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Effect of phenobarbitone on the distribution and elimination of imipramine in rats

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The effect of phenobarbitone on the steady state volume of distribution (Vd_{ss}) and the total body blood clearance ($CL_{tot,b}$) of imipramine and the serum concentration of its metabolite, desipramine was examined. The serum disappearance of imipramine after an 8 mg kg^{-1} i.v. dose followed a biexponential decline in both control and phenobarbitone-treated rats while the concentration of its metabolite increased in the phenobarbitone-treated rats then rapidly declined compared with that in control rats. Since $CL_{tot,b}$ was nearly equal to the hepatic blood flow (Q_H), Q_H may be the rate-determining step of imipramine elimination. In the control rats the Vd_{ss} of imipramine was large at 19·9 litre kg^{-1}. In the phenobarbitone-treated rats the pharmacokinetic parameters, biological half-life ($t\frac{1}{2}$) and Vd_{ss} significantly decreased to approximately 23–40% while $CL_{tot,b}$ increased to 126% of those in the control rats, although the latter difference was not statistically significant. The blood-to-plasma concentration ratios (R_B) of imipramine and desipramine decreased in the phenobarbitone-treated rats. The urinary excretion ratios of imipramine and desipramine, to the dose of imipramine over 8 h, were <1·5% in both groups. These ratios were not significantly changed in the phenobarbitone-treated rats. It was concluded that the significant decrease in $t\frac{1}{2}$ of the phenobarbitone-treated rats may not be attributed to the changes in $CL_{tot,b}$ and/or in the urinary excretion, but mainly to the decrease in Vd_{ss}.

The concomitant administration of barbiturates to patients receiving tricyclic antidepressants has been observed to result in a decreased plasma level of antidepressant (Hammer et al 1967; Burrows & Davies 1971; Silverman & Braithwaite 1972; Ballinger et al 1974). Similarly, in normal subjects, a decrease in steady state plasma concentration of nortriptyline was observed on coadministration of additional drugs including barbiturates (Alexanderson et al 1969). Though these phenomena are likely to be due to enzyme induction which produces the enhanced metabolic elimination of antidepressants, the literature is lacking in animal experiments to support this. Also reports concerning the effects of barbiturates on the tissue distribution of weakly basic drugs, including tricyclic antidepressants (Breyer-Pfaff et al 1978), are few. We have examined the effect of continuous phenobarbitone administration on the time-courses of imipramine and its metabolite, desipramine, and on their pharmacokinetic parameters, i.e. biological half-life ($t\frac{1}{2}$), total body blood clearance ($CL_{tot,b}$), volume of distribution (Vd) and urinary excretion ratio, in rats.

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Methods

Imipramine HCl, desipramine HCl and sodium phenobarbitone were kindly supplied by Yoshitomi Pharm. Co., Tokyo, Japan, Ciba Geigy Pharm. Co., and Sankyo Pharm. Co., respectively. [^{14}C]Imipramine (48 mCi mmol^{-1}) was purchased from the Radiochemical Research Centre (Amersham, UK); [^{3}H]-imipramine and [^{3}H]desipramine were purchased from New England Nuclear Co., Boston, Mass. These radioactive compounds were at least 98% pure by TLC. All other reagents were commercial products and of analytical grade.

Adult, male Wistar rats, 200–290 g, were used. They had free access to tap water and standard laboratory chow (CE-2, Clea Japan Inc., Tokyo, Japan) except before i.v. administration of imipramine when they were fasted for 24 h. For the phenobarbitone treatment, the animals received 1 g litre^{-1} in their drinking water for 6 days. Control rats received water. Under light ether anaesthesia, the femoral vein and artery were cannulated with PE-50 polyethylene tubing. The urinary bladder was cannulated with PE260 polyethylene tubing. Cannulated rats were kept in restraining cages with water, under normal housing conditions before experiments. After 3 h for recovery from ether anaesthesia, the rats were given 8 mg kg^{-1} of imipramine containing 40 μCi kg^{-1} of [^{14}C]imipramine in saline through the femoral vein cannula over 30 s. Blood samples (0·25 ml) then were obtained at 1, 2, 5, 10, 15, 30, 60, 120, 180, 240, 360 and 480 min in polyethylene centrifuge tubes. Urine was collected for 8 h. Body temperature was kept at 37 °C using a heat lamp. Serum was separated by

centrifugation for 3 min in a table-top microfuge (Beckman Instrument Co., Fullerton, CA).

