The Hepatic and Intestinal Metabolic Activities of P450 in Rats with Surgery- and Drug-Induced Renal Dysfunction

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Purpose. The hepatic and intestinal metabolic activities of P450 were evaluated in rats with surgery- and drug-induced renal dysfunction.
Methods. Renal failure was induced by five-sixths nephrectomy (NR), bilateral ureter ligation (BUL), the intramuscular injection of glycerol (GL), and the intraperitoneal injection of cisplatin (CDDP). Phenytoin 4-hydroxylation, debrisoquine 4-hydroxylation, and testosterone 6β-hydroxylation were estimated to evaluate the metabolic activities of cytochrome P450 (CYP) 2C, 2D, and 3A, respectively.
Results. The hepatic CYP3A metabolic activities were decreased by 65.9% and 60.2% in NR and GL rats, respectively. The hepatic CYP2C metabolic activity was decreased by 48.8% in CDDP rats. No alteration in hepatic drug-metabolizing activities was observed in BUL rats. On the other hand, the intestinal CYP3A metabolic activity was weakly increased in GL rats but not significantly altered in NR, CDDP, and BUL rats.
Conclusions. This study suggested (a) that only selected P450 metabolic activity in the liver is decreased in renal failure, (b) that extent of the decrease in hepatic metabolic activities of P450 is dependent on the etiology of renal failure, and (c) that alteration of CYP3A metabolic activity in the intestine is not always correlated with that in the liver.

KEY WORDS: renal failure; hepatic metabolism; intestinal metabolism; P450.

INTRODUCTION

Although renal failure is commonly thought to have its sole effect on the renal elimination of drugs, altered metabolic clearances of some drugs in patients with renal failure are reported (1,2). The hepatic clearances of metoprolol, theophylline, nifedipine, and lidocaine are not significantly affected in patients with renal dysfunction. Decreased hepatic clearances of propranolol, bufuralol, verapamil, captopril, erythromycin, and tacrolimus have been suggested in patients with renal dysfunction. However, the specific mechanisms of reduced hepatic metabolism in patients with renal dysfunction are unclear (1,2).

This study was designed to examine the diversity in the regulation of hepatic chtochrome P450 (CYP) enzymes in renal failure model rats. That is, we assessed the phenytoin 4-hydroxylation, debrisoquine 4-hydroxylation, and testosterone 6β-hydroxylation activity in rat liver microsomes. Phenytoin is used clinically as an antiepileptic drug, and CYP2C6 shows the highest activity of 4-hydroxylated phenytoin formation in rats (3). Debrisoquine is a well-known substrate for CYP2D isoforms, and debrisoquine 4-hydroxylation is catalyzed most effectively by CYP2D2 in rats (4). Testosterone is a typical substrate for CYP3A isoforms, and 6β-hydroxylation of testosterone is mainly catalyzed by CYP3A2 in the rat liver (5).

In addition, some reports show the enterocyte enzymes, CYP3A isoforms, are involved in the significant first-pass metabolism of some drugs in rats and humans (6,7). To our knowledge, however, there have been no systematic studies concerning the intestinal drug metabolism in renal dysfunction (6,8). In this study, therefore, we also assessed intestinal testosterone 6β-hydroxylation in rats with experimental renal dysfunction.

MATERIALS AND METHODS

Materials

Cisplatin (Platosin® injection, 0.5 mg/ml) was purchased from Kyowa Hakko (Tokyo, Japan). 5-(4-Hydroxyphenyl)-5-phenyl-hydantoin (4-hydroxylated phenytoin) was obtained from Aldrich Chemicals (Milwaukee, WI). 6β-Hydroxytestosterone was obtained from Sigma (St. Louis, MO). 4-Hydroxydebrisoquine was purchased from ICN (Aurora, OH). All other chemicals were of the highest grade available.

Animals

All animal experiments were performed in accordance with The Guidelines for Animal Experiments of Toyama Medical and Pharmaceutical University. Male Wistar rats (240–260 g, 8 weeks old) were purchased from Japan SLC Inc. (Hamamatsu, Japan). Before the experiments, the rats were housed in a temperature- and humidity-controlled room with free access to water and standard rat chow.

Induction of Renal Failure

Single-step five-sixths nephrectomy (NR) was performed according to the method of Ji *et al.* (9), and the rat was used for experiments 7 days after the operation. Bilateral ureter ligation (BUL) was performed according to the method of Giacomini *et al.* (10), and the rat was used for experiments 36 h after the operation. Sham-operated rats served as respective controls.

