Plasma α_1-Acid Glycoprotein Concentration in Rats with Chemical Liver Injury

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The influence of liver injury on the plasma concentrations of α_1-acid glycoprotein (AGP) and albumin was examined in several different models of chemically-induced liver injury. The plasma AGP concentration in carbon tetrachloride (CCl_4), allyl alcohol, bromobenzene, acetaminophen or *N*-nitrosodimethylamine-induced liver injury was increased to 2—3.5 times the normal level at 24 h after the intoxication. The plasma AGP concentration was unchanged in ethionine-induced liver injury and was markedly decreased in galactosamine-induced injury. The plasma albumin concentration was significantly decreased by the damage due to galactosamine, allyl alcohol or *N*-nitrosodimethylamine-induced liver injury, while no influence was observed by other hepatotoxin-induced liver injury.

The plasma protein binding of propranolol was also determined in relation to the plasma concentrations of AGP and albumin in all the experimental models. Propranolol binding, expressed as bound to free ratio, showed a good correlation with the AGP concentration ($r=0.940$; $p<0.001$), but not with the albumin concentration.

Keywords liver injury; α_1-acid glycoprotein; carbon tetrachloride; galactosamine; ethionine; allyl alcohol; acetaminophen; *N*-nitrosodimethylamine; bromobenzene

Introduction

The important role of plasma protein binding in the pharmacokinetics and pharmacodynamics of drug has been well recognized.[1] α_1-Acid glycoprotein (AGP), in addition to albumin, is an important plasma constituent that binds basic drugs.[1,2] AGP is an acute phase reactant protein produced by the liver that undergoes a striking change in plasma concentration in response to various pathophysiological conditions. There have been many reports that the protein binding of many basic drugs was increased due to an elevation of the plasma AGP level in patients suffering from diseases such as trauma, rheumatoid arthritis, scalding, myocardial infarction and cancer.[3] In patients with liver disease, however, results of studies on the plasma AGP level and/or plasma protein binding of basic drugs are complicated since the plasma AGP level shows a large variation in relation to its pathological status.[1a,2a,4] The AGP level may be raised in the plasma of patients with certain acute viral hepatitis but depressed in those with chronic liver disease or liver cirrhosis.[5] With regards to animal experimental models of liver disease, only a few studies on the plasma AGP level have been reported. In rodents with galactosamine-induced liver injury, a fall in the plasma AGP level has already been documented by some authors.[6] In contrast, we reported recently that rats with CCl_4 induced liver damage showed an elevated plasma level of AGP and a resultant increase in the protein binding of basic drugs.[7] In the present study, we investigated the concentrations of AGP and albumin in several models of chemically-induced liver injury. In addition, the plasma protein binding of propranolol, a model basic drug, was also examined in relation to the change of plasma AGP level.

Materials and Methods

Chemicals *dl*-[^{3}H]Propranolol (27 Ci/mmol) was purchased from Dupont/NEN Research Products. Unlabeled *dl*-propranolol-HCl, carbon tetrachloride (CCl_4) and galactosamine-HCl were purchased from Wako Pure Chemical Co., Ltd. (Osaka, Japan). Ethionine, *N*-nitrosodimethylamine, allyl alcohol, acetaminophen and bromobenzene were obtained from Nacalai Tesque, Inc. (Kyoto, Japan).

Animal Treatments Male Wistar rats weighing about 250 g were raised on a normal laboratory diet and fasted for 24 h prior to experiments. Acute liver injury was induced in the rats by the intraperitoneal administration of one of the following hepatotoxins. CCl_4 (1 ml/kg) and bromobenzene (1 ml/kg) were given as a 20% and a 50% solution, respectively, in olive oil. Galactosamine (500 mg/kg), ethionine (800 mg/kg), *N*-nitrosodimethylamine (50 mg/kg) and allyl alcohol (50 mg/kg) were dissolved in saline. Acetaminophen (900 mg/kg) was suspended in 20% glycerol. Blood samples were collected from the inferior *vena cava* by using a heparinized-syringe at 24 h after the intoxication and centrifuged at 3000 rpm to obtain plasma. Assessment of liver injury in the intoxicated rats was performed by plasma glutamic pyruvic transaminase (GPT) level.

