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The Toxicity of Thioxanthene Neuroleptics to Isolated Rat Liver Cells (39041)

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The thioxanthenes, a relatively new class of neuroleptics, are structurally related to the phenothiazines. Both classes contain 6,6,6-tricyclic structures, have 3-carbon side chains terminating with methylated amine groups and substituents at position 2 of the tricyclic structure. However, the thioxanthenes have a carbon replacing the nitrogen of the phenothiazines (1). When the amine side chain is attached to the tricyclic nucleus by an unsaturated bond, as is the case with the highly active compounds, the substituted thioxanthenes have *cis*- and *trans*-isomers. Of the two isomers, only the *cis*-forms of each compound are active neuroleptics (2–5).

The thioxanthene drugs have received widespread attention, as potent antipsychotics, which have been reported to cause fewer side effects than their phenothiazine analogs (3, 6). However, a recent report has implicated chlorprothixene as the causative agent in cholestatic jaundice (7) and the FDA has eight reports of hepatic dysfunction caused by thiothixene (five cases) or chloroprothixene (three cases) (H. D. Carnahan, personal communication). The small number of reports indicate that hepatic injury caused by these drugs was probably the result of an idiosyncratic response of the individual.

It has been postulated that liver damage caused by a therapeutic agent in a small number of patients may be, in part, the result of slight intrinsic toxicity of the drug combined with a generalized hypersensitivity of the recipient (8). One model used to test the intrinsic toxicity of drugs has been that of isolated hepatocytes (9). For example, chlorpromazine (CPZ) and erythromycin estolate, both of which produce jaundice in clinical circumstances, caused more enzyme leakage from isolated rat hepatocytes than did promazine (PZ) and erythromycin base (EB), respectively, which seldom (PZ) if ever (EB) cause jaundice (9, 10). Since the thioxanthenes have been reported to cause hepatic injury and are structurally related to the phenothiazines, studies were implemented to ascertain whether the thioxanthenes have cytotoxic effects on hepatocellular suspensions, and to compare their potencies with those of the corresponding phenothiazines.

Methods and materials. The *cis*- and *trans*-isomers of chlorprothixene were obtained from Hoffman La Roche. H. Lundbeck provided the *cis*- and *trans*-isomers of clopenthixol and flupenthixol; N-716, a thioxanthene analog of promazine having an unsaturated bond between the tricyclic nucleus and the aminopropylidene side chain; N-710, a promazine analog having a saturated exocyclic bridge to the aminopropyl side chain; and N-756A, the bromo-analog of chlorprothixene (11). The bromo-analog is the active form and is probably the *cis*-isomer (N. Lassen, H. Lundbeck, personal communication). Xanthiol and methixene were obtained from Pfizer and Dorsey Laboratories, respectively. Chlorpromazine and SKF 10812 (*trans*) were donated by Smith, Kline and French. Except for *trans*-chlorprothixene and N-710, all of the compounds were available as the hydrochloride salts. *Trans*-chlorprothixene was in the free base form and N-710 was provided as the oxalate.

Male CD rats (250–300 g) were obtained from Charles River Laboratories and permitted food and water *ad lib*. Isolated hepatocytes were prepared by a modification of the method of Berry and Friend (12) as described previously (13). After isolation, the hepatocytes were counted using a hemocytometer and diluted until there were 2×10^5 cells/ml. With the exception of *trans*-chlorprothixene, each drug was dissolved in Ca-free Hank's solution (pH 7.4) at two times the concentration to be studied. The *trans*-isomer of chlorprothixene was dissolved in Hank's containing 5% ethanol. This concentration of ethanol was found to exert no measurable effect on leakage of enzymes

from the isolated hepatocytes. One ml of the drug solution (or no drug) and the hepatocyte suspension were combined in test tubes and incubated, without shaking, at 37° for 30 min. The samples then were centrifuged to sediment the cells. The supernatant of each sample was decanted and designated as the medium. One milliliter of distilled water was added to each cell fraction and the tubes were agitated vigorously to resuspend the cells. The cell fractions were lysed by alternately freezing (dry ice in ethanol) and thawing (37° water bath) three times.

