Increase in the Plasma Protein Binding of Weakly Basic Drugs in Carbon Tetrachloride-Intoxicated Rats

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Plasma protein binding of weakly basic drugs such as propranolol and quinidine was determined in rats with carbon tetrachloride (CCl₄)-induced hepatic disease. Free fractions of propranolol and quinidine in the plasma of rats at 24 h after CCl₄-intoxication were decreased by 41 and 30%, respectively, compared to those of control rats. An addition of Tris (butoxyethyl) phosphate (TBEP), a specific displacer for basic drugs from α_1 -acid glycoprotein (AGP), to the plasma increased the free fractions of the basic drugs, resulting in no difference in the extent of the plasma free fraction of each drug between control and CCl₄-intoxicated rats.

Plasma concentration of AGP in CCl₄-intoxicated rats was elevated 2.7-fold of that in control rats at 24 h after the CCl₄ intoxication and reached a peak of 4.8-fold elevation at 48 h. A regression analysis revealed a high degree of positive correlation between ratios of bound to free fraction of propranolol and plasma concentrations of AGP. These results suggest that the plasma protein binding of the basic drugs was increased mainly due to the rise in the plasma AGP concentration in CCl₄-intoxicated rats.

Keywords plasma protein binding; α_1 -acid glycoprotein; hepatic disease; carbon tetrachloride; weakly basic drug

Introduction

Protein binding in the blood is an important determinant of modified drug pharmacokinetics in the disease state. In patients with hepatic diseases, the binding of many acidic drugs to plasma proteins is frequently decreased.¹⁾ The decreased plasma protein binding of drug has been related to either the reduction of concentrations of plasma proteins such as albumin and lipoproteins or the binding competition between acidic drugs and endogenous substances such as bilirubin and free fatty acids on binding sites of those plasma proteins.²⁾ In experimental model animals with D-galactosamine-induced hepatic disease, a reduction of albumin concentration and the resultant decrease in the binding of phenytoin to the plasma protein have been observed.³⁾

On the other hand, the plasma protein binding of basic drugs in patients with hepatic diseases is not conclusive despite many clinical reports.⁴⁾ Basic drugs associate with α_1 -acid glycoprotein (AGP) as well as with albumin and lipoproteins.⁵⁾ The plasma level of AGP has been reported to fluctuate even under ill conditions of the liver^{4,6)} in comparison with concentrations of albumin and lipoproteins which are mostly in the direction of reduction in patients with hepatic diseases.^{2a,b)} Therefore, AGP is considered to play a more important role than albumin and lipoproteins in the variability of the plasma binding of basic drugs in such patients.^{4,6)} However, little information on the change of the plasma protein binding of basic drugs in relation to the change of the concentrations of plasma proteins under the hepatic disease state is available.

This study was undertaken to examine the effect of experimental liver disease caused by carbon tetrachloride (CCl₄)-intoxication on the plasma protein binding of commonly used basic drugs such as propranolol and quinidine, and on the plasma concentration of AGP in rats. The plasma binding of salicylic acid, a model acidic drug, was also examined.

Materials and Methods

Chemicals *dl*-[³H]Propranolol (27 Ci/mmol) and [¹⁴C]salicylic acid (56 mCi/mmol) were purchased from Dupon/NEN Research Products.

Unlabeled *dl*-propranolol-HCl, quinidine sulfate and sodium salicylic acid were purchased from Wako Pure Chemical Co., Ltd. (Osaka, Japan). All other chemicals used were of reagent grade.

Animals Male Wistar rats weighing 250–300 g on a normal laboratory diet were used. CCl₄ was dissolved in olive oil (1:4, v/v). The mixture was intraperitoneally administered to rats at a dose of 0.5 ml per 100 g body weight. Control rats received an injection of olive oil alone. Blood was collected by a heparinized-syringe from a venae cava inferior of rats under ethyl ether anesthesia. The blood samples were centrifuged at 10000 rpm to obtain plasma.