Binding Experiments The protein binding of propranolol to plasma was measured by means of an ultrafiltration method as described in a previous paper.[7] *dl*-[^{3}H]Propranolol was added to the plasma, the pH of which was adjusted to 7.4 with 0.1 M phosphate buffer. After being incubated for 10 min at room temperature (20—23 °C), the plasma was filtered through a Molcut-II LGC (Millipore-Japan). Aliquots (50 μl) of the filtrate and the plasma were added into 6 ml of ACS-II scintillation fluid (Millipore-Japan), and radioactivity was counted in a liquid scintillation counter (Aloka Co., Ltd., Japan). The percentage binding was calculated from the following equation:

$$\text{percentage binding} = \{(C_p - C_t)/C_p\} \times 100$$

where C_t = radioactivity in the filtrate, C_p = initial radioactivity in plasma.

Analytical Methods The AGP concentration was measured by radial immunodiffusion according to the method of Mancini *et al.*[8] as described previously.[7] The immunodiffusion plates were made from Agarose-LGT (Nacalai Tesque Inc). The AGP assay measured the amount of AGP within the range from 500 ng to 5 μg in each test well. The plasma total protein concentration was measured by the method of Lowry *et al.*[9] The activities of GPT and glutamic oxaloacetic transaminase (GOT) were determined with Shino Test kits (Shino Test Co., Ltd., Tokyo, Japan). The albumin concentration was determined with Albumin B-Test kit (Wako Pure Chemicals Co., Ltd.).

Data Analysis Correlations between the protein binding expressed as the ratio of bound/free and the plasma concentrations of AGP or albumin were assessed by least-squares linear regression analysis. The significance of differences between groups was determined by Student's *t*-test.

Results

Plasma AGP, Albumin and Total Protein Levels Table I shows the plasma GPT level 24 h after the injection of the various hepatotoxins. In all treated rats, a significant increase in the GPT level was observed. The elevation of GOT occurred almost parallel to that found for GPT in all the models (not shown). The death of animals did not permit the use of higher doses than those employed for *N*-nitrosodimethylamine, acetaminophen and bromobenzene. The dose of ethionine used was chosen because of its

known maximal toxic effect on rat hepatocytes.[10] Figure 1 shows the profiles of relative plasma levels of AGP, albumin and total protein in the plasma of various models of liver injury in comparison with the control levels. The mean concentrations of AGP, albumin and total protein in control rats were 0.137±0.039, 37.2±0.7 and 76.9±1.3 mg/ml, respectively. The plasma AGP concentration in rats with galactosamine-induced liver injury was decreased by 45% compared to that of control rats. This is in good agreement with the data previously reported by Monnet *et al.*[6c] In contrast, in rats with CCl_4, acetaminophen, *N*-nitrosodimethylamine, allyl alcohol or bromobenzene-induced liver injury, the plasma AGP concentration was markedly raised compared to the control, with the percent increase being 246, 188, 166, 122 and 92%, respectively. The plasma AGP concentration was not significantly altered in rats with ethionine-induced liver injury.