Under light ether anaesthesia the carotid artery of the donor rat was cut and the liver was perfused through the inferior vena cava with ice-cold 0·9% NaCl for 2 min. Immediately thereafter, the liver was removed and homogenized in 3 vol of ice-cold 0·9% NaCl in a Teflon-glass homogenizer. The homogenate was centrifuged at 4 °C for 15 min in a refrigerated centrifuge (Hitachi 20PR-5, Hitachi Koki Co. Ltd, Tokyo, Japan) at 9000*g*. The supernatant fraction was then centrifuged at 105 000*g* for 60 min in a Hitachi 65P ultracentrifuge (Hitachi Koki Co. Ltd) at 4 °C. The microsomal pellets were resuspended in 6 ml of ice-cold 5 mM phosphate buffer (pH 7·4) and then centrifuged at 105 000*g* for 30 min. The microsomal pellets were suspended in a volume of 5 mM phosphate buffer (pH 7·4) to make a concentration equivalent to 2 mg of microsomal protein ml^{-1}.

The blood-to-plasma concentrations (R_B) of imipramine and desipramine at 1 h after i.v. administration of 8 mg kg^{-1} of imipramine were determined by the centrifugation method. Blood samples (0·25 ml) were obtained both in heparinized (50 units per tube) and non-heparinized polyethylene tubes. Serum and plasma were separated by centrifugation for 3 min in a table-top microfuge. No significant difference was observed between serum and plasma concentration of imipramine (or desipramine) in either tubes. In-vitro heparin treatment also had no effect. The values of R_B were calculated from the blood and plasma concentrations in heparinized tubes. The blood cell-to-plasma concentration ratio (C_{BC}/C_P) is given by the following equation:

$$C_{BC}/C_P = \{(R_B - 1) + H_t\}/H_t \qquad (1)$$

where H_t is the haematocrit value. Also the plasma total clearance ($CL_{tot,p}$) was converted into $CL_{tot,b}$ by the following equation:

