DECREASED IN VIVO METABOLISM OF DRUGS IN CHRONIC RENAL FAILURE

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ABSTRACT:

Chronic renal failure (CRF) is associated with a decrease in renal excretion of drugs, but its effects on the liver metabolism of xenobiotics are poorly defined. The objectives of this study were to determine the effects of CRF on hepatic cytochrome P450 (CYP450) and its repercussions on in vivo hepatic metabolism of drugs. Two groups of rats were studied: control paired-fed and CRF. CRF was induced by subtotal nephrectomy. Total CYP450 activity and protein expression of several CYP450 isoforms (CYP1A2, CYP2C11, CYP3A1, CYP3A2) were assessed in liver microsomes. In vivo cytochrome P450 activity was evaluated with breath tests using substrates for different isoenzymes: caffeine (CYP1A2), aminopyrine (CYP2C11), and erythromycin (CYP3A2). Creatinine clearance was reduced by 60% (P < .01) in rats with CRF. Compared with control paired-fed rats, total CYP450 activity was reduced by 40% in rats with CRF. Protein expression of CYP2C11, CYP3A1, and CYP3A2 was considerably reduced (more than 45%, P < .001) in rats with CRF, whereas the levels of CYP1A2 were unchanged. In rats with CRF, there was a 35% reduction in the aminopyrine (CYP2C11) and the erythromycin (CYP3A2) breath tests compared with control animals (P < .001). The caffeine (CYP1A2) breath tests remained comparable to controls. Creatinine clearance correlated with the aminopyrine and erythromycin breath tests (r² = 0.73 and r² = 0.81, respectively, P < .001). In conclusion, CRF is associated with a decrease in total liver CYP450 activity in rats (mainly in CYP2C11, CYP3A1, and CYP3A2), which leads to a significant decrease in the metabolism of drugs.

Reduction of renal function alters the clearance of many drugs mainly by decreasing their renal elimination (Lam et al., 1997; Matzke and Frye, 1997). However, in patients with chronic renal failure (CRF), drug metabolism in the liver can also be altered (Touchette and Slaughter, 1991). In rats with experimental CRF, several studies have shown a reduction in hepatic cytochrome P450 (CYP450) (Leber and Schutterle, 1972; Van Peer and Belpaire, 1977; Leber et al., 1978; Patterson and Cohn, 1984). Furthermore, recent studies in the rat have demonstrated that some of the liver CYP450 isoforms implicated in drug metabolism were down-regulated in CRF. Uchida et al. (1995) reported that in rats with CRF, the in vivo metabolism of several substrates is reduced in CRF rats (Gibson, 1986). However, very few data are available on the in vivo hepatic drug-metabolizing capacity in CRF rats. Uchida et al. (1995) reported that in rats with CRF, the in vivo N-demethylation of trimethadione (mediated through several isoforms of the CYP450) was decreased.

The objectives of this study were to determine the effects of CRF on hepatic cytochrome P450 and to evaluate the repercussions of CRF on the in vivo liver metabolism of drugs. To this purpose, we measured in control paired-fed and CRF rats 1) the liver cytochrome P450 total activity; 2) the main CYP450 isoforms involved in drug metabolism; and 3) the disposition of caffeine, aminopyrine, and erythromycin, reflecting mainly the in vivo activity of CYP1A2, CYP2C11, and CYP3A2, respectively (Watkins et al., 1989; Schaad et al., 1995; Bastien and Villeneuve, 1998).

Materials and Methods

Experimental Model. Male Sprague-Dawley rats (Charles River, Saint-Charles, Québec, Canada), weighing 200 to 300 g were housed at the Research Center Animal Care Facility and maintained on Purina rat pellets and water ad libitum up to the beginning of the experiments. Animals were allowed to acclimatize in our animal care facility for at least 3 days before any experimental work was undertaken. All the experiments were conducted according to the Canadian Council on Animal Care guidelines for care and use of laboratory animals.
Experimental Protocol. Studies were performed in two groups of six animals each: control and CRF. Chronic renal failure was induced by two stages five-sixth nephrectomy. Briefly, the rats underwent a two-third nephrectomy of the left kidney through a midline incision and 7 days later a right nephrectomy was performed. Rats from the control group underwent two sham laparotomies. Pentobarbital was used for anesthesia (60 mg/kg via i.p. injection). After surgery, CRF animals were fed Purina rat chow and water ad libitum. The food consumption of CRF rats was 20 to 30 g/day during the first week after the left partial nephrectomy. After the right nephrectomy, food consumption decreased to 10 to 15 g/day and remained stable for the rest of the experiment. Control paired-fed rats were fed the same amount of rat chow that was ingested by the CRF rats on the previous day, to assess the effect of CRF-induced malnutrition. Body weight was measured every other day for the duration of the study. Breath tests (see below) were performed between day 38 and day 40 after surgery. At day 41 after the nephrectomy, the rats were housed in metabolic cages and urine was collected for 24 h to determine the clearance of creatinine. Rats were sacrificed by decapitation at 42 days after nephrectomy. Blood was collected for the measurement of serum creatinine and urea.