Samples from the media and cells were taken and the level of glutamic-oxaloacetic transaminase (GOT) was measured (14). Each sample was run in triplicate. Initially, the drugs were tested at 0.5 *M* intervals from 10^{-5} to 10^{-3} *M* to obtain preliminary data on the approximate cytotoxic concentration. After establishing an approximate cytotoxic level, each compound was then tested at 0.1 *M* intervals around this concentration to obtain a more precise level. Ten experiments were conducted with each drug. CPZ, which has been shown to cause enzyme leakage in this model (10), was run with each experiment as a "positive" control.

Student's *t* test was utilized to determine differences between means and the 5% level was taken to be significant (15).

Results. With the exception of N-710, all of the thioxanthenes examined caused leakage of GOT from isolated hepatocytes. The most potent compound was SKF 10812, which had an adverse effect at 3×10^{-5} *M*. The *cis*- and *trans*-isomers of chlorprothixene and N-756A caused significant leakage at 6×10^{-5} *M*, while *cis*- and *trans*-flupenthixol exerted an effect at 8×10^{-5} *M* (Table I).

Exposure of hepatocytes to xanthiol or methixene, two thioxanthenes which have the amine side chain attached by a saturated bond, resulted in a significant efflux of cellular GOT at 9×10^{-5} and 2×10^{-4} *M*, respectively. The isomeric forms of clopenthixol and N-716 caused enzyme leakage at 2×10^{-4} *M* (Table II). N-710, in concentrations from 10^{-5} to 5×10^{-4} *M*, had no effect on the leakage of GOT in this model. Because of problems with solubility and enzyme inhibition, the effects of N-710, above 5×10^{-4} *M*, were not examined.

Discussion. The results of the present investigation provide evidence that these thioxanthenes can adversely affect the membrane of isolated rat hepatocytes and that this effect is related to the concentration of drug in the medium. As the concentration increased, so did leakage of GOT into the medium. The relative potency of the thioxanthenes in producing this effect was SKF 10812 > *cis*- = *trans*-chlorprothixene = N-756A > *cis*- = *trans*-flupenthixol > xanthiol > *cis*- = *trans*-clopenthixol = N-716 = methixene > N-710.

Among the thioxanthenes with a dimethylaminopropylidene side chain, SKF 10812, which possesses a trifluoromethyl group at position 2, is more potent than those with a halogen at that position [*cis*- and *trans*-chlorprothixene or N-756A (the bromo-analog of chlorprothixene)]. The unsubstituted member of this series, N-716, is one of the least potent thioxanthenes tested.

The effect of a chlorine at position 2 of the thioxanthene moiety resembles the effect of the same substituent on the phenothiazine or iminodibenzyl moiety. In each case, the chlorinated compound is much more potent than its repective unsubstituted compound, e.g. CPZ > PZ (10, 13).

A comparison of the unsubstituted tricyclic neuroleptics and antidepressants in causing leakage of GOT from hepatocytes reveals the potency of the thioxanthene (N-716) > dibenzylcycloheptene (amitriptyline [AT], nortriptyline [NT]) > iminodibenzyl (imipramine [IM] desmethylimipramine [DMI]) > phenothiazine (PZ). N-716, AT and NT have their amine side chains attached by a double bond, but PZ, IM and DMI have the methylated aminopropyl side chain connected by a carbon–nitrogen bond. Among the thioxanthene and ibenzylcycloheptene drugs, N-170 and protriptyline, in which the side chains are attached by a saturated carbon-carbon bond, are less potent in eliciting this response than N-716 and NT, the analogs in which the exocyclic linkages are unsaturated (Table II; 13). These observations suggest that the rigidity imposed

TABLE I. THE EFFECTS OF CHLORPROTHIXENE, FLUPENTHIXOL, SKF 10812 AND N-756A ON THE LEAKAGE OF AN INTRACELLULAR ENZYME (GOT) INTO THE MEDIA[a].

