Effect of Carbon Tetrachloride on Thioridazine Action and Metabolism.* (26693)

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(Introduced by F. W. Schueler)
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In an earlier study(1) we found that thioridazine (2-methylmercapto-10-[2-(N-methyl-2-piperidyl) ethyl] phenothiazine HCl, Mellaril, TP-21 had a marked effect on hepatic hemodynamics which was dose-dependent. Before proceeding further with these studies, it seemed desirable to obtain some preliminary information regarding the metabolic aspects of this compound. Since the literature suggests that the phenothiazines have their chief biotransformation site in the liver(2,3), we have studied the effect of experimentally induced liver damage on two pharmacological actions of thioridazine. In addition we have attempted to relate these effects to the persistence of this phenothiazine in the body.

Material and methods. Control and liver damaged male Swiss albino mice (18-30 g) were compared as to acute lethality, hypothermia and disposition of thioridazine. Liver damage was produced by administering 1 ml/kg of CCl_4 (20% v/v in olive oil) subcutaneously. Control animals received olive oil alone (0.1 ml per 20 g). Two groups of mice were sacrificed 24 hours after pretreatment with CCl_4 or oil; sections of liver and kidneys were removed and fixed in 10% formalin. Sections stained with hematoxylin and eosin were examined for histopathologic changes.† This 24-hour treatment with CCl_4 was the standard method employed throughout the experiment for producing liver damage.

Acute lethality determinations were carried out on control and liver damaged animals using 25, 50, 100, 200, and 300 mg/kg (i.p.) doses of thioridazine. Ten mice were used at each dosage level. The number of deaths occurring 24, 48, and 72 hours later were recorded. LD_{50} values were estimated for each time interval, using the method of Litchfield and Wilcoxon(4). Similar experiments were carried out using 80, 110, 140, 170 and 200 mg/kg (i.p.) doses of pentobarbital, Na.

The hypothermic response of mice to 10 mg/kg (i.p.) of thioridazine was assessed by measuring rectal temperatures, in groups of 5 mice, at hourly intervals, with a Yellow Springs Tele-Thermometer Model 44TK; a small animal probe was inserted to a depth of 1.5 cm. Statistical comparisons of the control *vs.* the liver damaged groups were carried out by means of Student's t-test(5).

Whole body concentrations of thioridazine were determined 4 and 18 hours after its administration (50 mg/kg, i.p.). The whole animal was homogenized in a Waring Blendor in a volume of water 4 times the animal weight. The homogenate was run through the following analytical procedure which is a modification of the method of Salzman and Brodie(2).

Two ml of tissue homogenate were added to a 50 ml glass-stoppered tube, containing 1 ml of water and about 25 mg of $NaHCO_3$. Next 10 ml of n-heptane (previously washed with 1N H_2SO_4,1N NaOH, and H_2O) containing isoamyl alcohol (2% v/v) were added. The tubes were stoppered, shaken for 10 minutes, and centrifuged. Eight ml of the organic phase were then transferred to a second tube containing 4 ml of 1N H_2SO_4. This tube was shaken for 10 minutes and centrifuged. The aqueous acid phase was then transferred to a cuvette and the absorption determined on a Beckman DU Spectrophotometer, set at a wavelength of 263 mμ. A reagent blank was carried through the same procedure by substituting water for the mouse homogenate. Using this procedure, tissue blanks were found to be negligible and

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TABLE I. Comparison of Median Lethal Doses of Thioridazine and Pentobarbital in Oil-Treated and CCl_4-Treated Mice.

Time, hr	Oil-treated LD_{50}, mg/kg	CCl_4-treated LD_{50}, mg/kg
	Thioridazine	
24	243 (187-316)*	148 (99-224)
48	195 (141-269)	120 (83-174)
72	148 (102-214)	96 (67-138)
	Pentobarbital	
24	132 (116-150)	125 (106-135)
48	132 (116-150)	125 (106-135)

* 95% confidence limits.

the O.D. readings required no corrections. After quantitation at 263 mμ absorption spectra were taken on selected samples over the range of 220-320 mμ. To assess the specificity of the method, the n-heptane phase (from the first extraction step) from several mice of a given group was pooled. Equal aliquots of this pooled extracted were then washed with one each of the following phthalate or phosphate buffers(6): pH 3.0, 5.0, 6.5, and 8.0. Then, the washed heptane phases were transferred to other extraction tubes and extracted with 1N H_2SO_4 as before. Thus the ratio:

$$\frac{\text{O.D. reading of } H_2SO_4 \text{ after buffer wash}}{\text{O.D. reading of } H_2SO_4 \text{ without buffer wash}}$$

gives values characteristic for thioridazine. The method is similar in principle to that employed by Fujimoto *et al.*(7). To facilitate discussion, these ratios will hereafter be referred to as "partition ratios."