Preparation of Liver Microsomes. Livers were excised immediately after sacrifice and microsomes were isolated by differential centrifugation (Cinti et al., 1972). Samples were maintained at 4°C during microsome preparation. Briefly, 5 g of liver was homogenized (using a Potter-Elvehjem tissue grinder; Wheaton, Vineland, NJ) in 25 ml of 0.25 M sucrose and centrifuged at 12,000g. The supernatant 1 M CaCl2 was added (10% v/v) and further centrifuged at 27,000g. The pellet containing the microsomes was stored at -80°C in 0.1 M Tris, pH 7.4, 20% glycerol, 10 mM EDTA up to analysis.

Determination of Total CYP450 Activity. Microsomal protein content was determined by the method of Lowry et al. (1951) using bovine serum albumin as standard protein. Total CYP450 activity was measured from the difference spectrum of the reduced protein (Omura and Sato, 1964).

Western Blot Analysis. The following CYP450 isoforms were assayed by Western blot analysis: CYP1A2, CYP2C11, CYP3A1, and CYP3A2. Forty micrograms of proteins was electrophoresed in a 7.5% polyacrylamide gel containing 0.1% SDS, and the separated proteins were electrophoretically transferred to nitrocellulose (Laemmli, 1970; Towbin et al., 1979). Immuno-bLOTS for respective isoforms were performed in 5% low-fat milk in PBS and transferred to nitrocellulose. CYP1A2 was detected using a polyclonal antibody (swine anti-goat IgGMI). CYP3A2 was detected using a goat anti-rat 3A2 (Gentest Corporation). A monoclonal mouse anti-rat 3A1 (Oxford Biochemical Research Inc., Oxford, MI) and a monoclonal mouse anti-rat 3A1 (Oxford Biochemical Research Inc., Oxford, MI). CYP3A2 was detected using a goat anti-rat 3A2 (Gentest Corporation). Immune complexes were revealed by secondary antibody (swine anti-goat IgG from Biosource International, Camarillo, CA, and goat anti-mouse IgG from Sigma Chemical Co., St. Louis, MO) coupled to peroxidase and the Luminol Immune complexes were revealed by secondary antibody (swine anti-goat IgGMI). CYP3A2 was detected using a goat anti-rat 3A2 (Gentest Corporation). CYP1A2 was detected using a monoclonal mouse anti-rat 3A1 (Oxford Biochemical Research Inc., Oxford, MI), and CYP3A2 was detected using a monoclonal mouse anti-rat 3A2 (Gentest Corporation). Immune complexes were revealed by secondary antibody (swine anti-goat IgG from Biosource International, Camarillo, CA, and goat anti-mouse IgG from Sigma Chemical Co., St. Louis, MO) coupled to peroxidase and the Luminol derivative of Lumi-Light Western blotting substrate (Roche Biochemical, Laval, Québec, Canada). Immune reaction intensity was determined by computer-assisted densitometry on exposed Biomax MR film.

Breath Tests. Under isoflurane anesthesia, rats received 0.25 µCi of a radioactive substrate administered i.v. in the dorsal penile vein. The animals were then placed in glass breathing chambers. The breath test apparatus used was similar to that described by Villeneuve et al. (1978). The air exhaled was directed through a system of tubes attached to the restraining glass chamber by action of vacuum pressure. The air was passed through a dehumidifying column of Drierite to remove water vapor and bubbled through a solution of 10 ml of methanol-ethanolamine 2:1 (v/v), which trapped 14CO2 exhaled by the rat. Collections of exhaled 14CO2 were performed at 15-min intervals for 2 h, in interchangeable glass scintillation vials containing the trapping solution. Ten milliliters of scintillation fluid (Hionic fluor) was added to each vial, and the radioactivity was determined using a beta counter (Wallac 1409). As described by Villeneuve et al. (1978), the air exhaled was directed through a system of tubes attached to the restraining glass chamber by action of vacuum pressure. The air was passed through a dehumidifying column of Drierite to remove water vapor and bubbled through a solution of 10 ml of methanol-ethanolamine 2:1 (v/v), which trapped 14CO2 exhaled by the rat. Collections of exhaled 14CO2 were performed at 15-min intervals for 2 h, in interchangeable glass scintillation vials containing the trapping solution. Ten milliliters of scintillation fluid (Hionic fluor) was added to each vial, and the radioactivity was determined using a beta counter (Wallac 1409). For each rat, breath tests were performed on consecutive days, in a randomized order, using substrates for the different CYP450 isoforms: caffeine (CYP1A2), aminopyrine (CYP2C11), and erythromycin (CYP3A2). In the caffeine and erythromycin breath tests rats received 0.25 µCi of a tracer dose of the 14C-labeled substrates. For the aminopyrine breath test, animals received a dose of 50 mg/kg unlabeled aminopyrine together with the 14C-tracer dose. All breath tests done from the second day on were immediately preceded by a 45-min evaluation of the residual excretion of 14CO2 from the substrate injected on the previous day. This "background" radioactivity, which did not exceed 0.6%, was subtracted from the counts obtained on that day. The rate of 14CO2 exhalation over 2 h was expressed as the percentage of administered radiolabel recovered in breath plotted as a function of time (per 15-min intervals). Results are presented as the cumulative amount of 14CO2 exhaled over 2 h.

