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Adverse Effects of Chlorpromazine Metabolites on Isolated Hepatocytes (39833)[1]

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Treatment of patients with chlorpromazine (CPZ) occasionally results in jaundice, an adverse reaction generally attributed to hypersensitivity (1–3). However, clinical and experimental observations suggest that CPZ may possess some slight intrinsic toxicity (4, 5). Other studies have also demonstrated that CPZ has deleterious effects on Chang human liver cells, liver slices, and the perfused rat liver. Furthermore, these studies have shown that CPZ exerted greater effects than did promazine, a closely related compound which rarely causes jaundice (6–9).

CPZ is subject to a wide variety of metabolic transformations including S-oxidation, N-demethylation, and ring hydroxylation (10–13). These metabolic products may exert greater or lesser effects than the parent compound, as biotransformation may activate or inactivate a drug (14). For example, two of these metabolites, desmethylchlorpromazine and 7-hydroxychlorpromazine, have been reported to possess neuroleptic activity, while others, e.g., chlorpromazine sulfoxide, do not (15–18). In addition, it has been reported that the *in vivo* toxicity of CPZ increases as the compound is progressively demethylated (19). Since several CPZ metabolites seem to have pharmacological properties, it is quite possible that they may also have effects on the liver. Therefore, it seemed of interest to examine the effects of these metabolites in a model system in which CPZ is also tested, to ascertain whether their effects on isolated hepatocytes correlate with their reported *in vivo* effects.

Methods and materials. Chlorpromazine (CPZ), didesmethylchlorpromazine (Nor_2-CPZ), chlorpromazine sulfoxide (CPZSO), desmethylchlorpromazine sulfoxide (Nor_1-CPZSO), and didesmethylchlorpromazine sulfoxide (Nor_2-CPZSO), as their hydrochloride salts, were donated by Smith, Kline, and French. Dr. A. A. Manian (Pharmacology Section, NIH) provided a sample of 7-hydroxychlorpromazine (7-OH-CPZ), and Rhodia supplied samples of 8-hydroxychlorpromazine (8-OH-CPZ) and desmethylchlorpromazine HCl (Nor_1-CPZ). Since these compounds are sensitive to light (18), the compounds were stored in air-tight containers in a desiccator in the dark. Immediately prior to use, a sample of the test compound was weighed, dissolved in Hanks' solution, and added to the hepatocyte suspension.

Male CD rats (250–300 g) were obtained from Charles River Laboratories and were kept in the animal facility for at least 1 week prior to use. The animals were maintained in animal care facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

Hepatocytes were isolated by a slight modification (20) of the method of Berry and Friend (21). After isolation, the cells were suspended in Ca-free Hanks' solution (pH 7.4). They were counted using a hemocytometer and were diluted to provide 2×10^5 cells/ml.

The drugs were dissolved in the Hanks' buffer at twice the final concentration. One milliliter each of the hepatocyte suspension and the drug solution (Hanks' only in the control) were combined and shaken gently to mix the contents. Then, the samples were incubated in test tubes, without shaking, for 30 min at 37°. Afterward, the samples were centrifuged to sediment the cells, and the supernatant was decanted and designated as the medium. Distilled water (1 ml) was added to each cell sample, and the tubes were vigorously agitated to resuspend the cells. To release cellular enzymes, the cell samples were lysed by alternatively freezing (dry ice in ethanol) and thawing (37° water

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bath) three times. Aliquots of medium and cell samples were taken, and the level of asparate aminotransferase (GOT) in each sample was determined (22). Ten experiments were conducted on each drug unless otherwise noted, and each assay was run in triplicate. Prior to each drug study, the effects of the drug on the activity of a pure GOT preparation (Sigma G-1751) were tested. At the concentrations used in these studies, none of the compounds inhibited GOT activity by more than 10%.

In a separate series of experiments, the effects of calcium on the response of the hepatocytes to CPZ were examined. After isolation, the cells were counted and suspended in the buffer. The suspension was divided, and calcium (1.12 μmole/ml) was added to one group. Then, CPZ, in varying concentrations, was added to both groups of cells. Incubation and analysis were conducted as described above.