Results. Histologically, livers from CCl_4-treated animals exhibited extensive central coagulative necrosis and pronounced depletion of glycogen. The kidneys, on the other hand, exhibited no cellular necrosis. Some minimal nonspecific changes in cytoplasmic staining were noted; however, similar findings were seen in kidneys from oil-treated animals. It was concluded that this dose of CCl_4 had marked effect on the liver but little or no effect on kidney morphology.

The lethality data are depicted in Table I. Control animals exhibited the "delayed death" phenomenon, *i.e.*, deaths occurred over a period of 72 hours after administration of thioridazine. The number of "delayed deaths" was sufficient to cause progressive lowering of the LD_{50} estimate through this period; the 72-hour value was significantly lower than the 24-hour value. It can be seen that CCl_4 treatment increases the lethal potency of thioridazine in that at all time intervals LD_{50} values for the CCl_4-treated groups were lower than in the respective oil-treated controls; this difference was greatest at 24 hours ($p<.05$). Although LD_{50} is reduced by CCl_4 treatment, this procedure does not seem to alter the occurrence of "delayed deaths." All LD_{50} curves were found to be mutually parallel.

In the lower part of Table I data are included for comparative purposes, showing that CCl_4 treatment did not alter 24- and 48-hour acute LD_{50} values for pentobarbital. These animals however did show pronounced prolongation of sleeping time. The temporal decline in the LD_{50} estimate observed with the phenothiazine was not demonstrated with this particular barbiturate.

Fig. 1 illustrates the results obtained in

TABLE II. Partition Ratios of Ultraviolet Absorbing Material Recovered from Whole Body Homogenates of Mice Receiving Thioridazine.

Group	Optical density ratio and stand. error after buffer equilibration* pH 8.0	pH 6.5	pH 5.0	pH 3.0
Thioridazine added to whole body homogenate *in vitro*	.80 ± .03	.66 ± .03	.50 ± .03	.30 ± .03
Whole body homogenate from oil-treated mice receiving thioridazine	1.04† ± .06	.78 ± .09	.61 ± .08	.39 ± .03
Whole body homogenate from CCl_4-treated mice receiving thioridazine	.94 ± .08	.61 ± .06	.45 ± .05	.33 ± .01

* Each value represents mean of 3 determinations.
† Significantly different from control value ($P < .05$).

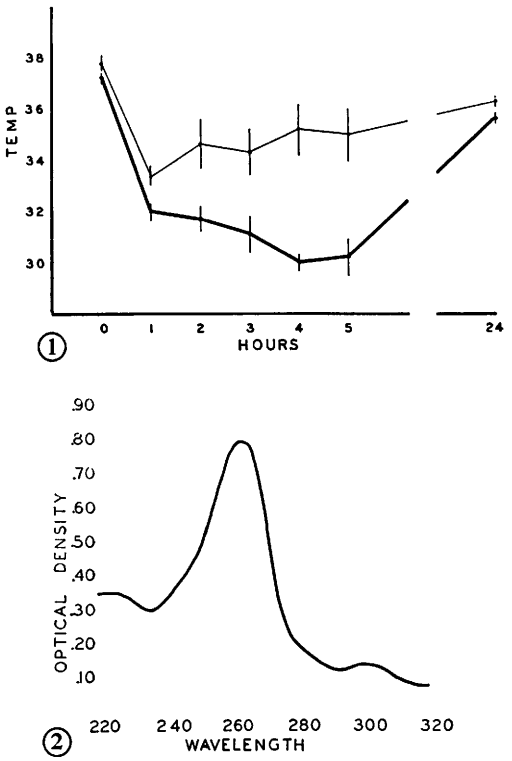

FIG. 1. Effect of CCl_4 treatment on hypothermia produced by thioridazine (10 mg/kg, i.p.). Ordinate is rectal temperature (°C); abscissa is time after administration of thioridazine. Upper line depicts oil-treated control animals; lower line, CCl_4-treated animals. Each point represents mean of 5 animals; vertical lines represent stand. errors.

FIG. 2. Ultraviolet absorption spectrum of thioridazine HCl (10 μg/ml) in 1 N H_2SO_4.

the hypothermic studies. CCl_4 treatment resulted in both enhanced lowering of body temperature and prolongation of the effect. The temperature differences between the 2 groups were statistically significant from the 2nd hour through the 5th hour, the maximum difference occurring in the 4th hour ($p < .005$).