Glycerol dissolved in saline (50% v/v, 10 ml/kg) was injected into the leg muscle after a 24-h period of water deprivation to produce glycerol-induced renal failure model (GL) rats (11,12). Cisplatin (Platosin® injection, 0.5 mg/ml), 10 mg/kg, was injected intraperitoneally to produce cisplatin-induced renal failure model (CDDP) rats (6,12–14). These rats were used for experiments 72 h after the injection of chemicals. Saline-treated rats served as controls.

Hepatic Metabolism with Liver Microsomes

The liver was removed from the rat and homogenized with 4 volumes of ice-cold 1.15% potassium chloride dissolved in isotonic phosphate buffer (pH 7.4) in a Potter-Elvehjem homogenizer. The homogenate was centrifuged at

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9000 *g* for 20 min. The supernatant was transferred and centrifuged again at 105,000 *g* for 60 min. The precipitate was resuspended with 3 volumes of the original liver weight of ice-cold 1.15% potassium chloride. The mean yield of hepatic microsomal protein in 48 rats was 10.1 mg protein/g tissue. The microsomal suspensions were stored at −85°C until use.

The activities of phenytoin 4-hydroxylation, debrisoquine 4-hydroxylation, and testosterone 6β-hydroxyration were determined to evaluate the metabolic activities of CYP2C, 2D, and 3A subfamilies, respectively. The reaction mixture, consisting of liver microsome (1 mg protein) and 1 mM NADPH in potassium phosphate buffer (pH 7.4), was preincubated for 5 min at 37°C. The reaction was started by the addition of drug solution. The final concentration of phenytoin, debrisoquine, and testosterone were 100 μM, 100 μM, and 300 μM, respectively. The reaction was conducted for 30 min at 37°C. Metabolic activities of microsomes obtained from 1 g of liver (μ mol/min/g tissue) are calculated by assessing metabolite formation.

Intestinal Metabolism with Isolated Intestine

The isolated intestine was used for the evaluation of intestinal CYP3A metabolic activity (6,15,16). Rats were anesthetized with diethylether, and a 30-cm length of the small intestine was removed. Isotonic phosphate buffer (pH 6.5) containing disodium hydrogen phosphate (123 mM), sodium dihydrogen phosphate (163 mM), and glucose (5 mM) was continuously bubbled with 95% O_2/5% CO_2. The intestinal tissues were incubated for 60 min at 37°C in 30 ml of buffer containing 300 μM testosterone. The buffer solution was collected at the end of the incubation, and the intestinal tissue was homogenized with 9 volumes of the ice-cold isotonic phosphate buffer. Metabolic activities in 1 g of intestine (μmol/min/g tissue) were calculated by assessing metabolite formation.

Analytic Method

The plasma concentrations of urea nitrogen, creatinine, and glutamic oxaloacetic transaminase (GOT) were measured with kits obtained from Wako Pure Chemical Industries Ltd (Osaka, Japan). The protein content of the hepatic microsomal suspension was determined using the Bio-Rad protein assay dye reagent (Bio-Rad, Munich, Germany). The concentration of 4-hydroxylated phenytoin was determined by HPLC as described by Komatsu *et al.* (17). The concentration of 4-hydroxydebrisoquine was determined by HPLC as described by Kronbach (18). The concentration of 6β-hydroxytestosterone was determined by HPLC as reported by Ikeda *et al.* (19).

Data Analysis

Values are expressed as means ± SE for n animals. Statistical difference between mean values was calculated using a nonpaired *t* test provided that the variances were similar. If this was not the case, the Mann-Whitney U-test was applied. Values of p less than 0.05 (two-tailed) were considered to be statistically different.

RESULTS

Four kinds of renal failure models were used in this study. Figure 1 shows the biochemical parameters in the renal failure model rats. The plasma concentrations of creatinine and urea nitrogen in rats with renal failure were 1.5- to 9.4-fold higher than those in respective controls. The plasma GOT values in rats with renal failure were only slightly different from those in controls, though the difference in GOT between GL and control rats was statistically significant.