Drug concentration (*M*)	Chloprothixene				Flupenthixol				SKF-10812		N-756A	
	cis		*trans*		*cis*		*trans*		*trans*		*cis*	
	Med	Cells	Med	Cells	Med	Cells	Med	Cells	Med	Cells	Med	Cells
10^{-5}									128 ±15	107 ±9		
2×10^{-5}									112 ±18	100 ±11		
3×10^{-5}									235 ±61[b]	66 ±10[b]		
4×10^{-5}									315 ±67[c]	42 ±7[c]		
5×10^{-5}	102 ±9	112 ±15	120 ±18	109 ±11	94 ±11	134 ±19	104 ±10	116 ±17	315 ±60[c]	59 ±16[c]	114 ±3	95 ±17
6×10^{-5}	148 ±18[b]	115 ±20	168 ±25[b]	99 ±22	110 ±11	154 ±31	120 ±12	132 ±23	352 ±55[c]	34 ±7[c]	154 ±9[c]	76 ±13[b]
7×10^{-5}	205 ±21[c]	81 ±10[b]	180 ±19[c]	88 ±21	130 ±10	99 ±14	136 ±12	115 ±17			193 ±12[c]	60 ±9[c]
8×10^{-5}	240 ±18[c]	66 ±19[c]	218 ±27[c]	77 ±13[b]	159 ±14[c]	81 ±12[b]	150 ±12[b]	99 ±15			227 ±26[c]	51 ±10[c]
9×10^{-5}	243 ±19[c]	63 ±19[c]	226 ±19[c]	57 ±19[c]	190 ±18[c]	70 ±12[c]	166 ±10[c]	85 ±13[b]			236 ±31[c]	41 ±8[c]
10^{-4}	243 ±10[c]	42 ±12[c]	224 ±17[c]	56 ±17[c]	184 ±24[c]	64 ±12[c]	193 ±13[c]	61 ±15[c]			259 ±29[c]	57 ±9[c]
CPZ (5×10^{-4})	299 ±23[c]	18 ±17[c]	280 ±34[c]	31 ±6[c]	330 ±60[c]	52 ±12[c]	330 ±60[c]	52 ±10	350 ±56[c]	38 ±8	289 ±38[c]	47 ±9[c]
Hank's	100 ±7	100 ±10	100 ±17	100 ±10	100 ±17	100 ±17	100 ±17	100 ±17	100 ±10	100 ±10	100 ±13	100 ±17

[a] Values are expressed as per cent of controls. The control values in i.u./liter (Medium ±-SE; Cell ± SE) for the respective drugs are: *cis*-chlorprothixene (88 ± 6; 139 ± 13), *trans*-chlorprothixene (84 ± 14; 159 ± 15) *cis*- and *trans*-flupenthixol (70 ± 12; 135 ± 23), SKF 10812 (60 ± 6; 100 ± 10) and N-756A (90 ± 12; 275 ± 48).

[b] $P < 0.05$.

[c] $P < 0.01$.

by the double bond has some importance in causing the phenomenon.

Replacement of the dimethylaminopropylidene side chain with a hydroxyethylpiperazinylpropylidene moiety results in a decrease in activity. *Trans*-flupenthixol and the *cis*- and *trans*-isomers of clopenthixol are 5–6 times less potent than SKF 10812, *cis*- and *trans*-chlorprothixene, respectively. However, as in the dimethylaminopropylidene series, the trifluoromethyl compounds (*cis*- and *trans*-flupenthixol) which possess the piperazinyl side chain are more potent than their halogenated analogs (*cis*- and *trans*-clopenthixol).

Generally, the thioxanthenes exerted an effect on isolated rat liver cells at lower concentrations than did their corresponding phenothiazines. Except for the isomers of clopenthixol, which exerted their effect at the same concentration as their phenothiazine analog (perphenazine), the thioxanthenes were 4–8 times more active than are their phenothiazine analogs (Fig. 1).

With the phenothiazines, there was some correlation between activity in this model and neuroleptic potency, e.g. CPZ > PZ, but with the thioxanthenes, this did not appear to be the case. Although only the *cis*-thioxanthenes possess high neuroleptic activity, both the *cis*- and *trans*-isomers of each compound were equipotent in causing this adverse effect

TABLE II. THE EFFECTS OF CLOPENTHIXOL, N-716, XANTHIOL AND METHIXENE ON THE LEAKAGE OF AN INTRACELLULAR ENZYME (GOT) INTO MEDIA.[a]

