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TOXICITY OF TRICYCLIC ANTIDEPRESSANTS TO ISOLATED RAT HEPATOCYTES

CHARLES O. ABERNATHY, LORINC LUKACS and HYMAN J. ZIMMERMAN

Veterans Administration Hospital, Washington, D.C. 20422, U.S.A.

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Abstract—A series of tricyclic antidepressant drugs was tested for cytotoxicity as measured by enzyme leakage from isolated rat hepatocytes. The relative potency appeared to be: chlorimipramine > amitriptyline ≥ nortriptyline > desipramine > protriptyline > imipramine. The presence of a chloro-substituent in position R_3 increased apparent cytotoxicity, and the dibenzylcycloheptene nucleus seems to be more toxic than the corresponding phenothiazine or imminodibenzyl moieties.

A wide variety of chemicals has been reported to cause liver damage. Some (e.g. carbon tetrachloride) produce injury consistently and are classified as intrinsic or predictable hepatotoxins. Others, which cause hepatic injury in a small proportion of recipients, are considered to depend on an idiosyncratic response of the individual rather than toxic effect of the drug *per se*. One form of idiosyncratic response appears to depend on hypersensitivity (allergy) to the drug [1].

Previous studies suggest that some drugs may possess a slight degree of toxicity that only becomes manifest when combined with a generalized hypersensitivity. These studies have demonstrated that certain drugs, which can produce clinically evident liver damage, have greater cytotoxic effect on liver cells than closely related drugs which do not produce hepatic injury in humans. Chlorpromazine (CPZ) and erythromycin estolate (EE), for example, cause more enzyme leakage from hepatocyte suspensions than does promazine or erythromycin base [2–5]. Furthermore, CPZ and EE also have been shown to interfere with the excretion of bile and sulfobromophthalein by the isolated perfused rat liver [6, 7].

There have been reports of hepatic damage by several tricyclic antidepressants. Amitriptyline [8–10], imipramine [11–13] and a combination of imipramine and desipramine [14] or imipramine and cyproheptadine [15] have all caused jaundice in patients. Accordingly, studies on the effects of this series of compounds on hepatocytes *in vitro* were undertaken.

Since isolated heptocytes possess significant metabolizing capacity [16, 17], the possible effects of metabolites of the respective drugs also warranted consideration. To evaluate this possibility, the effects of pretreating rats with phenobarbital sodium prior to isolation of hepatocytes, on the response of the cells to the tricyclic antidepressants, were evaluated.

METHODS AND MATERIALS

Amitriptyline-HCl, and protriptyline-HCl were supplied by Merck & Co., while nortriptyline-HCl was received from Eli Lilly & Co. Imipramine-HCl and chlorimipramine-HCl were donated by CIBA Pharmaceuticals. USV Pharmaceuticals and Smith, Kline & French provided samples of desipramine-HCl and chlorpromazine-HCl respectively.

Female Sprague–Dawley rats (230–260 g) were purchased from Flow Research Laboratories and given free access to food and water. In the phenobarbital studies, rats were given the drug (i.p., 100 mg/kg, daily) for 5 days, while control rats received saline only.

Isolated hepatic cells were prepared by a slight modification of the method of Berry and Friend [18] as detailed below. The hepatic portal vein was cannulated (PE-205 tubing, Intramedic, Clay-Adams) and the liver was perfused with Ca-free Hanks' solution for 10 min. During this period, the liver was removed from the animal and all non-hepatic tissue was carefully removed. Afterward, the liver was transferred to a perfusion chamber maintained at 37° (Metalloglass, Boston) and perfused for 5 min at low pressure (5 cm H_2O) with 150 ml enzyme solution (0·05% collagenase and 0·1% hyaluronidase in Ca-free Hanks' solution). Then, the liver was perfused for 25 min at high pressure (30 cm H_2O) with the same solution. The enzyme solution was removed and the liver was rinsed at high pressure for 10 min with Ca, Mg-free Hanks' solution.