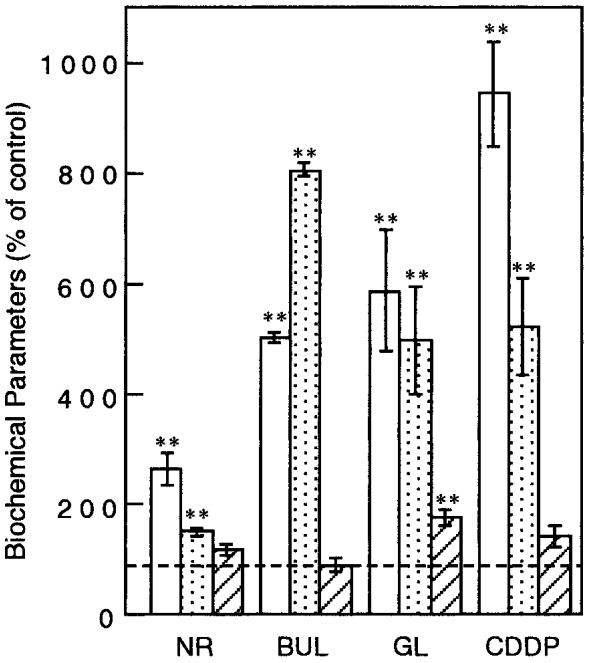

Fig. 1. Biochemical parameters in plasma of rats with various renal failure models. Open, dotted, and hatched columns show serum urea nitrogen, serum creatinine, and GOT concentrations, respectively. Values (% of control) are expressed as mean ± SE for seven animals. **p < 0.01 compared with controls.

The hepatic and intestinal drug-metabolizing activities in NR and BUL rats are shown in Fig. 2A and Fig. 2B, respectively. The hepatic metabolic activities of CYP2C and CYP2D enzymes were not altered in NR rats compared with sham-operated controls. On the other hand, the hepatic CYP3A activity in NR rats was 65.9% less than that in sham-operated controls. In contrast, the intestinal metabolic activity of the CYP3A subfamily was not altered in NR rats (Fig. 2A). No differences in the metabolic activities of each CYP isoform were observed between BUL and sham-operated controls. In addition, the difference in the intestinal CYP3A activity between BUL and sham-operated controls was not statistically significant (Fig. 2B).

Figure 2C shows the hepatic and intestinal metabolic activities in GL rats. The metabolic activities of hepatic CYP2C and CYP2D enzymes were not altered in GL rats, whereas the metabolic activity of hepatic CYP3A was significantly decreased in GL rats as compared with controls. On the other hand, the intestinal CYP3A activity was slightly increased in GL rats. It should be noted that the hepatic weight of GL rats was increased by 21.3% as compared with control rats and that the weight of excised intestine of GL rats was decreased by 32.3% as compared with controls.

Figure 2D shows the hepatic and intestinal drug-metabolizing activities in CDDP rats. Phenytoin 4-hydroxylation activity in CDDP rats was 48.8% lower than that in controls. The hepatic metabolic activity of CYP2D and

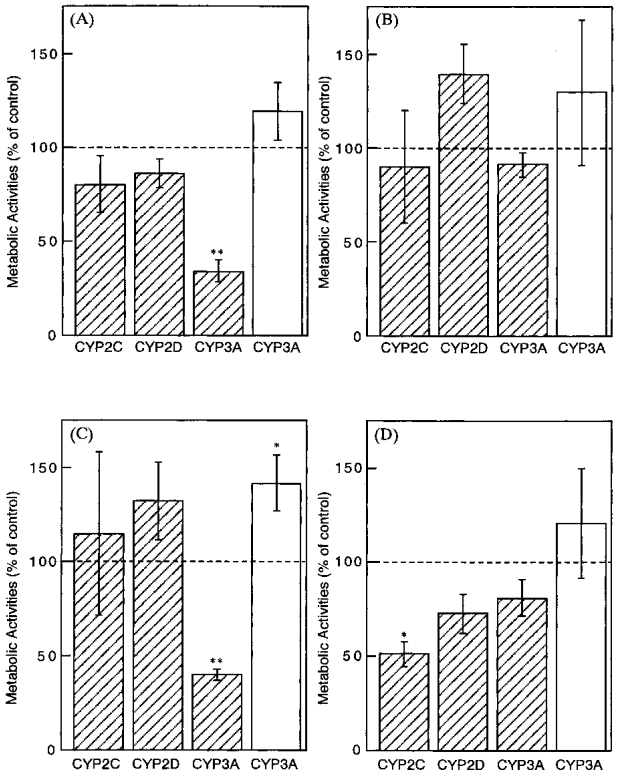

Fig. 2. Hepatic and intestinal drug-metabolizing activities of P450s in NR (A), BUL (B), GL (C), and CDDP rats (D). Metabolic activity of the CYP2C subfamily was assessed by phenytoin 4-hydroxylation. Metabolic activity of the CYP2D subfamily was assessed by debrisoquine 4-hydroxylation. Metabolic activity of the CYP3A subfamily was assessed by testosterone 6β-hydroxylation. Hatched columns show metabolic activities in the liver, and open columns show metabolic activities in the intestine. Metabolic activities (% control) are expressed as mean ± SE for seven to nine animals. *$p < 0.05$, **$p < 0.01$ compared with the control values (100%), respectively.