Drug concentration (*M*)	Clopenthixol *cis* Med	Clopenthixol *cis* Cells	Clopenthixol *trans* Med	Clopenthixol *trans* Cells	N-716 Med	N-716 Cells	Xanthiol Med	Xanthiol Cells	Methixene Med	Methixene Cells
9×10^{-5}							173 ±3[c]	88 ±9		
10^{-4}	113 ±14	104 ±16	124 ±13	112 ±12	82 ±10	112 ±19	160 ±6[c]	71 ±5[c]	100 ±11	91 ±12
2×10^{-4}	243 ±15[c]	54 ±15[c]	183 ±14[c]	87 ±18	189 ±34[c]	62 ±6[c]	280 ±7[c]	33 ±3[c]	193 ±23[c]	48 ±18[c]
3×10^{-4}	270 ±24[c]	54 ±17[c]	207 ±17[c]	64 ±12[c]	231 ±41[c]	35 ±4[c]	309 ±6[c]	38 ±6[c]	260 ±18[c]	37 ±8[c]
4×10^{-4}	267 ±22[c]	55 ±17[c]	228 ±21[c]	65 ±14[c]	236 ±41[c]	34 ±3[c]	273 ±16[c]	39 ±5[c]	273 ±15[c]	39 ±12[c]
5×10^{-4}	289 ±21[c]	36 ±9[c]	252 ±20[c]	56 ±9[c]	266 ±47[c]	34 ±4[c]	311 ±10[c]	29 ±3[c]	282 ±15[c]	28 ±4[c]
CPZ 5×10^{-4}	357 ±38[c]	23 ±5[c]	357 ±38[c]	23 ±5[c]	289 ±38[c]	47 ±9[c]	249 ±9[c]	45 ±8[c]	249 ±9[c]	45 ±8[c]
Hank's	100 ±15	100 ±11	100 ±15	100 ±11	100 ±13	100 ±17	100 ±5	100 ±8	100 ±5	100 ±8

[a] Values are expressed as per cent of controls. The control values in i.u./liter (Medium ± SE; Cells ± S.E.) for the respective drugs are; *cis*- and *trans*-clopenthixol (46 ± 7; 84 ± 9), N-716 (90 ± 12; 279 ± 48), xanthiol (45 ± 2; 126 ± 10) and methixene (45 ± 2; 126 ± 10).

[b] $P < 0.05$.

[c] $P < 0.01$.

on hepatocyte suspensions. Correlation of the deleterious effects of the thioxanthenes on the membranes of isolated rat hepatocytes, with an adverse effect on the liver in clinical situations remains to be established.

Summary. A series of thioxanthenes was tested for cytotoxicity to isolated rat hepatocytes, as measured by the loss of an intracellular enzyme (GOT) to the surrounding medium. The relative order of potency was found to be: SKF 10812 > *cis*- = *trans*-chlorprothixene = N-756A > *cis*- = *trans*-flupenthixol > xanthiol > methixene = *cis*- = *trans*-clopenthixol = N-716 > N-710. The presence of an unsaturated exocyclic bond increased the apparent toxicity as did the presence of a substituent (trifluoromethyl, chlorine, bromine) at the two position of the tricyclic nucleus. The trifluoromethyl substituted thioxanthenes were three to four times more potent than their halogenated analogs, but there were no differences in potency among the halogenated (chlorine or bromine) thioxanthenes. Compounds which had a dimethylaminopropyli-

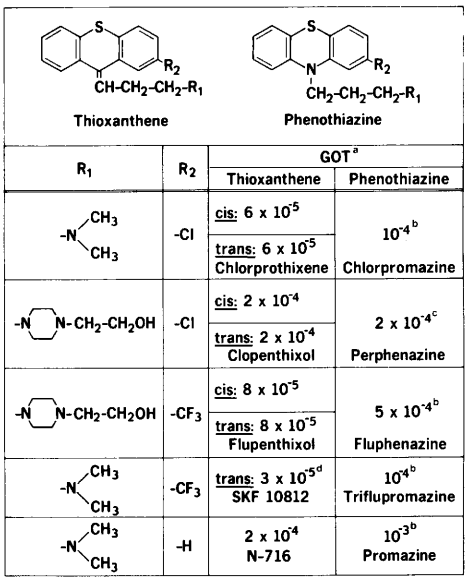

FIG. 1. Comparison of the relative potencies of some thioxanthenes and their phenothiazine analogs. [a] *M* concentration of drug that causes efflux of intracellular GOT to medium. [b] Data from Zimmerman and Kendler, 1970. [c] Unpublished results. [d] *cis*-analog not available.

dene side chain were five to six times stronger in causing enzyme leakage than were their analog which had a hydroxyethylpiperazinylpropylidene side cain. Although only the *cis*-isomers of each compound are highly active neuroleptics, both isomers were equipotent at causing the efflux of GOT from rat hepatocytes.

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