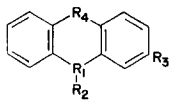
The liver was removed from the chamber and macerated, placed in 50 ml enzyme solution and gently agitated for 15 min at 37° to further digest the clumps of cells that remained. The resulting suspension was filtered through two layers of nylon and centrifuged at 500 *g* for 5 min to precipitate the cells and remove the enzyme solution. The cells were resuspended in Ca-free

Table 1. Cytotoxicity of tricyclic antidepressant drugs to isolated hepatocytes as measured by the leakage of a cellular enzyme (glutamic oxalacetic transamine, GOT) into the medium*

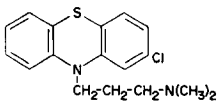
Drug conen (M)	Amitriptyline		Nortriptyline		Protriptyline		Imipramine		Desipramine		Chlorimipramine	
	Medium	Cells	Medium	Cells	Medium	Cells	Medium	Cells	Medium	Cells	Medium	Cells
8×10^{-5}											113 ± 16	70 ± 12
9×10^{-5}											140 ± 20†	68 ± 8†
10^{-4}	86 ± 20†	101 ± 26	60 ± 9‡	116 ± 19	61 ± 6†	130 ± 29	64 ± 9†	124 ± 26	64 ± 10†	123 ± 22	175 ± 33‡	57 ± 9†
2×10^{-4}	139 ± 29	102 ± 31	79 ± 14	94 ± 24							249 ± 36‡	30 ± 6‡
3×10^{-4}	208 ± 32‡	78 ± 25†	171 ± 27†	51 ± 15†					89 ± 20	82 ± 14	263 ± 32‡	23 ± 3‡
4×10^{-4}	320 ± 65‡	29 ± 6†	237 ± 31‡	23 ± 6‡					135 ± 34	68 ± 16†	295 ± 23‡	21 ± 2‡
5×10^{-4}	313 ± 65‡	23 ± 6‡	306 ± 40‡	18 ± 3‡	106 ± 13	122 ± 28	89 ± 10	116 ± 27	193 ± 31‡	69 ± 18†	328 ± 32‡	23 ± 3‡
6×10^{-4}	385 ± 81‡	19 ± 6‡	269 ± 40‡	14 ± 3‡	124 ± 17	119 ± 31	110 ± 19	85 ± 31	242 ± 45‡	26 ± 6‡		
7×10^{-4}	387 ± 80‡	16 ± 5‡			169 ± 27†	81 ± 21	124 ± 23	88 ± 33	262 ± 43‡	15 ± 3‡		
8×10^{-4}					197 × 44‡	62 ± 22†	154 ± 27	72 ± 33				
9×10^{-4}					222 ± 54‡	52 ± 13†	179 ± 29†	61 ± 25†				
10^{-3}	364 ± 79‡	16 ± 3‡	290 ± 34‡	16 ± 2‡	249 ± 38‡	44 ± 12‡	227 ± 28‡	48 ± 18‡	300 ± 38‡	14 ± 2‡	265 ± 46‡	28 ± 7‡
CPZ (5×10^{-4})	304 ± 63‡	28 ± 10‡	291 ± 32‡	21 ± 6‡	288 ± 37‡	18 ± 3‡	275 ± 35‡	18 ± 3‡	299 ± 32‡	21 ± 6‡	334 ± 31‡	32 ± 10‡
Controls	100 ± 24	100 ± 25	100 ± 15	100 ± 12	100 ± 19	100 ± 18	100 ± 17	100 ± 18	100 ± 15	100 ± 14	100 ± 15	100 ± 10

* Values are expressed as per cent of controls. The control values in i.u./liter (medium ± S. E., cells ± S. E.) for the experiments with the respective drugs are: amitriptyline (59 ± 12, 98 ± 25), nortriptyline (82 ± 12, 117 ± 15), protriptyline (78 ± 18, 128 ± 23), imipramine (88 ± 15, 127 ± 23), desipramine (75 ± 11, 114 ± 15) and chlorimipramine (64 ± 10, 87 ± 9).

† $P < 0.05$. ‡ $P < 0.01$.