Binding Experiments The extent of the binding of drug to plasma proteins was determined through an ultrafiltration procedure. 0.9 ml of plasma were mixed with 0.1 ml of 1.0 M phosphate buffer, pH 7.4 containing a drug. The final concentration of drug in the plasma sample was 1 μ g/ml. In the case of the competitive binding study with Tris (butoxyethyl) phosphate (TBEP), 5 μ l of an ethanol solution of TBEP was added to each milliliter of plasma sample to give a final TBEP concentration of 200 μ g/ml. After being incubated for 10 min at room temperature (20–23 °C), an aliquote (50 μ l) was removed from each plasma compartment by filtration through Molcut II LGC (Millipore-Japan) of molecular sieve 10000. The amount of drug in the filtrate was assessed by measuring either radioactivity for propranolol and salicylic acid or intensity of fluorescence for quinidine. The ratio of the free fraction of drug in plasma was calculated as follows:

$$F = C_f / C_p$$

Where C_f = a drug concentration in filtrate

C_p = a drug concentration in plasma

Analytical Method AGP was purified from rat serum collected at 48 h after a turpentine injection according to the procedure described by Urban *et al.*⁷⁾ The amount of AGP in the plasma was measured by a single radial immunodiffusion analysis with purified AGP as a standard.⁸⁾ The protein assay measured the amount of AGP within the range of 500 ng to 5 μ g in each test hole. Lipoproteins were isolated from plasma by adding potassium bromide and centrifuging at 105000 *g* for 48 h.⁹⁾ The amount of protein was measured by the method of Lowry *et al.*¹⁰⁾ The albumin concentration was determined with an Albumin B-Test kit (Wako Pure Chemicals Co., Ltd.). Glutamic pyruvic transaminase (GPT) concentration was determined with a Shino Test kit (Shino Test Co., Ltd., Tokyo, Japan).

Data Analysis Statistical analysis was performed with a Student's *t* test. Values are presented as the means $\pm$ S.E.

Results

In Vitro Plasma Protein Binding of Propranolol, Quinidine, and Salicylic Acid The plasma protein binding of two basic drugs (propranolol and quinidine) and one acidic drug (salicylic acid) was determined in control and CCl₄-intoxicated rats 24 h after the intraperitoneal injection of

Fig. 1. Binding of Various Drugs to the Plasma Proteins from the Control and CCl₄-Intoxicated Rats

Animals were sacrificed 24 h after the injection. Plasma samples containing each drug at a concentration of 1 µg/ml were ultrafiltered. Plasma free fractions of the drug were expressed as ratios of the amount of drug in the filtrate to the total amount of drug. Values are the means ± S.E. of six to nine animals. Significantly different from control; ^{a)} $p < 0.01$. QN, quinidine; PPL, propranolol; SA, salicylic acid. □, control; ▨, CCl₄.

Fig. 2. Inhibitory Effect of TBEP on the Binding of Various Drugs to the Plasma Proteins from the Control and CCl₄-Intoxicated Rats

The same plasma samples as those used in Fig. 1 were subjected to the experiments. Plasma samples containing each drug and TBEP (200 µg/ml) were ultrafiltered. Other experimental conditions were the same as those in Fig. 1. Values are the means ± S.E. of six to nine animals. Significantly different from control; ^{a)} $p < 0.05$. QN, quinidine; PPL, propranolol; SA, salicylic acid. □, control; ▨, CCl₄.

olive oil and CCl₄-olive oil mixture, respectively. Plasma samples containing each drug at a concentration of 1 µg/ml were ultrafiltered and the amounts of drug in the filtrates were measured (Fig. 1). The plasma free fraction of salicylic acid in the CCl₄-intoxicated group was increased by 71% compared to that in the control group. In contrast, plasma free fractions of propranolol and quinidine in the intoxicated group were decreased by 41 and 30% compared to those in the control group, respectively. The binding experiment was also performed in the presence of TBEP (200 µg/ml) which is known to displace several basic drugs from AGP.¹¹⁾ The results are shown in Fig. 2. By an addition of TBEP to the plasma samples, plasma free fractions of propranolol and quinidine in the CCl₄-intoxicated group increased more than those in the control group. As a consequence, the difference in the plasma protein binding of the two basic drugs between the control and CCl₄-intoxicated groups was

TABLE I. The Plasma Concentrations of Total Proteins, Albumin, Lipoproteins and AGP in Control and CCl₄-Intoxicated Rats

Constituent	Concentration (mg/ml plasma)	
	Control	CCl ₄
Total proteins	73.2 ± 1.34	70.2 ± 1.13
Albumin	41.7 ± 0.07	40.6 ± 0.07
Lipoproteins	1.21 ± 0.073	0.62 ± 0.042 ^{a)}
AGP	0.168 ± 0.012	0.460 ± 0.025 ^{a)}

Animals were sacrificed 24 h after the injection. Values are the means ± S.E. of six to nine animals. Significantly different from control; ^{a)} $p < 0.001$.

