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*Departments of *Pharmacology and ‡Psychiatry University of Health Sciences/The Chicago Medical School, and §Psychiatry Service North Chicago Veterans Administration Medical Center North Chicago, IL 60064, U.S.A.*

ARON D. MOSNAIM*†
MARION E. WOLF‡§
UZOMA P. MADUBUIKE*

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* Send correspondence to: Aron D. Mosnaim, PH.D., Department of Pharmacology, UHS/The Chicago Medical School, 3333 Green Bay Road, North Chicago, IL 60064.

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Effect of cimetidine on paracetamol activation in mice

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Cimetidine is now known to inhibit the cytochrome P-450 mediated metabolism of a range of drugs, both in animals and in man [1]. Since paracetamol toxicity results from the metabolic conversion of the drug to a reactive, arylating intermediate by cytochrome P-450, it is possible that administration of cimeditine may result in a reduction in the hepatotoxicity associated with paracetamol overdose. A number of reports [2–5] have recently demonstrated a protective effect of cimetidine against paracetamol toxicity in animals and inhibition of the oxidative metabolism of paracetamol was implicated as the mechanism. However, it has recently been shown that one week pretreatment with cimetidine (1 g/day) [6] or co-administration of cimetidine (2 g) [7] did not alter the metabolism of a therapeutic dose of paracetamol in healthy human volunteers. Thus, the effect of larger doses of cimetidine on the metabolism of a potentially hepatotoxic dose of paracetamol has been investigated in the C3H mouse, which we have previously shown [8] to be a good model for paracetamol metabolism studies.

Materials and methods

Paracetamol was obtained from the Sigma Chemical Co. (St. Louis, MO), cimetidine from Smith, Kline and French (Sydney, Australia) and piperonyl butoxide from Chemical Dynamics Corp (South Plainfield, N.J.). Experiments were carried out using male C3H mice (23–27 g). Food and water were allowed *ad libitum*. Paracetamol, 200 mg/kg, (30 ml/kg in 0.9% saline) was administered by gavage. Cimetidine, either 50 mg/kg or 100 mg/kg (0.3 ml/kg in 0.9% saline), was administered intraperitoneally (i.p.) 30 min prior to and 1 hr after the paracetamol dose. Piperonyl butoxide (1 g/kg, i.p.) was administered as a single dose 0.5 hr before the paracetamol. The control group of animals was administered a similar volume of saline i.p. Groups of 6 mice received each treatment and animals were placed in individual metabolic cages for the collection of urine for 24 hr after the paracetamol dose. Urine was analysed for unchanged paracetamol and its glucuronide, sulphate, cysteine and mercapturic acid conjugates by high performance

liquid chromatography [8]. The cysteine and mercapturic acid conjugates of paracetamol were summed and reported as total glutathione (GSH)-derived conjugates; the excretion of these metabolites reflects the activity of the oxidative metabolic pathway less the amount of reactive metabolite bound to cellular constituents other than GSH [9]. To confirm cimetidine protected against paracetamol-induced hepatotoxicity, blood was collected 7 hr after administration of paracetamol (200 mg/kg), both from control and cimetidine-treated groups (N = 6) of animals, for the measurement of plasma alanine transaminase (ALT) [10]. All results are expressed as mean ± S.E. The significance of differences between treatment groups was evaluated using Students *t*-test for unpaired samples.

Results and discussion

The dose of paracetamol used in this study caused a significant degree of hepatotoxicity as evidenced by increased plasma ALT values 7 hr after paracetamol administration (control group ALT, 37 ± 6 U/l; paracetamol-treated group ALT, 1960 ± 291 U/l : $P < 0.001$). Both doses of cimetidine employed here markedly reduced the elevation in ALT due to paracetamol (cimetidine, 100 mg/kg, ALT 225 ± 42 U/l: cimetidine, 200 mg/kg, ALT 192 ± 30 U/l). Metabolic data are summarised in Table 1 and show that treatment of animals with either 100 or 200 mg/kg (in two divided doses) of cimetidine had no effect on the pattern of urinary paracetamol metabolites after a 200 mg/kg dose of paracetamol. By contrast, pretreatment of animals with piperonyl butoxide significantly decreased the fraction of GSH-derived conjugates excreted in the urine. The ratio of metabolite excreted in urine to unchanged paracetamol, commonly called the metabolic ratio, reflects the clearance to that metabolite [8]. Reconsideration of the data in Table 1 in terms of metabolic ratios showed that piperonyl butoxide decreased paracetamol apparent oxidative clearance by 72% ($P < 0.01$), although the actual degree of inhibition is likely to be higher as piperonyl butoxide decreases the covalent binding of the reactive metabolite [9]. Clearance to the glucuronide was reduced 50% ($P < 0.01$) by piperonyl butoxide but clearance to the sulphate was not significantly altered. Inhibition of paracetamol oxidative metabolism is consistent with protection against paracetamol-induced hepatotoxicity observed in animals pretreated with piperonyl butoxide [9]. It should be noted that piperonyl butoxide has previously been shown to inhibit paracetamol glucuronidation *in vivo* [8, 9]. Cimetidine at either dose level did not affect any of the paracetamol metabolic clearances, as measured by metabolic ratios. In particular, the metabolic ratio for the GSH-derived conjugates was not altered suggesting that neither the rate of production nor the degree of conjugation with GSH of the paracetamol reactive metabolite was affected by cimetidine.