Tricyclic Drug Nucleus



Chlorpromazine

DRUG	R_1	R_2	R_3	R_4
AMITRIPTYLINE	C=	$CH\text{-}CH_2\text{-}CH_2\text{-}N(CH_3)_2$	H	$CH_2\text{-}CH_2$
NORTRIPTYLINE	C=	$CH\text{-}CH_2\text{-}CH_2\text{-}N\text{-}HCH_3$	H	$CH_2\text{-}CH_2$
PROTRIPTYLINE	C-	$CH_2\text{-}CH_2\text{-}CH_2\text{-}N\text{-}HCH_3$	H	CH=CH
IMIPRAMINE	N-	$CH_2\text{-}CH_2\text{-}CH_2\text{-}N(CH_3)_2$	H	$CH_2\text{-}CH_2$
DESIPRAMINE	N-	$CH_2\text{-}CH_2\text{-}CH_2\text{-}N\text{-}HCH_3$	H	$CH_2\text{-}CH_2$
CHLORIMIPRAMINE	N-	$CH_2\text{-}CH_2\text{-}CH_2\text{-}N(CH_3)_2$	Cl	$CH_2\text{-}CH_2$

Fig. 1. Structures of drugs used in this study.

Hanks' solution and recentrifuged once to remove cell debris. The precipitate was suspended and diluted until there were 2×10^5 cells/ml.

The drugs used in this study (Fig. 1) were dissolved in Ca-free Hanks' solution at twice the concentration to be studied. Then the drug solution or control (Hanks' solution without drug) and cell suspension were combined (1·0 ml each). These samples were incubated in test tubes without shaking for 30 min at 37°. After this time, the samples were removed and centrifuged to sediment the cells. The supernatant was decanted and designated as the medium. One ml distilled water was added to the cell samples and the tubes were agitated to resuspend the cells. The cells were lysed by alternatively freezing (dry-ice and ethanol) and thawing (37° water bath) three times. Aliquots of the medium and cell fractions were taken and glutamic oxalacetic transaminase (GOT) levels were determined

Table 2. Relative potencies of tricyclic antidepressants as compared to CPZ on hepatocytes from normal rats

Drug concn (5×10^{-4} M)	Medium*	Cells*
Amitriptyline	313 ± 65	23 ± 6
CPZ	304 ± 63	28 ± 10
Nortriptyline	306 ± 40	18 ± 3
CPZ	291 ± 32	21 ± 6
Protriptyline	106 ± 13†	122 ± 28†
CPZ	288 ± 37	18 ± 3
Imipramine	89 ± 10†	116 ± 27†
CPZ	275 ± 35	18 ± 3
Desipramine	193 ± 31†	69 ± 18‡
CPZ	299 ± 32	21 ± 6
Chlorimipramine	328 ± 32	23 ± 3
CPZ	334 ± 31	32 ± 10

* Relative enzyme level as compared to controls, when control values = 100 per cent.
† $P < 0.01$.
‡ $P < 0.05$.

[19]. To determine the approximate cytotoxicity, every compound was checked at concentrations from 10^{-5} to 10^{-3} M (0·5 M intervals). Then, to establish a more precise level, each drug was tested at 0·1 M intervals around the approximate cytotoxic concentration. Each assay was run triplicate and ten experiments were conducted with each drug. CPZ has been shown to cause enzyme leakage in this model [2, 4], and it was employed as a "positive" control.

The data were analyzed using Student's *t*-test to determine differences between means [20].

RESULTS

Each of the tricyclic drugs studied had an adverse effect on hepatocytes, as measured by leakage of enzymes from the cells to the media. Chlorimipramine was the most potent, causing cell damage at 9×10^{-5} M, while amitriptyline and nortriptyline each caused significant leakage at 3×10^{-4} M. Desipramine affected the cells at 5×10^{-4} M and protriptyline at 7×10^{-4} M. Imipramine was the least potent, as it required a concentration of 9×10^{-4} M to cause leakage (Table 1).