Fig. 3. Changes in the Plasma Concentrations of AGP and GPT in the Rat after the CCl₄-Intoxication

Animals were sacrificed at definite times after the injection. Values are the means ± S.E. of six to eight animals. The AGP level: ○, control group; ●, CCl₄ group. The GPT levels (▲) were expressed as ratios of the plasma concentrations in the CCl₄-intoxicated rats to those in normal rats.

Fig. 4. Relationship between the Ratios of Bound to Free Fraction of Propranolol and the Concentration of AGP in the Plasma from Rats

Animals were sacrificed at various intervals after the injection. ○, control group; ●, CCl₄ group. Regression line $y = 13.95x + 0.415$; $r = 0.978$; $p < 0.001$.

completely cancelled. On the other hand, the difference of the plasma protein binding of salicylic acid between the two groups remained in the same degree even after the TBEP treatment. These results suggest that the increased plasma protein binding of the basic drugs in CCl₄-intoxicated rats was mainly due to the increase in the plasma AGP level.

Plasma Concentrations of Total Protein, Albumin, Lipoproteins and AGP Table I shows the concentrations of total protein, albumin, lipoproteins and AGP in the plasma from control and CCl₄-intoxicated rats 24 h after an injection.

The concentrations of total protein and albumin were not significantly altered by the CCl₄-intoxication. The concentration of lipoproteins in the intoxicated group was lowered by half compared to the control value. On the other hand, there was a marked elevation in AGP concentration in the intoxicated group. The mean values of AGP concentration were 168 µg/ml in the control group and 460 µg/ml in the intoxicated group, representing, a 2.7 fold increase in the level of the glycoprotein in the latter group. Following the CCl₄-intoxication, the AGP concentration reached its peak of 4.8 fold higher than the control value at 48 h and then declined gradually toward the control level over the following 72 h (Fig. 3). The level of GPT reached a maximum after 24 h and then fell down.

To assess the effect of the change in AGP concentration on the plasma protein binding of the basic drugs, the binding of propranolol to the plasma protein was determined simultaneously with the measurement of the plasma AGP concentration in all the experimental animals. The ratios of bound to free fraction of propranolol were plotted *versus* the AGP concentration (Fig. 4). These plots showed a good linear line with a correlation coefficient of $r = 0.978$.

Discussion

In this study we examined the plasma protein binding of two commonly used basic drugs such as propranolol and quinidine, and one acidic drug, salicylic acid in experimental hepatic disease rats caused by CCl₄-intoxication. The plasma protein binding of salicylic acid was decreased in CCl₄-intoxicated rats, which is consistent with the results obtained in patients with hepatic diseases.¹⁾ The decrease in plasma protein binding of the acidic drug seems to be due to the appearance of endogenous binding inhibitors.²⁾ In contrast to the acidic drug, the plasma protein binding of propranolol and quinidine was markedly increased in CCl₄-intoxicated rats. The competitive binding study with TBEP was performed since the ligand can selectively displace basic drugs from binding sites on AGP.¹¹⁾ The difference in the plasma protein binding of the basic drugs between control and intoxicated rats was completely abolished by an addition of TBEP into the plasma. This result suggested that the increased plasma protein binding of the basic drugs was largely due to an elevation in the plasma AGP level in CCl₄-intoxicated rats. In fact, the rise in the plasma AGP concentration following the CCl₄ intoxication was demonstrated by using an immunological method (Table I). Goldberg *et al.* reported previously an acute-phase response in hepatic disease rats which were caused by oral intubation of CCl₄.¹²⁾ However, the plasma protein binding of the drug was not referred to in their paper. We found a high degree of correlation between the

ratios of bound to free fraction of propranolol and the AGP concentration (Fig. 4). This finding further confirmed AGP as a major determinant of the plasma protein binding of basic drugs in rats with CCl₄-induced hepatic disease. It has been recognized that the increase in AGP level occurs in pathological states associated with inflammatory disease.¹³⁾ It is known that CCl₄-intoxication causes acute necrosis of parenchymal cells in the liver and the lesion is followed by infiltration of reticuloendothelial cells.¹⁴⁾ Hence, the rise in plasma AGP level in CCl₄-intoxicated rats seems to be a reflection of changes in the immunoregulatory system. Hepatic diseases in humans are likely caused by many factors which are different from CCl₄-intoxication. The present study at least showed the possibility that liver cells produce AGP despite severe damage.