In contrast to piperonyl butoxide, our results demonstrate that the protective effect afforded by cimetidine in animals [2–5] poisoned with paracetamol is unlikely to be due to decreased formation of the paracetamol reactive metabolite. These data are consistent with the lack of effect of therapeutic doses of cimetidine on the metabolism of a therapeutic dose of paracetamol in humans [6, 7]. Since cimetidine would appear to be unable to facilitate GSH synthesis, its mechanism in protecting against paracetamol-induced hepatotoxicity in male C3H mice remains obscure. Some compounds have, however, been shown to decrease the extent of cell damage when added to isolated hepatocytes in which covalent binding of the paracetamol reactive metabolite had already occurred [11]. It is possible that cimetidine may similarly affect those cellular processes which occur subsequent to reactive metabolite covalent binding.

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Department of Clinical Pharmacology
Flinders Medical Centre
Bedford Park
Adelaide, South Australia 5042

JOHN O. MINERS
ROGER DREW
DONALD J. BIRKETT

* Address all correspondence to: Dr. J. O. Miners, Department of Clinical Pharmacology, Flinders Medical Centre, Bedford Park, South Australia 5042.

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Table 1. Effect of cimetidine on the pattern of urinary paracetamol metabolites*

Treatment	Mean fractional excretion (% recovered dose)† G§	S	GSH	P	Mean % dose recovered‡
Control	58.9	9.0	18.9	13.2	94.9
(N = 6)	±2.3	±0.7	±1.2	±1.1	±3.3
Cimetidine, 100 mg/kg	59.7	9.9	17.4	13.0	101.4
(N = 6)	±1.9	±0.8	±1.4	±0.9	±4.7
Cimetidine, 200 mg/kg	56.4	10.1	19.4	14.1	95.5
(N = 6)	±3.0	±0.7	±1.7	±1.4	±4.0
Piperonylbutoxide	53.0	13.1	9.9‖	24.0‖	92.1
(N = 6)	±6.2	±3.1	±1.3	±3.0	±3.2

* Paracetamol dose administered to all animals was 200 mg/kg. Details of dosing for treatments shown in Materials and Methods.

† Expressed as percentage of total paracetamol-derived products recovered.

‡ Total paracetamol-derived products recovered expressed as percentage of the dose administered.

§ G = glucuronide, S = sulphate, GSH = glutathione-derived conjugates, P = unchanged paracetamol.

‖ Significantly different to control phase, $P < 0.01$.

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Evidence for the preferential interaction of micellar chlorpromazine with human serum albumin

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Although the interaction of chlorpromazine with human serum albumin has been the subject of several quantitative studies, there is considerable conflict about the stoichiometry and strength of the interaction [1–5], and also an indication [6] that the binding curve is sigmoidal. In an attempt to determine whether micelle formation by chlorpromazine might be a contributing factor to the seemingly diverse binding behaviour, an equilibrium dialysis study of the interaction between human serum albumin and the drug has been performed in acetate–chloride buffer, pH 5.5, I 0.154, conditions operative for the only quantitative characterization of chlorpromazine in terms of micelle size and association equilibrium constant [7]. It is concluded that albumin, like tubulin [8, 9], binds the micellar form of chlorpromazine preferentially to a single site; but that the existence of additional protein sites with weaker affinity for the drug precludes precise quantitative characterization of the system.