$$CL_{tot,b} = CL_{tot,p}/R_B \qquad (2)$$

The concentrations of imipramine and desipramine in serum, plasma and blood were determined by the modified method of Nagy & Treiber (1973). For each analysis, a constant amount of [^{3}H]imipramine (20 000 d min^{-1}), and [^{3}H]desipramine (20 000 d min^{-1}) as the internal standard, and 2 ml of 0·1 M NaOH were added to all samples (0·1 ml) in glass stoppered tubes, then 4 ml of n-heptane containing 3% of isoamylalcohol was added before extraction. The tube was shaken for 10 min in a mechanical shaker at approximately 300 min^{-1}, and then centrifuged at 3000 rev min^{-1} (1300*g*) for 10 min. Emulsions were broken by placing the tube on a dry ice-acetone mixture solution followed by thawing and recentrifugation. The organic phase was transferred into another glass-stoppered tube and the extraction procedure was repeated once. The combined organic phase (7 ml) was shaken with 3 ml of 0·1 M NaOH for 10 min and centrifuged. The organic phase (6 ml) was then evaporated under vacuum to a dry material at room temperature (20 °C). The dry material was dissolved in 120 μl of ethanol solution containing non-labelled imipramine and desipramine (each 10 μg ml^{-1}) and 0·1 ml was applied to a TLC plate (E. Merck, Darmstadt, West Germany) precoated with silica gel 60 F 254. The plate was developed with a chloroform–ether–n-propanol–ammonium hydroxide (25–28%) (85 : 15 : 20 : 1 v/v) solvent system by ascending technique in a dark room. After the solvent front had reached 13 cm from the base line, the plate was dried in a cold air stream and the separated imipramine and desipramine zones were visualized under an uv lamp (λ = 254 nm). The spots attributable to imipramine and desipramine were scraped into scintillation vials containing 1 ml of ethanol and 10 ml of scintillation cocktail (0·1 g of POPOP, 4·0 g of DPO and 500 ml of Triton X-100 $litre^{-1}$ of toluene). The R_F values of imipramine and desipramine were 0·73 and 0·29, respectively. The concentrations of [^{14}C]imipramine and desipramine and [^{3}H]imipramine and desipramine were determined in a Packard Tri-Carb counter (Packard Instruments Corp., Downers Grove, IL). The precision of the method, evaluated by estimation of 5 serum samples with varying concentrations from 0·83–3·3 μg ml^{-1} of imipramine and desipramine, was 5% for both drugs. The lower limit of sensitivity was approximately 5 ng ml^{-1} for both drugs, if 0·1 ml of serum was used for the extraction. The serum concentration of phenobarbitone was determined spectrophotometrically (Lin et al 1973). The amount of cytochrome P-450 was determined by the method of Omura & Sato (1964a, b). The protein concentration was determined by burette method using bovine serum albumin as the standard (Gornall et al 1949). The imipramine concentration data for individual animals were fitted to the equation $C_t = Ae^{-\alpha t} + Be^{-\beta t}$ for the serum concentration C_t at time t by non-linear least squares regression using the 'SALS' program (Nakagawa et al 1978). Pharmacokinetic constants were determined from the two-exponential equation constants, i.e., A, α, B and β, using conventional equations (Gibaldi & Perrier 1975). All means are presented with the standard error (the mean ± s.e.). Student's *t*-test was used to determine significant difference between the control and phenobarbitone-treated groups.

Results and discussion

As shown in Table 1, the liver wet weight per unit body weight significantly increased in phenobarbitone-treated rats, while the kidney wet weight was unchanged. The content of cytochrome P-450 significantly increased from 0·903 ± 0·025 nmol (mg protein)$^{-1}$ (n = 5) in the control rats to 1·65 ± 0·053 (n = 5) ($P < 0·05$). This finding is in agreement with others (Ohnhaus et al 1971; Ohnhaus & Locher 1975; Yates et al 1978). The serum level of phenobarbitone was below 1 μg ml^{-1}, which is less than the sensitivity of the method of Lin et al (1973).

Table 1. Imipramine pharmacokinetic parameters in control and phenobarbitone-treated rats.[a,b]

	Control	Treated
Body weight (g)	230 ± 2·0	250 ± 25·2
Liver weight (g kg^{-1})	35·9 ± 0·7	48·1 ± 0·9[c]
Kidney weight (g kg^{-1})	8·26 ± 0·14	8·78 ± 0·22
V_1 (litre kg^{-1})[d]	5·55 ± 1·20	2·00 ± 0·50[c]
V_2 (litre kg^{-1})[d]	14·4 ± 0·39	2·71 ± 0·50[c]
Vd_{ss} (litre kg^{-1})[d]	19·9 ± 4·95	4·70 ± 0·99[c]
Vd (litre kg^{-1})[d]	21·9 ± 5·20	5·48 ± 1·22[c]
$CL_{tot,b}$ (ml min^{-1} kg^{-1})[e]	55·4 ± 4·22	69·7 ± 4·15
$t\frac{1}{2}$ (min)	173 ± 15·7	66·8 ± 7·09[b,c]
Urinary excretion ratio of imipramine[f]	1·20 ± 0·28	0·47 ± 0·04
Urinary excretion ratio of desipramine[f]	0·53 ± 0·13	0·27 ± 0·02

[a] Dose: 8 mg kg^{-1}.
[b] Results are mean ± s.e. of four control and three phenobarbitone-treated rats.
[c] Significantly different ($P < 0·05$) from the control rats.
[d] The volume of distribution was calculated by conventional equations (Gibaldi & Perrier 1975) using two exponential equation constants from serum disappearance curves.
[e] The total body blood clearance ($CL_{tot,b}$) was calculated by the equation: $CL_{tot,b}$ = Dose $(AUC_p\ R_B)^{-1}$, where R_B is the blood-to-plasma concentration.
[f] Relative to 8 h excretion of imipramine dose as %.