The data were analyzed using a paired *t* test, comparing the GOT level in each drug sample to its respective control or to another drug sample that was tested on liver cells from the same suspension (23).

Results. CPZ, at concentrations of 9×10^{-5} *M* or greater, caused leakage of GOT, but the demethylated metabolites of CPZ, Nor_1-CPZ and Nor_2-CPZ, caused significant release of the enzyme from hepatocytes at concentrations of 6×10^{-5} and 3×10^{-5} *M*, respectively. The hydroxy derivatives, 7-OH-CPZ and 8-OH-CPZ, led to an efflux of GOT at a concentration of 2×10^{-4} *M* (Table 1). CPZSO had no measurable effect on isolated liver cells in concentrations ranging from 10^{-5} to 10^{-3} *M*. Due to the limited supply of Nor_1-CPZSO and Nor_2-CPZSO, only three experiments were run with these two compounds (data not given). In that limited set of experiments, neither compound appeared to exert an effect.

For direct comparisons, CPZ, Nor_1-CPZ, and Nor_2-CPZ were tested on suspensions of liver cells prepared from the same liver. As with the earlier series of experiments, the effects of the demethylated metabolites were greater than those of CPZ, and the didesmethyl analog exerted its effects at a lower concentration than did the desmethyl compound (Table 2).

The presence of calcium in the incubation medium had no observable effect on the CPZ-induced release of GOT. In both groups, CPZ caused an efflux at 9×10^{-5} *M*, and the potency of CPZ was similar in each set (Table 3).

Discussion. The results of the present investigation have demonstrated that the effects of CPZ on isolated hepatocytes are similar to those exerted by CPZ on Chang liver cells (8) and on liver slices (6). Furthermore, several metabolites of CPZ have adverse effects in this model similar to those of CPZ, and the presence of calcium in the medium did not modify the adverse effects of CPZ. The relative order of potency for the compounds studied was: Nor_2-CPZ > Nor_1-CPZ > CPZ > 7-OH-CPZ = 8-OH-CPZ $\gg$ CPZSO, Nor_1-CPZSO, and Nor_2-CPZSO. Demethylation of the terminal amino group appeared to increase toxicity, while hydroxylation caused a slight decrease. Oxidation of the sulfur yielded inactive compounds in this model.

The relationship of Nor_1-CPZ to CPZ, with respect to their effects on isolated liver cells, is quite similar to that of desipramine to imipramine. With both types of tricyclic psychotherapeutic agents, demethylation of the dimethylaminopropyl side chain results in a threefold increase in toxicity to isolated hepatocytes [Table 2; Ref. (20)]. It is also interesting to note the relationship between the *in vivo* toxicity and the adverse effects of CPZ metabolites in this model. It has been reported that progressive demethylation of CPZ increases the toxicity to rats (19) and, as shown in this study, also increases toxic effects on hepatocytes (Table 2).

Hydroxylation of the tricyclic nucleus gave rise to compounds that were slightly less toxic toward liver cells than was CPZ in these studies, although one of the compounds, 7-OH-CPZ, has been reported to possess neuroleptic activity (16, 24). Based on the results of this study, it might be anticipated that demethylation of the hydroxylated compounds would give chemicals that were more active. Unfortunately, those compounds were not available for study.

Sulfoxidation of CPZ led to loss of the toxicity for liver cells, just as it affects the

TABLE 1. THE EFFECTS OF CPZ METABOLITES ON EFFLUX OF GOT FROM ISOLATED RAT LIVER CELLS.[a]