A decreased plasma albumin concentration was found in rats with liver damage due to allyl alcohol (23% of the control), galactosamine (20%) or *N*-nitrosodimethylamine (11%)-induced liver injury. In correlation with the decreased albumin concentration in these groups of rats, there was also a fall in the plasma total protein concentration relative to the control by 23, 17 and 8%, respectively. This decrease in total protein concentration was explained by damaging of the function of the polyribosomes on the hepatocyte

TABLE I. Plasma GPT Levels in Control Rats and Several Different Models of Chemical Liver Injury

Control	23± 3[a]	Allyl alcohol	657±187[c]
Galactosamine	900±186[d]	CCl_4	1130±140[d]
Ethionine	83± 18[c]	Acetaminophen	64± 14[b]
N-Nitroso-dimethylamine	115± 21[d]	Bromobenzene	86± 24[b]

Animals were sacrificed 24 h after the injection of galactosamine (500 mg/kg), ethionine (800 mg/kg), *N*-nitrosodimethylamine (50 mg/kg), allyl alcohol (50 mg/kg), CCl_4 (1 ml/kg), acetaminophen (900 mg/kg) or bromobenzene (1 ml/kg). *a*) GPT values (Karmen unit). Values are the means±S.E. of 4 to 10 animals. Significantly different from the control; *b*) $p<0.05$, *c*) $p<0.01$, *d*) $p<0.001$.

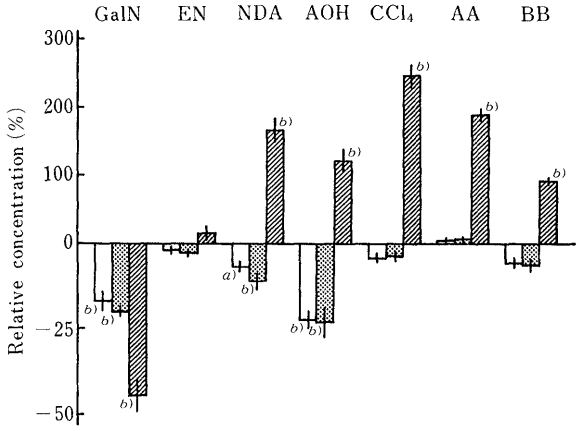

Fig. 1. Relative Plasma Concentrations of Total Protein, Albumin and AGP in Several Models of Chemically-Induced Liver Injury

The relative concentration (%) were calculated from the equation:

$$\% = \{(P_i - P_c)/P_i\} \times 100$$

where P_i is the plasma protein concentration in the rats with chemically-induced hepatic injury and P_c is the plasma protein concentration in the control rats. Values are the means±S.E. of 4 to 10 animals. Significantly different from corresponding plasma protein concentration in the control: *a*) $p<0.05$, *b*) $p<0.01$. GalN: galactosamine, EN: ethionine, NDA: *N*-nitrosodimethylamine, AOH, allyl alcohol; AA, acetaminophen; BB, bromobenzene. □, total protein; ▨, albumin; ▨, AGP.