The mean percentage recoveries of thioridazine added to water, liver, and whole animal homogenates were 87, 93, and 84% respectively when run through the extraction procedure. Fig. 2 depicts the ultraviolet absorption spectrum of thioridazine (10 μg/ml in 1N H_2SO_4). It is characterized by a maximum at 263 mμ.

The 4-hour whole body concentration of thioridazine determined in mice with liver damage was found to be significantly higher ($p < .05$) than in the oil-treated controls (39.3 ± (SE) 2.5 mg/kg *vs* 26.4 ± 3.0 mg/kg). This was also found to be true at 18 hours (40.4 ± 0.9 mg/kg *vs* 28.4 ± 2.0 mg/kg).

Although the data suggest that no disappearance of thioridazine occurred between 4 and 18 hours, it should be borne in mind that these results were obtained in separate experiments employing different batches of mice and were designed only to show the effect of liver damage. In another experiment using a single batch of mice designed to study the time-disappearance relationship of this drug in normal mice, it was found that mean whole body concentrations were 46.8, 25.9, and 17.9 mg/kg at 1, 4, and 18 hours respectively. Since in all cases the initial dose administered was 50 mg/kg, these data demonstrate that thioridazine, or metabolic products which are indistinguishable from it on the basis of ultraviolet spectrum, persist in the body for a relatively long time.

Mean partition ratios, and their standard errors, for thioridazine which are pertinent for assessing specificity of the method are given in Table II. It can be seen that the products extracted from oil-treated mice deviated somewhat in their partition characteristics when compared to the *in vitro* control values. However, only the difference at pH 8 was statistically significant. This suggests that the extraction procedure may extract some metabolic products as well as parent compound. The ratios obtained from the CCl_4 group more closely parallel those of the parent compound, although they are not significantly different from the oil-treated group.

Discussion. The lethality data show that CCl_4 treatment increases the lethal potency of thioridazine. This suggests that the presence of liver damage delays thioridazine degradation to a point where normally sublethal doses become lethal.

The lethality results were particularly striking when the effect was compared to those obtained with pentobarbital. The response to this agent is known to be affected by liver damage(8). Indeed, in the present experiments prolonged CNS depression was noted. On the other hand, the finding that

lethal potency was unaltered, for pentobarbital, was initially surprising. However, after reading the literature cited by Maynert and Van Dyke(8) indicating that liver damage increases the toxicity of certain barbiturates, it was seen that most investigators have termed prolongation of anesthesia as "toxicity" rather than estimating LD_{50} values, as was done here. This phenomenon may in part be peculiar to agents which have an immediate toxic effect like pentobarbital in that benactizine prolongs pentobarbital sleeping time without altering the LD_{50} of pentobarbital(9).

The reason that CCl_4 enhances the lethal potency of thioridazine may be related to the fact that this latter substance is long acting and produces "delayed deaths" in contrast to pentobarbital. The reason for "delayed deaths" is not clear. A number of contributory factors have been suggested by other investigators working on phenothiazines(10, 11).

The results of the hypothermia studies show that at least one pharmacological action beside lethality is intensified and prolonged by CCl_4 treatment. These data are highly suggestive that liver damage and subsequent delay in biotransformation produce these effects.

More directly, by comparing whole body concentrations of thioridazine from oil-treated animals with those from CCl_4-treated animals, it is evident that liver damage delays thioridazine degradation. This is further supported by the partition ratio data, in that those obtained from CCl_4-treated animals more closely approximated control values for unchanged thioridazine. Although the analytical method seems fairly specific for thioridazine, the fact that the partition ratios from the control mice do not match precisely those of the parent compound suggests that perhaps some contribution from metabolic products is occurring. Of these the more likely products would be sulfoxide and N-demethylated derivatives. The former compound should be distinguishable from the parent compound in that this modification causes a shift in the spectrum of other phenothiazines(1,12). No such shifts were found in the extracts analyzed in the present study. N-demethylated derivatives, however, have approximately the same spectrum as parent phenothiazines and could contribute to the thioridazine measured(12). This may explain why even though no shift in the spectra were found the partition ratios did not give values exactly like thioridazine.

Summary. CCl_4 treatment was found to increase both lethal and hypothermic potency of thioridazine. The dose of CCl_4 employed produced liver damage but no kidney damage. A method is described for determining thioridazine in tissues. Whole body concentrations of this phenothiazine were found to be significantly higher in mice treated with CCl_4.

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