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Effect of pregnancy or renal dysfunction on differences in plasma protein binding of phenytoin in peripheral blood and blood exiting the liver of rats

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The in vivo activity of hepatic drug metabolizing enzyme systems is reflected by the hepatic intrinsic metabolic clearance of drugs. The intrinsic hepatic clearance is determined on the basis of a drug's total hepatic clearance as represented by the concentration changes (referable to the liver) of free plus protein-bound drug in blood, the fraction of that concentration which is not bound to proteins, and the hepatic blood perfusion rate (Wilkinson and Shand, 1975). If a substantial portion of drug in the blood is contained in or on the erythrocytes, the rate of release of drug from erythrocytes to plasma relative to the residence time of the blood in the liver may also have to be taken into consideration.

It is customary, particularly in the case of relatively slowly cleared drugs, to determine the plasma or serum (rather than whole blood) clearance of a drug, either following administration of a bolus dose or at steady-state during constant rate infusion, by obtaining blood samples from a peripheral vein for measurement of drug concentrations and free fraction (protein binding) in plasma or serum. However, the plasma protein binding of drugs is often affected by the presence of endogenous binding inhibitors in the plasma, for example during pregnancy (Stock et al., 1980) and renal dysfunction (Craig et al., 1976; Sjöholm et al., 1976), and it is possible that the concentration of these inhibitors, and therefore the protein binding of a drug, may be different in peripheral and hepatic blood. This can be so because the liver may extract some endogenous binding displacers from the circulation, an example being the substantial hepatic extraction of endogenous non-esterified fatty acids (Chou et al., 1983), and/or because the liver may form endogenous displacers

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which then enter the blood. These processes would result in a gradient of displacer activity, and consequently of a drug's free fraction in plasma, across the liver. Under these conditions, intrinsic hepatic clearance calculations based on the drug's free fraction value in peripheral plasma would be incorrect.

The change in plasma or serum protein binding of phenytoin across the liver has been estimated in normal rats, in rats in late pregnancy, and in rats with experimental renal dysfunction by comparing phenytoin-free fractions in plasma or serum of blood taken at the same time from a peripheral and the hepatic vein. Phenytoin is particularly suitable for this purpose because it is relatively extensively protein bound in plasma and because its free fraction value is essentially independent of concentration over a wide range (Stock et al., 1980). Female Sprague–Dawley rats, non-pregnant ($\approx$ 200 g), 20 days pregnant (normal gestation time 21–22 days), or with renal dysfunction induced by an i.v. injection of uranyl nitrate (Giacomini et al., 1981), 2 or 5 mg/kg five days earlier, were used in the study of transhepatic protein binding changes. Simultaneous blood samples were obtained from the hepatic vein and from the femoral vein as previously described (Chou et al., 1983; Yokota et al., 1976). The blood was centrifuged immediately and permitted to clot for 2 or 3 h at 4°C. The serum was then separated and immediately dialyzed for 6 h at 37°C against an equal volume of pH 7.4 isotonic sodium and potassium phosphate buffer containing [^{14}C]phenytoin, 0.5 μg/ml, and non-radioactive phenytoin, 8 μg/ml. Previous studies had shown that diffusion equilibrium is attained under these conditions (Chou and Levy, 1981). Aliquots of the serum and buffer phases were added to scintillation fluid (Ultrafluor, National Diagnostics, Somerville, NY) and counted in a scintillation spectrometer with external quench correction. In another experiment, the blood was collected in plastic syringes containing sodium edetate, 2 mg/ml blood, plasma was separated immediately, phenytoin was added to produce a concentration of 25 μg/ml, 1 ml was ultrafiltered for 45 min (Chou and Levy, 1982), and plasma and filtrate was assayed by high-performance liquid chromatography (Chou and Levy, 1981). Preliminary experiments showed that phenytoin binding to the ultrafiltration membrane was not detectable.

In vivo and in vitro (by equilibrium dialysis) phenytoin-free fraction values in serum were compared in a separate experiment on female Lewis rats with uranyl nitrate-induced renal dysfunction. The animals received an i.v. injection of phenytoin, 2–8 mg/kg. Blood (for serum) from the aorta and cerebrospinal fluid (CSF) were obtained (Chou and Levy, 1981) 15 min later and were assayed by gas chromatography (Joern, 1981). The in vivo free fraction was determined as the ratio of phenytoin concentrations, CSF/serum. The in vitro free fraction was determined by equilibrium dialysis, but without addition of phenytoin to the buffer phase.

Results of the studies on non-pregnant (normal) and pregnant rats are summarized in Table 1. With respect to the serum-free fraction values determined by equilibrium dialysis, the results of the present study show that protein binding of phenytoin is consistently less in hepatic vein than in femoral vein serum. This suggests hepatic extraction of displacer(s) from the blood although contributions by the lungs and other interposed tissues cannot be ruled out. It was shown in an earlier

TABLE 1

PHENYTOIN SERUM OR PLASMA PROTEIN BINDING CHANGES ACROSS THE LIVER IN PREGNANT AND NON-PREGNANT SPRAGUE-DAWLEY RATS

Rats [a]	Number of animals	Method [b]	Free fraction of phenytoin		Free fraction ratio (hepatic/femoral)
			Hepatic vein	Femoral vein	
Non-pregnant	11	Equilibrium dialysis (serum)	0.119 ± 0.008	0.131 ± 0.008 [c]	0.919 ± 0.088
Pregnant	11	Equilibrium dialysis (serum)	0.267 ± 0.049	0.299 ± 0.037 [c]	0.887 ± 0.097
Non-pregnant	6	Ultrafiltration (plasma)	0.123 ± 0.012	0.136 ± 0.014	0.912 ± 0.113
Pregnant	6	Ultrafiltration (plasma)	0.181 ± 0.021	0.218 ± 0.021 [c]	0.832 ± 0.108

[a] The pregnant rats were studied on the 20th day of gestation.