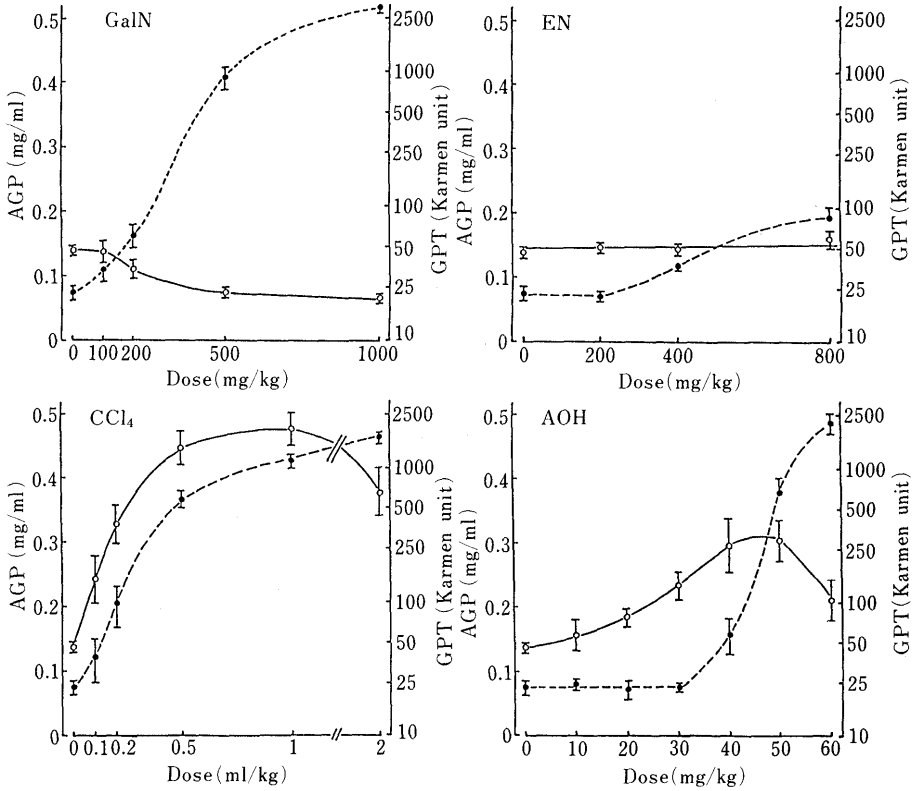

Fig. 2. The Plasma Concentrations of AGP and GPT in the Rats Treated with Various Doses of CCl_4, Allyl Alcohol, Galactosamine or Ethionine

Animals were sacrificed 24 h after the injection of hepatotoxin. Values are the means±S.E. of 7 to 8 animals. ○, AGP level; ●, GPT level. GalN, galactosamine; EN, ethionine; AOH, allyl alcohol.

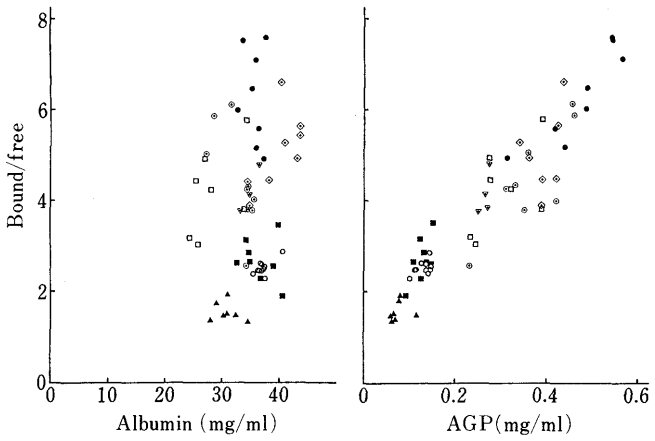

Fig. 3. Relationship between the Propranolol Bound/Free Ratio and the Plasma Concentration of AGP and Albumin in Control Rats and Several Different Models of Chemical Liver Injury

The same plasma samples as those shown in Fig. 1 were subjected to the experiments. ○, control; ▲, galactosamine; ■, ethionine; ⊙, *N*-nitrosodimethylamine; □, allyl alcohol; ●, CCl_4; ◇, acetaminophen; ▽, bromobenzene. Regression line for AGP: $y=11.9x+0.71$, $r=0.940$, $p<0.001$.

rough endoplasmic reticulum in animals treated with galactosamine.[6,11] The plasma albumin concentration was scarcely affected in rats with liver injury due to CCl_4, acetaminophen and bromobenzene.

Changes in Plasma AGP and GPT Levels as a Function of Hepatotoxin Dose To get an indication of whether the elevation of plasma AGP concentration is related to the development of liver necrosis or is induced directly by the toxic effect of the chemicals themselves, plasma levels of AGP and GPT were simultaneously determined after treatment with various doses of CCl_4, allyl alcohol, galactosamine or ethionine (Fig. 2). The AGP concentration in the plasma of CCl_4-treated rats was increased almost parallel with the elevation of GPT activity. In allyl alcohol-treated rats, however, the increase in AGP began from a dose at which a rise in GPT did not occur. Such a study with acetaminophen, bromobenzene or *N*-nitrosodimethylamine also showed no correlation between the patterns of elevation of AGP and GPT. On the other hand, galactosamine-treated rats showed no initial rise but a fall in the plasma AGP concentration with increasing liver injury. No significant change in AGP levels was observed at any doses in ethionine-treated rats. These indicate that distinct liver injury is not a prerequisite for the hepatic production of AGP.