The serum disappearance of impramine and desipramine after intravenous administration of 8 mg kg^{-1} of imipramine in the control and phenobarbitone-treated rats is shown in Fig. 1 and it is seen that the disappearance of imipramine followed biexponential curves in both the control and treated rats. In the latter there was an increase in the serum concentration of imipramine at the distribution phase (α-phase) and a decrease in those of both imipramine and desipramine at the elimination phase (β-phase) (Fig. 1, panel a). The pharmacokinetic constants computed are listed in Table 1. In the control rats, the Vd_{ss} and $CL_{tot,b}$ were large at 19·9 litre kg^{-1} and 55·4 ml min^{-1} kg^{-1}, respectively. In the phenobarbitone-treated rats, a significant decrease was observed in $t\frac{1}{2}$ and the volumes of distribution, i.e. Vd, V_1, V_2 and Vd_{ss}. The $CL_{tot,b}$ increased to 126% of that of the control rats, although the difference was not statistically significant. The serum concentration of desipramine after i.v. administration of 8 mg kg^{-1} of imipramine increased and rapidly declined compared with that of the control rats.

The biological half-life ($t\frac{1}{2}$) can be expressed by:

$$t\tfrac{1}{2} = \ln 2\ Vd_{\beta}/CL_{tot,b} \quad (3)$$

The values of hepatic blood flow (Q_H) for adult, male Wistar rats were reported to be 52 ml min^{-1} kg^{-1} in the control rats and 69 ml min^{-1} kg^{-1} in the phenobarbitone-treated rats (Yates et al 1978). These values were nearly equal to the $CL_{tot,b}$ of imipramine, i.e. 55 ml min^{-1} kg^{-1} in the control rats and 70 ml min^{-1} kg^{-1} in the phenobarbitone-treated rats (Table 1). Moreover, using the single pass rat liver perfusion model, Erlandsen & Gram (1982) have shown imipramine to be eliminated by hepatic metabolism with a high extraction ratio (E_H = 0·93 at the inflow concentration of 10^{-5} M). These findings suggest that Q_H is the rate-determining step of imipramine elimination. Thus, equation 3 can be

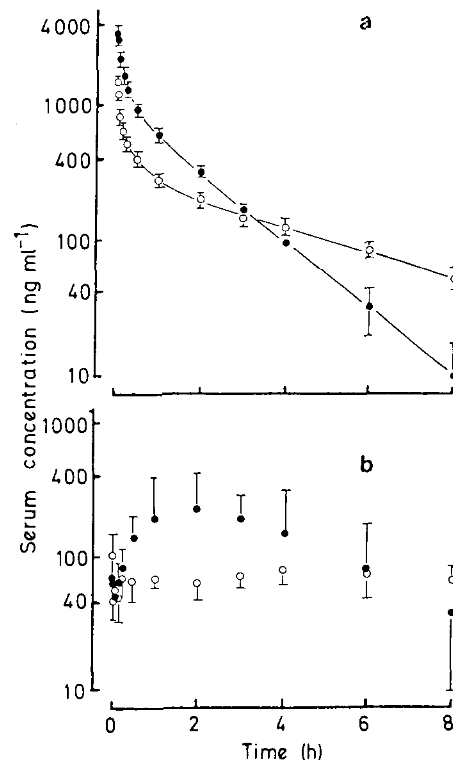

Fig. 1. Serum disappearance curves of imipramine (panel a) and desipramine (panel b) after i.v. administration of 8 mg kg^{-1} of imipramine. Each point and vertical bar represent the mean and s.e. of four control and three phenobarbitone-treated rats. Curves were calculated by the SALS method (Nakagawa et al 1978) using a digital computer. Key: (○) control; and (●) phenobarbitone-treated rats.