The results from the phenobarbital-pretreated rats are not included, as this regimen had minimal effect on the cytotoxic potency of these compounds. The cytotoxicity of nortriptyline and chlorimipramine was lowered significantly but only slightly to 4×10^{-4} M, and 2×10^{-4} M, respectively, while that of desipramine, protriptyline and imipramine was increased slightly to 4×10^{-4} M, 6×10^{-4} M and 7×10^{-4} M, respectively. Pretreatment with phenobarbital did not alter the apparent cytotoxicity of amitriptyline.

Since each experiment contained a CPZ "positive" control, the efficacy of each compound at 5×10^{-4} M was compared to the effect of CPZ at the same concentration. With chlorimipramine, amitriptyline and nortriptyline, there was no difference, but protriptyline, imipramine and desipramine were less cytotoxic than CPZ (Table 2). Pretreatment with phenobarbital did not change this relationship.

DISCUSSION

The results of this study indicate that this group of compounds can cause damage to hepatocytes in the following order of potency: chlorimipramine > amitriptyline ≥ nortriptyline > desipramine > protriptyline > imipramine. Chlorimipramine, the 3-chloro derivative of imipramine, is the most cytotoxic of these compounds and ten times more potent than imipramine. The chemical relationship of chlorimipramine to imipramine and the relative cytotoxicity of the two resemble that of chlorpromazine to promazine. In both instances, a chloro-substituent in position R_3 (equivalent to the 2 position of CPZ) leads to a distinct increase in toxicity to hepatocytes [3] (Table 1).

Comparison of iminodibenzyl compounds that do not contain a chlorine reveals that the secondary

amine (desipramine) has greater potency than the tertiary amine (imipramine). Desipramine is a metabolic product of imipramine and has been suggested to be the active form [21]. The results of the present investigation raise the possibility that desipramine may be responsible for liver damage caused by the administration of imipramine.

With the dibenzylcycloheptene drugs, a double bond between the R_1 and R_2 (amitriptyline and nortriptyline) seems to enhance cytotoxic activity; a saturated link at this position yields a less active compound (protriptyline). Methylation of the aminopropyl side chain appears to have no significant effect, since the tertiary and secondary amines are equipotent in this model.

The dibenzylcycloheptene nucleus with the methylated aminopropyl group appears to be more cytotoxic than the corresponding phenothiazine or iminodibenzyl compound, as amitriptyline is more potent than promazine [2, 4] or imipramine (Table 1). From these observations, one might anticipate that a compound with a chloro-substituent at R_3 of amitriptyline would be more cytotoxic than either chlorimipramine or CPZ. This compound, however, has not been available for testing.

Phenobarbital pretreatment had a slight effect or no effect on the toxicities of these drugs. The minor changes in cytotoxic potency and the limited conditions of the studies permit no inference to be drawn regarding the relative role of the drugs or their metabolites.

Each of the drugs exerted an adverse influence on hepatocyte membranes, as measured by leakage of cellular enzymes into the media, and appeared to be toxic to hepatocytes. These results are consistent with the hypothesis [1] that the hepatic injury in humans which has been attributed to hypersensitivity may be in part dependent on slight intrinsic toxicity of the respective agent.

The concentrations of drugs employed are higher than those found in patients who are given the agents. Under therapeutic conditions, the plasma levels of amitriptyline, nortriptyline and desipramine can reach 10^{-6} M, while imipramine can reach 10^{-5} M [22, 23]. In rabbits, the liver/plasma ratio of imipramine is 10 [24], while in rats that of desipramine is 100–200 [25]. This level of imipramine is one-tenth that needed to cause damage to isolated hepatocytes, but that of desipramine is about one-half. If amitriptyline or nortriptyline were concentrated 10- to 100-fold by the liver, the level attained would be one-tenth to one-half those needed to cause enzyme leakage. Furthermore, in unpublished studies in our laboratories, amitriptyline and nortriptyline, at 5×10^{-5} M, were found to adversely affect bile flow and sulfobromophthlein excretion by the isolated perfused rat liver. Nevertheless, the relevance of the adverse effects of these agents demonstrated in these models *in vitro* to hepatic injury in clinical circumstances remains to be established.

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