References

- 1) W. D. Hooper, F. Bochner, M. J. Eadie and J. H. Tyher, *Clin. Pharmacol. Ther.*, **15**, 276 (1973); T. F. Blaschke, P. J. Mettin, K. L. Melmon and M. Rowland, *ibid.*, **17**, 685 (1975); M. Perez-Mateo and S. Erill, *Eur. J. Clin. Pharmacol.*, **11**, 225 (1977).
- 2) a) T. F. Blaschke, *Clin. Pharmacokinet.*, **2**, 32 (1977); b) J. J. Thiessen, E. M. Sellers, P. Denbeigh and L. Dolman, *J. Clin. Pharmacol.*, **16**, 345 (1976); c) R. Gugler, D. W. Shoeman and D. L. Azarnoff, *Pharmacology*, **12**, 160 (1974); d) K. M. Kessler, R. C. Leech and J. F. Spann, *Clin. Pharmacol. Ther.*, **25**, 204 (1979).
- 3) T. Itoh, Y. Sawada, T. H. Lin, T. Iga and M. Hanano, *J. Pharmacobio-Dyn.*, **11**, 289 (1988).
- 4) a) K. M. Pfafsky, O. Borga, I. Odar-Cederlof, C. Johansson and F. Sjoqvist, *N. Engl. J. Med.*, **299**, 1435 (1978); b) K. M. Pfafsky, *Clin. Pharmacokinet.*, **5**, 246 (1980); c) C. Aguirre, R. Caloo and J. M. Rodriguez, *Int. J. Clin. Pharmacol. Ther. Toxicol.*, **26**, 566 (1988).
- 5) O. G. Nilsen, P. Leren, I. Aakesson and S. Jacobsen, *Biochem. Pharmacol.*, **27**, 871 (1977); E. Pike, P. Kierulf, B. Skuterud, J. E. Bredesen and P. K. Lunde, *Br. J. Clin. Pharmacol.*, **16**, 233 (1983).
- 6) E. Echizen, S. Saima, N. Umeda and T. Ishizaki, *Clin. Pharmacol. Ther.*, **40**, 274 (1986); J. Bonde, N. A. Graudal, L. E. Pedersen, S. Balslov, H. R. Angelo, T. L. Svendsen and J. P. Kampmann, *Eur. J. Clin. Pharmacol.*, **31**, 73 (1986).
- 7) J. Urban, C. Danny and S. Gerhard, *J. Biol. Chem.*, **254**, 10565 (1979).
- 8) G. Mancini, A. O. Carbonara and J. F. Heremans, *Immunochemistry*, **2**, 235 (1965).
- 9) E. Pike, B. Skuterud, P. Kierulf and P. K. M. Lunde, *Clin. Pharmacol. Ther.*, **32**, 599 (1982).
- 10) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
- 11) O. Borga, K. M. Pfafsky and O. G. Nilsen, *Clin. Pharmacol. Ther.*, **22**, 539 (1977); N. Terao and D. O. Shen, *J. Pharmacol. Exp. Ther.*, **227**, 369 (1983).
- 12) M. Goldberg, F. M. Fouad and G. Ruhenstroth-Bauer, *J. Clin. Chem. Clin. Biochem.*, **21**, 125 (1983).
- 13) K. M. Pfafsky, O. Borga, I. Odar-Cederlof, C. Johansson and F. Sjoqvist, *N. Engl. J. Med.*, **299**, 1435 (1978).
- 14) T. Kanaghinis, A. F. Aygerinos, P. Scliros, N. Kalantzis, J. Hatzioannou, P. Nikolopoulou, D. Anagnostou, A. Katsas, J. Demopoulos, G. Rekoumis and D. Stathakos, *Am. J. Gastroenterol.*, **77**, 512 (1982).