rearranged as follows:

$$t\tfrac{1}{2} = \ln 2\ Vd_{\beta}/Q_H \quad (4)$$

Consequently, the decreases in $t\frac{1}{2}$ of the phenobarbitone-treated rats may not be due to the increase in Q_H, but mainly to the decrease in Vd. The effect of phenobarbitone on the plasma elimination of propranolol in dogs was investigated by Vu et al (1983) who found that chronic barbiturate treatment caused significant decreases in both $t\frac{1}{2}$ and Vd of propranolol after i.v. and oral doses, while little change was observed in Q_H and the systemic clearance, a phenomenon similar to the pharmacokinetic behaviour of imipramine found by us. Breyer-Pfaff et al (1978) reported that oral pretreatment with phenobarbitone for 5 days slightly enhanced the elimination of desipramine and decreased its brain-to-plasma, kidney-to-plasma and liver-to-plasma concentration ratios. This finding suggests that the decrease in Vd of imipramine (Table 1) may be due to the decrease in its distribution to each tissue or organ.

Table 2 demonstrated the changes in the distribution of imipramine and desipramine to blood cells at 1 h after

Table 2. Effect of phenobarbitone on the distribution of imipramine and desipramine to blood cells in rat.[a]

	Imipramine[b]		Desipramine[b]	
	Control	Treated	Control	Treated
R_B[c]	1·67 ± 0·10	0·90 ± 0·07[d]	1·76 ± 0·45	0·77 ± 0·07[e]
C_{BC}/C_p[f]	2·60 ± 0·26	0·77 ± 0·18[d]	2·81 ± 1·06	0·44 ± 0·17[e]

[a] Results are given as the mean ± s.e. of five control and four phenobarbitone-treated rats.
[b] Values of imipramine and desipramine at 1 h after i.v. administration of 8 mg kg^{-1} of imipramine.
[c] Blood-to-plasma concentration ratio.
[d] Significantly different ($P < 0·05$) from the control rats.
[e] Significantly different ($P < 0·10$) from the control rats.
[f] Calculated by equation 1 in text.

i.v. administration of 8 mg kg^{-1} of imipramine. The values of R_B and C_{BC}/C_p ratios significantly decreased in phenobarbitone-treated rats.

In-vitro studies by Brinkschulte & Breyer-Pfaff (1982) demonstrated that α_1-acid glycoprotein (α_1-AGP) isolated from plasma of phenobarbitone-treated rats bound more strongly to desipramine than did that from the control rats, and that the former contained a higher percentage of *N*-acetylneuraminic acid than the latter. Recently, Vu et al (1983) reported that after chronic phenobarbitone-administration to dogs, the plasma unbound fraction (fu) of propranolol fell from 0·152 to 0·024 and that the increased plasma protein binding was highly correlated with the elevated concentration of non-precipitable glycoproteins, suggesting that α_1-AGP is the primary binding protein for propranolol in dog serum (Bai & Abramson 1982; Abramson et al 1983). As preliminary evidence, we reported an increase in the serum concentration of α_1-AGP in phenobarbitone-treated rats (Lin et al 1983, 1984; Sugiyama et al 1985). In the present study, the enhanced binding of imipramine to α_1-AGP may be one of the factors responsible for the decrease in both Vd and C_{BC}/C_p in the phenobarbitone-treated rats.

From Table 1, the urinary excretion ratios of imipramine and desipramine to dose after i.v. administration of imipramine were <1·5% of dose administered in both the control and phenobarbitone-treated rats thereby showing that the urinary excretion of imipramine is not a main step of the elimination process. These ratios were not significantly altered by phenobarbitone treatment.

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