Materials and methods

Chlorpromazine hydrochloride was obtained from Sigma Chemical Co. and the human serum albumin (25 mg/ml solution) was kindly donated by the Red Cross Blood Transfusion Service in Brisbane. In order to remove the slight colouration resulting from residual haemoglobin and erythrocytic fragments, the albumin concentrate was further purified by ion-exchange chromatography on carboxymethyl-cellulose (Whatman CM32) pre-equilibrated with 10 mM sodium phosphate buffer, pH 5.5. Prior to binding studies the purified albumin solution, which passed unretarded through the ion-exchange column, was dialysed for 24 hr at 4° against acetate–chloride buffer, pH 5.5 (0.01 M sodium acetate/0.144 M sodium chloride, pH adjusted with acetic acid).

Dialysis sacs (Visking 18/32) containing human serum albumin (5 ml, 10 mg/ml) and chlorpromazine (20 μM–5 mM) in the acetate–chloride buffer were placed in the same buffer (450 ml) containing an identical concentration of chlorpromazine. Dialysis was allowed to proceed at 25° for 36 hr, after which the concentration of chlorpromazine in the diffusate, $[S]_0$, was determined spectrophotometrically on the basis of a molar absorptivity of 4400 $M^{-1}cm^{-1}$ at 300 nm [10]; and the total concentrations of albumin and drug in the inner solution ($[\bar{A}]_i$, $[\bar{S}]_i$) obtained by combining absorbance measurements at 300 nm and 280 nm in the expressions

$$A_{280} = 36{,}300[\bar{A}]_i + 1995[\bar{S}]_i \quad (1a)$$

$$A_{300} = 3940[\bar{A}]_i + 4400[\bar{S}]_i. \quad (2a)$$

The molar absorptivity of human serum albumin at 280 nm (36,300 $M^{-1}\,cm^{-1}$) is based on a molecular weight of 66,000 [11] and an absorption coefficient ($A^{1\%}_{1cm}$) of 5.50 [12]: magnitudes of the molar absorptivities of chlorpromazine at 280 nm and of albumin at 300 nm were deduced from spectral measurements designed to test the additivity of absorbances in albumin–chlorpromazine mixtures at these wavelengths. A value of the Klotz [13] binding function, r, was then determined from the expression $r = ([\bar{S}]_i - [S]_i)/[\bar{A}]_i$, where $[S]_i$, the concentration of free chlorpromazine in the albumin–drug mixture, was obtained from that of the diffusate ($[S]_0$) by allowance [14] for the Donnan redistribution of ions.

Solutions of chlorpromazine (0.05–4.85 mg/ml) in acetate–chloride buffer, pH 5.5, I 0.154, were subjected to frontal gel chromatography [15] on a column (0.9 × 12.5 cm) of Sephadex G-25, pre-equilibrated at 25° with the same buffer. The column effluent, maintained at a flow-rate of 0.75 ml/min, was divided into 1.5 ml fractions which were then assayed spectrophotometrically at 300 nm after appropriate dilution. The weight-average elution volume, V_w, was determined from the centroid [16] of the advancing profile, and converted to the corresponding partition coefficient, σ_w, by the expression $\sigma_w = (V_w - V_0)/(V_t - V_0)$, the void ($V_0$) and total ($V_t$) volumes of the column having been taken as the elution volumes of serum albumin and potassium chromate, respectively.

Results and discussion

Results of equilibrium dialysis experiments with free chlorpromazine concentrations in the range 20 μM–5 mM are presented in Scatchard format in Fig. 1, the most characteristic feature of which is the existence of a minimum in the vicinity of $r = 1$, and presumably, therefore, of a maximum, since the limiting value of $r/[S]_i$ as $[S]_i \rightarrow \infty$ is zero: although not established unequivocally, the present results suggest that this maximum occurs at $r = 12$–15. The general form of the plot resembles that for the interaction of chlorpromazine with brain tubulin [17], a system for which the unusual binding curve reflects the preferential interaction of micellar drug with a single site on the protein acceptor [8, 9]. A similar interpretation of Fig. 1 would be conditional upon chlorpromazine undergoing pronounced micellization at concentrations in the vicinity of 5 mM, a phenomenon for which there is certainly evidence for the drug in 0.154 M NaCl [7, 18]. The micellar characteristics of chlorpromazine under the present conditions (acetate–chloride, pH 5.5, I 0.154) have therefore been examined by gel chromatography on Sephadex G-25 [9].