[b] Serum was dialyzed at 37°C against an equal volume of a buffer solution of phenytoin, 8.5 μg/ml. Plasma containing phenytoin, 25 μg/ml, was ultrafiltered at 37°C.

[c] Significantly different from free fraction in hepatic vein serum or plasma ($P < 0.02$ by paired t-test).

TABLE 2

PHENYTOIN SERUM PROTEIN BINDING CHANGES ACROSS THE LIVER IN FEMALE SPRAGUE-DAWLEY RATS WITH AND WITHOUT EXPERIMENTAL RENAL DYSFUNCTION

Rats [a]	Number of animals	Free fraction of phenytoin [b]		Free fraction ratio, (hepatic/femoral)	Femoral vein conc. (mg/100 ml)	
		Hepatic vein	Femoral vein		Creatinine	Urea nitrogen
Normal	10	0.120 ± 0.015	0.134 ± 0.018 [c]	0.896 ± 0.094	0.99 ± 0.23	16.7 ± 2.6
Renal dysfunction	10	0.303 ± 0.021	0.318 ± 0.030 [c]	0.953 ± 0.057	8.86 ± 2.72	272 ± 92

[a] Renal dysfunction was induced by injection of uranyl nitrate, 5 mg/kg, 5 days before the experiment.

[b] Determined by equilibrium dialysis at 37°C against an equal volume of a buffer solution of phenytoin, 8.5 μg/ml.

[c] Significantly different from free fraction in hepatic vein serum ($P < 0.05$ by paired t-test).

study that the in vivo and in vitro phenytoin-free fraction values (the latter determined by equilibrium dialysis) are essentially identical in normal rats (Chou and Levy, 1981). However, it has recently been established that phenytoin-free fraction values obtained by equilibrium dialysis of plasma from *pregnant* rats are artifactually increased by in vitro lipolysis (Chou and Levy, 1982, 1984). The experiment was therefore repeated but the free fraction values were determined by immediate ultrafiltration of plasma, a procedure that minimizes lipolysis and yields results that are in reasonable agreement with in vivo free fraction values (Chou and Levy, 1982, 1984). The results obtained with plasma from non-pregnant rats are almost identical to those obtained by equilibrium dialysis of serum. The free fraction values determined by ultrafiltration of plasma from pregnant rats yielded lower free fraction values than those determined by equilibrium dialysis of serum, as expected. However, the free fraction values were again lower in hepatic vein plasma than in femoral vein plasma, consistent with hepatic extraction of displacer activity. The relative increase in serum or plasma protein binding of phenytoin across the liver and other interposed tissues tended to be more pronounced in the pregnant animals (who exhibited less phenytoin binding than the controls) as compared to the controls, but the difference between the two groups of animals is not statistically significant. It was previously established, and confirmed in some animals in the present study, that there is no measurable difference in total protein and albumin concentrations between femoral vein and hepatic vein serum or plasma (Chou et al., 1983).

The results of the study of rats with experimental renal dysfunction are summarized in Table 2. The serum protein binding of phenytoin was less extensive in these animals than in normal controls. Both groups showed a small, but statistically significant increase in protein binding (lower free fraction values) in hepatic vein as compared to femoral vein serum. Unlike serum from pregnant rats, serum from rats

TABLE 3

COMPARISON OF IN VIVO AND IN VITRO PHENYTOIN-FREE FRACTION VALUES IN SERUM OF FEMALE LEWIS RATS WITH EXPERIMENTAL RENAL DYSFUNCTION [a]

Variable	Results
No. of animals	22
Total phenytoin serum conc. (μg/ml)	0.675 to 3.68
Creatinine serum conc. (mg/100 ml)	5.83 ± 2.00 (n = 16)
Urea nitrogen serum conc. (mg/100 ml)	137 ± 37 (n = 16)
Phenytoin-free fraction in serum	
in vitro (equilibrium dialysis [b])	0.241 ± 0.048
in vivo (CSF/serum conc. ratio)	0.235 ± 0.030
Ratio of phenytoin-free fraction estimates (in vitro/in vivo)	1.04 ± 0.21

[a] Female Lewis rats received an i.v. injection of phenytoin, 2–8 mg/kg, CSF and blood samples were obtained 15 min later. Renal dysfunction was produced by an injection of uranyl nitrate, 2 or 5 mg/kg, 5 days earlier.

[b] At 37°C for 6 h.

with experimental renal dysfunction does not undergo binding alterations with respect to phenytoin during equilibrium dialysis. This is shown in Table 3 by the excellent agreement between the phenytoin-free fraction values obtained in vivo and in vitro (by equilibrium dialysis).

This investigation has shown that the protein binding of phenytoin is consistently less extensive in serum or plasma from peripheral blood than in serum or plasma from blood exiting from the liver. Since albumin concentrations in these fluids are independent of sampling site (Chou et al., 1983), the results are consistent with hepatic extraction of endogenous displacers and consequent transhepatic alterations of drug–protein binding in plasma. While the magnitude of these protein binding changes is small in the case of phenytoin under the experimental conditions of this investigation, more pronounced effects may be encountered with other drugs, other species, and in different diseases. These considerations apply not only to endogenous displacing agents but also to drug interactions involving alterations in plasma protein binding, particularly if one of the interactants is subject to extensive hepatic extraction.

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