactivity concentrations at the 90 min time interval were used for these calculations.

Distribution of radioactivity. Table 2 shows the distribution of radioactivity in bile, urine and various body compartments at the end of the 90 min collection period. Total recovery obtained by adding these components averaged about 90% of the administered dose. Only urinary radioactivity in the thioridazine group was significantly different from control. [^{14}C] in the urine of this group was approximately one-quarter that of control. The tissue compartment in Table 2 was calculated by assuming 12% of the body weight to be fat and 40% to be muscle. No significant difference between control and treated groups was found either when the tissues were grouped into one compartment as in Table 2 or when treated individually (i.e. in terms of radioactivity levels expressed as imipramine hydrochloride, μg g^{-1} tissue).

Absorption. Approximately 40–50% of the administered radioactivity was found in the stomach 90 min after the oral dosing. No significant differences were found between groups in either stomach or small intestinal retention of radioactivity (Table 2). [^{14}C] concentration ratios between stomach contents and plasma also showed no significant difference between groups (untransformed mean ± "s.e." for control =

Table 2. *Distribution of radioactivity 90 min after [^{14}C] imipramine administration.*

Compartment	Control	Pretreatment Diazepam	Thioridazine
Oesophagus	0·40 ± 0·08	0·22 ± 0·06	0·76 ± 0·31
Stomach (tissue & contents)	42·0 ± 4·4	38·8 ± 9·1	51·3 ± 8·1
Small intestine (tissue & contents)	4·53 ± 0·84	3·59 ± 0·90	5·31 ± 1·7
Σ Tissues (except GI tract)	16·7 ± 2·1	20·2 ± 4·6	19·0 ± 4·7
Bile (90 min collection)	24·9 ± 3·1	25·6 ± 3·3	13·6 ± 3·3
Urine (90 min collection)	1·61 ± 0·15	1·54 ± 0·48	0·40 ± 0·13**

See Fig. 1 for dosing schedule. Each value is the mean ± "s.e." of untransformed percentages of the total dose administered. The arcsin transformation was applied before determination of statistical significance (see Methods).
** Differs from control at $P < 0·01$.

1493 ± 265, diazepam = 1500 ± 429 and thioridazine = 2084 ± 510). These figures indicate that enormous gradients exist between stomach contents and plasma thus confirming that imipramine is poorly absorbed from an acid medium (Bickel & Weder, 1969). A significant reduction from control in the [^{14}C] concentration ratio of small intestinal contents to plasma occurred in the diazepam pretreated group (untransformed mean ± "s.e." for control = 50·6 ± 5·7, diazepam = 30·4 ± 3·2, $P < 0·05$, and thioridazine = 61·6 ± 20). The thioridazine group did not differ significantly from control in this respect.

Physiological indices. To ascertain whether or not some of the pharmacodynamic effects of the tranquillizers could be attributed to alterations in the rats' physiological status, measurements were taken of urinary and biliary volumes, and of intestinal content weight. Table 3 shows a significant elevation in the total weight of the gastrointestinal contents in the thioridazine-pretreated group. This effect was not found when the stomach, small intestine and large intestine were analysed separately. No differences between groups were found in the volume of bile and urine produced during the 90 min collection period. However, urinary volume from the thioridazine pretreated group was only about one-third that of control and might offer at least a partial explanation for the highly significant reduction in urinary radioactivity noted above.

Study with non-cannulated rats. To determine whether or not inhibition of stomach emptying by thioridazine was responsible for reduced biliary outflow of radioactivity at 20 and 30 min in the bile cannulated rats, another experiment was made on un-

Table 3. *Physiological status as determined by weight of gastrointestinal contents (g kg^{-1} body weight) and 90 min volumes of bile and urine (ml kg^{-1} body weight).*

	Control	Pretreatment Diazepam	Thioridazine
Stomach contents	4·15 ± 0·41	3·93 ± 0·75	5·31 ± 0·90
Small intestinal contents	12·9 ± 0·84	14·2 ± 0·67	14·1 ± 0·75
Large intestinal contents	9·38 ± 0·71	10·5 ± 0·66	11·6 ± 0·82
Total gastrointestinal contents	26·4 ± 0·90	28·6 ± 1·4	31·0 ± 1·2*
Bile	4·92 ± 0·12	4·90 ± 0·35	4·75 ± 0·47
Urine	5·89 ± 1·5	7·70 ± 2·0	2·0 ± 0·47

Dosing schedule is the same as in Fig. 1. Each value is the mean ± s.e. of contents weights (g kg^{-1} body weight) or of bile or urine volumes (ml kg^{-1} body weight).
* Differs from control at $P < 0·05$.

operated, unrestrained animals. The mean untransformed percentage ($\pm$ "s.e.") of administered radioactivity recovered in the stomach 20 min after oral administration of [^{14}C]imipramine was 54·6 $\pm$ 5·0 for the thioridazine pretreated rats and 25·7 $\pm$ 2·8 for the controls. Arcsin transformations show this difference to be significant at $P < 0{\cdot}001$. Furthermore, the average weight ($\pm$ s.e.) of the stomach contents (in g kg^{-1} body weight) was 7·19 $\pm$ 0·90 for the thioridazine pretreated group compared to 3·80 $\pm$ 0·53 for the control group (difference significant at $P < 0{\cdot}01$).