Plasma Protein Binding of Propranolol in Different Models of Liver Injury To assess the effect of changes in the protein concentrations of AGP and albumin on the plasma binding of propranolol, the protein binding of the drug at the concentration of 1 μg/ml was determined simultaneously with the measurement of the plasma protein concentrations in all the liver injury models. The binding of propranolol, expressed as the bound/free ratio, is plotted against the AGP and albumin concentrations in Fig. 3. There was a significant correlation between the AGP concentration and the propranolol binding ratio ($r=0.940$; $p<0.001$) but not between the latter and the albumin concentration. These results indicate that the variations in the protein binding of propranolol were largely dependent on the changes in the AGP concentrations, with albumin having only a minor role.

Discussion

While changes in the plasma AGP concentration have been adequately investigated for some diseases,[3] such knowledge with regard to liver disease is still limited. In the present study, we therefore determined the plasma concentrations of AGP and albumin in rats with various types of chemical liver injury. We found that the acute liver injury caused by chemicals such as CCl_4, acetaminophen, *N*-nitrosodimethylamine, allyl alcohol and bromobenzene was associated with a rise in the plasma AGP concentration. The hepatic production of acute-phase reactant proteins is triggered by cytokines released by activated phagocytic cells at the site of inflammation.[12] We previously reported[7a] that the rise of plasma AGP in rats with CCl_4-induced liver injury might be explained as follows: the hepatotoxin first caused the necrosis of liver parenchymal cells and the resultant infiltration of inflammatory cells led to the secretion of cytokines. However, in the present study, an increase in the AGP concentration occurred without any elevation of GPT activity in rats with allyl alcohol-induced liver injury. This finding suggests that the rise in plasma AGP concentration in rats with chemically-induced hepatic injury might not be related to the development of liver necrosis, but rather to the toxic effect of the chemicals themselves. It is well documented that all the hepatotoxins which induced a rise in plasma AGP level in the present study produce reactive intermediates including active oxygen species stemming from microsomal monooxygenase metabolism or from the cytosolic alcohol dehydrogenase reaction in the case of allyl alcohol.[13] We also tested two hepatotoxins, galactosamine and ethionine, which cause liver injury independently of the formation of oxidative free radicals.[11,14] Both of them did not cause a rise in the plasma AGP concentration. We therefore propose that oxidative stress might be implicated in the increased production of AGP in the liver.

Although there is a general consensus that the plasma protein binding of acidic drugs is decreased in liver disease, information on the binding of basic drugs are irregular in this disease.[1a,2a,4] To our knowledge, there have been no reports on the effect of liver disease on the plasma protein binding of basic drugs and plasma protein components in animal models of toxic liver damage. There was a large variation in the plasma concentrations of AGP, albumin and total protein among the different model liver injuries. The models can be classified into four categories: category I (CCl_4, acetaminophen and bromobenzene) showed a high plasma AGP level and a normal plasma albumin level; category II (*N*-nitrosodimethylamine and allyl alcohol) showed a high AGP level and a low albumin level; category III (ethionine) showed normal AGP and albumin levels and category IV (galactosamine) showed low levels of AGP and albumin. Thus, these four categories are suitable for exploring the influence of changing concentrations of AGP and albumin on the plasma protein binding of weak basic drugs in liver disease. Albumin is responsible for about two thirds of the plasma binding of propranolol in rats.[2b,c,7a] However, little influence of the albumin concentration on propranolol binding was observed in the liver damaged rats. In contrast, the plasma protein binding of propranolol

bound/free ratio was lineally related to the changes in the plasma AGP concentration. These results indicate that changes in the plasma protein binding of propranolol can be predicted solely on the base of the AGP concentration in rats with chemical liver injury.

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