CYP3A also tended to be decreased in CDDP rats compared with controls. In contrast, the activity of intestinal drug metabolism was not changed in CDDP rats. However, it should be noted again that the hepatic weight in CDDP rats was decreased by 20.1% as compared with control rats, and that the weight of excised intestine in CDDP rats was decreased by 33.3% as compared with controls.

DISCUSSION

A wide variety of animal models have been developed in an attempt to mimic conditions seen in human renal failure; however, no single animal model would be completely satisfactory because the etiologies and development of renal failure are diverse (13). Therefore, this study was designed to evaluate the differential effect of etiology of renal failure on hepatic drug metabolism. NR rats have been widely used to study the progression of renal damage resulting from reduced renal mass (9). The BUL model causes severe uremia, shows a high incidence of fatalities, and survives only 2 days (10). The GL rat is a model of acute trauma in which intramuscular injection of 50% glycerol causes rapid myoglobinuria, oliguria, and a rapid reduction in glomerular filtration rate (11). An injection of uranyl nitrate has been found to be the most effective and easiest method to produce renal dysfunction in laboratory animals (10,13,15). However, changes in government regulations on the production and use of radioactive substances make the compound less available. As a result, cisplatin has been chosen as an inducer of renal failure because of its ability to produce kidney damage and its identical site and mechanism of action on the kidneys as uranyl nitrate (6,12–14).

The metabolic activity of CYP3A in the liver was significantly decreased in NR rats (Fig. 2A), though the increases of serum creatinine and urea nitrogen in NR rats were smaller than those in other renal failure model rats (Fig. 1). There are some reports that used NR rats to elucidate hepatic metabolism in renal failure (20). Our results on hepatic drug-metabolizing activities in NR rats are consistent with those obtained by other investigators (20). That is, mRNA level and protein expression of only selected cytochrome P450 isoforms, i.e., CYP3A2, are decreased in the liver of NR rats, but CYP2C6 and 2D are not altered in NR rats compared with sham-operated controls (20).

There are few reports concerning the metabolic activity in rats with renal failure other than the NR model, although alterations of hepatic CYP isozymes in NR rats have been extensively studied (20). In this study, the hepatic drug-metabolizing activities were also evaluated in BUL, GL, and CDDP rats as well as in NR rats. As a result, it was thought that the hepatic metabolic activities in experimental renal dysfunction model rats are largely dependent on the etiology of renal failure as well as on the molecular forms of P450 (Fig. 2). That is, phenytoin 4-hydroxylation was altered only in CDDP rats, whereas debrisoquine 4-hydroxylation was not altered significantly in any renal failure model used. Testosterone 6β-hydroxylation in the liver microsome was significantly decreased only in NR and GL rats.

Testosterone 6β-hydroxylation in the intestine was not decreased in any renal failure model rat, though the hepatic CYP3A activities were significantly decreased in NR and GL rats (Fig. 2). The reason for different alterations of metabolic activities in the liver and intestine is unknown. The mRNA of members of the CYP3A subfamily, CYP3A1, 3A2, 3A9, 3A18, and 3A23, has been detected in rat liver, but CYP3A1 is thought to be an allelic variant of CYP3A23 (21). On the other hand, some investigators did not detect any mRNA for CYP3A2 in rat enterocytes and suggested that the P450 isoenzyme expressed in rat enterocyte is likely to be CYP3A9, which may cross-react with CYP3A2 peptide antibodies (5,16). To reveal the mechanism(s) of different regulation of metabolic activities in the liver and intestine, it may be necessary to determine the intestinal CYP3A isoform that is involved in the testosterone 6β-hydroxylation.

In conclusion, it was found in this study (a) that only selected P450 metabolic activity in the liver is decreased in renal failure, (b) that extent of the decrease in hepatic metabolic activities of P450 is dependent on the etiology of renal failure, and (c) that an alteration of CYP3A metabolic activity in the intestine is not always correlated with that in the liver. The results obtained in this study would provide useful information to investigate pharmacokinetics and to study the regulations of cytochrome P450 in renal dysfunction.

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