DISCUSSION

Absorption. The data from the non-cannulated study indicates that thioridazine exerts a strong inhibitory effect on the stomach emptying of imipramine. This is the most likely cause of the reduction in radioactivity of the bile at the 20 and 30 min intervals after [^{14}C]imipramine administration in the thioridazine-pretreated rats from the bile fistula experiment. The mechanism whereby thioridazine slows the gastic emptying of imipramine remains unclear. However, Sun & Shay (1959) have presented evidence which shows that chlorpromazine in small doses acts centrally to reduce vagal drive to the gastric mucosa. An inhibition of vagal drive would also explain the increased weight of the gastrointestinal contents found in the thioridazine group (Table 3). Since constipation is a well known side-effect of imipramine (Lambert, 1973), this effect of thioridazine might have been taking place over and above an imipramine-induced reduction in gastrointestinal motility.

Metabolism. It has been suggested that neuroleptics inhibit the metabolism of tricyclic antidepressants in man. Gram & Overø (1972) found that patients treated with perphenazine, haloperidol or chlorpromazine excreted less radioactivity into the urine than controls after a test dose of [^{14}C]imipramine. Crammer & Rolfe (1972) reported a fourfold increase in the plasma concentrations of imipramine and desipramine, and decrease in the urinary excretion of free and conjugated hydroxylated metabolites after chlorpromazine administration. They concluded that inhibition of metabolism was likely to be occurring at the level of hydroxylation of imipramine and desipramine. Marked elevations in plasma concentrations of nortriptyline in rats occurred when thioridazine is given concurrently, regardless of the route of administration (oral., i.p. or i.v.) (Fuller, Snoddy & Slater, 1974). Doses of only 10 mg kg^{-1} of either drug were used to obtain this effect by the oral route.

The present study shows no difference in the concentrations of the major hydroxylated metabolites in the 90 min bile collection. Most of these would have been in the conjugated form before incubation with Glusulase (Bickel & Minder, 1970a). Hence, if thioridazine was blocking either the hydroxylation or conjugation mechanisms, the biles of the thioridazine group should have shown a reduction of hydroxylated metabolites relative to imipramine or desipramine which are transported into the bile by a different mechanism (Bickel & Minder, 1970b). Since no such reduction was observed (Table 1) it appears that thioridazine under the conditions of this experiment had no effect on either the hydroxylation or conjugation processes. Similarly, the lack of effect on the percentage of desipramine in the bile rules out an effect on the demethylation pathway. It would appear therefore that the metabolic interaction postulated for other tricyclic antidepressant-neuroleptic combinations does not occur with imipramine and thioridazine, at least with the dosing schedule and species of rat we used.

The presence of a higher percentage of radioactivity in the coloured band just preceding the t.l.c. solvent front in the biles of the thioridazine-pretreated group (Table 1) may simply mean that the pathways of metabolism involved were operating more efficiently during the earlier time intervals after imipramine dosing. Since blockage of stomach emptying by thioridazine would have drastically reduced the concentrations of imipramine reaching the liver at this time, the chances of enzyme saturation were lower.

Urinary excretion. The highly significant decrease in urinary radioactivity in the thioridazine-pretreated animalsh as no simple explanation. A large part of the decrease could be attributed to the much smaller (significant only at $P < 0{\cdot}1$) volume of urine produced by these animals. Another partial explanation might be that urinary radioactivity is simply reflecting a similar reduction in biliary excretion of the drug. Lower levels of biliary radioactivity indicate that there are fewer polar metabolites being produced (due to an inhibition of stomach emptying) and therefore less drug can be eliminated by the kidneys.

Effect of diazepam. Diazepam had little effect on the pharmacokinetics of imipramine showing only a minor decrease in the [^{14}C] concentration ratio of the small intestinal contents to that of plasma. This alteration appears to be of minimal importance since it did not have any significant repercussions on the total amount of drug absorbed nor did it alter the shape of the curve in Fig. 1 relating biliary radioactivity to time. The lack of an effect on imipramine metabolism by diazepam (10 mg kg^{-1}) agrees well with data reported in man (Silverman & Braithwaite, 1972; Gram, Overø & Kirk, 1974).

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