Drug concentration (M)	CPZ (10)[b]		7-OH·CPZ (10)		8-OH·CPZ (8)		Nor_1-CPS (10)		Nor_2-CPZ (10)	
	Medium	Cells	Medium	Cells	Medium	Cells	Medium	Cells	Medium	Cells
10^{-5}							95 ± 16	108 ± 19	63 ± 4[d]	97 ± 12
2×10^{-5}							84 ± 14	111 ± 20	68 ± 3[d]	93 ± 11
3×10^{-5}							86 ± 13	107 ± 19	168 ± 14[d]	47 ± 12[c]
4×10^{-5}							98 ± 14	97 ± 20	234 ± 14[d]	39 ± 9[d]
5×10^{-5}							125 ± 18	82 ± 18	250 ± 28[d]	32 ± 7[d]
6×10^{-5}					115 ± 12	157 ± 33	154 ± 26[d]	71 ± 16		
7×10^{-5}	98 ± 17	106 ± 21			124 ± 19	172 ± 35	202 ± 23[d]	61 ± 16[d]		
8×10^{-5}	116 ± 20	92 ± 20			154 ± 35	111 ± 23				
9×10^{-5}	180 ± 31[c]	75 ± 17[c]			194 ± 56	82 ± 19				
10^{-4}	209 ± 35[d]	64 ± 16[d]	100 ± 10	102 ± 5	175 ± 43	63 ± 20				
2×10^{-4}	321 ± 43[d]	24 ± 4[d]	133 ± 14[c]	89 ± 5	321 ± 53[d]	40 ± 14[d]				
3×10^{-4}	327 ± 41[d]	18 ± 3[d]	188 ± 22[c]	63 ± 5[d]						
4×10^{-4}	339 ± 38[d]	15 ± 4[d]	224 ± 22[d]	62 ± 7[d]						
5×10^{-4}	288 ± 46[d]	18 ± 4[d]	218 ± 20[d]	54 ± 8[d]						
Control	100 ± 17	100 ± 20	100 ± 10	100 ± 10	100 ± 7	100 ± 26	100 ± 17	100 ± 20	100 ± 7	100 ± 4

[a] Values are expressed as percentages of controls. The control values in international units per liter (medium ± SE; cells ± SE) for the respective drugs are: CPZ and Nor_1-CPZ (56 ± 9.3; 119 ± 23.9), Nor_2-CPZ (38 ± 2.8; 92 ± 13.3), 8-OH·CPZ (33 ± 2.4; 88 ± 22.8), and 7-OH·CPZ (51 ± 5.2; 123 ± 12.1).
[b] Values in parentheses are numbers of experiments.
[c] $P < 0.05$ from controls.
[d] $P < 0.01$ from controls.

TABLE 2. COMPARISON OF THE EFFECTS OF THE DEMETHYLATED ANALOGS OF CHLORPROMAZINE (CPZ) ON THE LEAKAGE OF ASPARTATE AMINOTRANSFERASE FROM ISOLATED HEPATOCYTES.[a]

	CPZ		Nor_1-CPZ		Nor_2-CPZ	
Drug concentration (M)	Medium (8)[b]	Cells (7)	Medium (4)	Cells (3)	Medium (8)	Cells (7)
10^{-5}					43 ± 7[c]	120 ± 16
2×10^{-5}					50 ± 9	116 ± 16
3×10^{-5}			40 ± 2	130 ± 20	81 ± 7[c,e]	79 ± 11[c,e]
4×10^{-5}	38 ± 9[c]	126 ± 19	43 ± 3	122 ± 17	107 ± 18[c,d,e]	62 ± 11[c,d,e]
5×10^{-5}	42 ± 8	107 ± 13	55 ± 7	115 ± 16	145 ± 19[c,d,e]	48 ± 12[c,d,e]
6×10^{-5}	55 ± 8	104 ± 13	70 ± 8	94 ± 17		
7×10^{-5}	51 ± 9	103 ± 14	94 ± 10[c,d]	79 ± 22[c]		
8×10^{-5}	59 ± 6	99 ± 17				
9×10^{-5}	86 ± 7[c]	83 ± 14[c]				
10^{-4}	102 ± 9[c]	76 ± 13[c]				
Control	57 ± 9	108 ± 13	46 ± 2	131 ± 23	57 ± 9	108 ± 13

[a] Aspartate aminotransferase levels are expressed in international units per liter ± SE.
[b] Values in parentheses are number of experiments in each group.
[c] $P < 0.05$ from controls.
[d] $P < 0.05$ from CPZ.
[e] $P < 0.05$ from Nor_1-CPZ.

TABLE 3. THE EFFECTS OF CHLORPROMAZINE (CPZ) ON ISOLATED HEPATOCYTES IN THE PRESENCE OR ABSENCE OF CALCIUM.[a]

	GOT Levels (IU/liter + SE)			
	No calcium		Calcum (1.12 μmole/ml)	
Molar concentration of CPZ (M)	Medium	Cells	Medium	Cells
7×10^{-5}	21 ± 1	104 ± 17	22 ± 2	85 ± 10
8×10^{-5}	22 ± 2	100 ± 14	24 ± 2	76 ± 8
9×10^{-5}	29 ± 3[b]	84 ± 16	37 ± 4[c]	63 ± 11
10^{-4}	34 ± 2[c]	72 ± 13[b]	45 ± 5[c]	61 ± 10[b]
2×10^{-4}	74 ± 7[c]	62 ± 12[b]	98 ± 12[c]	47 ± 11[c]
3×10^{-4}	122 ± 11[c]	39 ± 14[c]	129 ± 12[c]	24 ± 7[c]
4×10^{-4}	141 ± 9[c]	17 ± 5[c]	148 ± 14[c]	15 ± 6[c]
5×10^{-4}	143 ± 13[c]	18 ± 5[c]	156 ± 10[c]	14 ± 5[c]
Control	20 ± 2	85 ± 11	19 ± 3	79 ± 9

[a] Nine sets of experiments were conducted.
[b] $P < 0.05$ from controls.
[c] $P < 0.01$ from controls.

pharmacological effects (16–18, 24). Limited data on the demethylated analogs of CPZSO indicates that they are inactive also.

Guttman and Friedman (25) have reported that the anti-motility activity of several phenothiazines toward *Tetrahymena pyriformis* parallels their clinical effectiveness, and that addition of calcium (0.6 μmole/ml) to the medium leads to a 90% reversal of CPZ's effect in that model. In the present study, calcium, at a higher concentration (1.12 μmole/ml), had no measurable effect on the response of the hepatocytes to CPZ (Table 3). We also found that the addition of calcium did not alter the effects of amitriptyline, nortriptyline, or chlorprothixene on liver cells (unpublished data). These results suggest that the presence of calcium does not modify the effects of tricyclic drugs on isolated hepatocytes.

The present study provides evidence that several metabolites of CPZ exert deleterious effects, as judged by the loss of GOT, on isolated liver cells. These results are consistent with, but not proof of, the hypothesis that certain drugs, which cause jaundice in a small percentage of patients, possess a slight intrinsic toxicity (26). However, no direct proof of a causal relationship between the effects of drugs on hepatocytes and hepatic

injury in clinical situations has been established. Nevertheless, if the *in vitro* model has a bearing on *in vivo* hepatotoxicity, the differences in the activity of several metabolites of CPZ in the *in vitro* model may have clinical significance. Relevant to this consideration is the effect of pretreatment with other drugs on the metabolism of CPZ. It has been reported that pretreatment of rats with phenobarbital causes an increase in the production of the hydroxylated intermediates, while administration of SKF-525A or methylphenidate increases the formation of the demethylated and sulfoxide metabolites (27). As many patients are on multiple drug therapy, the effects of drugs given concomitantly with CPZ might affect the potential for toxicity of CPZ.

Summary. Chlorpromazine (CPZ) and several of its metabolites were tested for *in vitro* cytotoxicity, as measured by the efflux of aspartate aminotransferase to the surrounding medium, toward isolated rat hepatocytes. Exposure of liver cells to CPZ, at a concentration of 9×10^{-5} M, led to enzyme leakage. The demethylated metabolites, mono- and didesmethyl-CPZ, were three and six times, respectively, more potent than CPZ. Hydroxylation of the tricyclic ring at the 7 or 8 position gives rise to compounds that were slightly less active than the parent compound, while oxidation of the sulfur atom resulted in inactive analogs. The presence of calcium in the medium had no apparent effect on the response of the hepatocytes to CPZ.

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