Other Assays. Blood and urine chemistries were determined with a Hitachi 717 autoanalyzer (Boehringer Mannheim Canada, Laval, Québec, Canada).

Statistical Analysis. The results are expressed as mean ± S.E. Differences between groups were assessed by using an unpaired Student’s t test. The threshold of significance was P < .05.

Results

Biochemical Parameters and Body Weight in Control and CRF Rats. Table 1 presents the characteristics of the two groups. Compared with control rats, CRF animals had higher levels of plasma creatinine as well as urea and lower values of creatinine clearance (P < .001). There was no difference in body weight between control and CRF rats.

Liver Total CYP450 Activity in Control and CRF Rats. In CRF rats, total CYP450 activity was significantly reduced by 40% compared with the control group (Table 1). A significant correlation with creatinine clearance was found for total liver CYP450 activity (r2 = 0.7, P < .001). Similar correlations were found with blood urea and creatinine.

Protein Expression of Liver CYP450 Isoforms in Control and CRF Rats. In CRF rats, there was a marked decrease in the protein expression of several CYP450 isoforms, compared with control (Fig. 1). The levels of CYP2C11, CYP3A1, and CYP3A2 in CRF rats were reduced by 45, 85, and 45%, respectively (P < .001). The levels of CYP1A2 were not modified in CRF rats compared with control.

Effect of CRF on Cytochrome P450 Activity Measured In Vivo Using 14C-Breath Tests. In rats with CRF, there was a 35% reduction in the aminopyrine (CYP2C11) and the erythromycin breath tests (CYP3A2) compared with control animals (P < .001) (Fig. 2). The aminopyrine (CYP1A2) breath test remained comparable to controls (Fig. 2).

A correlation was sought between breath test results and the severity of CRF as determined by creatinine clearance. Creatinine clearance correlated with the aminopyrine and erythromycin breath tests in the rats of both experimental groups (r2 = 0.73 and r2 = 0.81, respectively, P < .005). There was also a significant correlation between breath tests and liver protein expression of CYP450 isoforms. The correlation between the aminopyrine breath test and liver CYP2C11 was r2 = 0.73 (P < .001), whereas the correlation between the erythromycin breath test and liver CYP3A2 was r2 = 0.62 (P < .001).

Discussion

This study demonstrates that in the rat, CRF induces a decrease in liver total CYP450 content secondary to reduced protein expression of

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**Table 1**

**Table 1** Characteristics of the control paired-fed and CRF rats

<table>
<thead>
<tr>
<th></th>
<th>Control Paired-Fed</th>
<th>CRF</th>
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<tbody>
<tr>
<td>Body weight (g)</td>
<td>385 ± 19</td>
<td>348 ± 8</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>53 ± 3</td>
<td>152 ± 13*</td>
</tr>
<tr>
<td>Serum urea (mmol/l)</td>
<td>6.4 ± 1.1</td>
<td>25.2 ± 2.5*</td>
</tr>
<tr>
<td>Creatinine clearance (µl/100 g b. wt./min)</td>
<td>370 ± 33</td>
<td>75 ± 17*</td>
</tr>
<tr>
<td>Liver total CYP450 content (nmol/mg protein)</td>
<td>0.56 ± 0.02</td>
<td>0.34 ± 0.04*</td>
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</tbody>
</table>

*P < .01 compared with control paired-fed animals.
some but not all CYP450 isoforms. The repercussions on the in vivo metabolism of drugs are important because we observed a 35% decrease of aminopyrine and erythromycin demethylation mediated by the CYP2C11 and CYP3A family, respectively.

Several studies have demonstrated that animals with CRF present decreased hepatic drug metabolism (Gibson, 1986). Because the CYP450 is the major enzymatic system involved in drug metabolism, most studies have focused on liver CYP450. There is an 18 to 42% decrease in liver total CYP450 in rats with CRF (Leber and Schutterle, 1972; Van Peer and Belpaire, 1977; Leber et al., 1978; Patterson and Cohn, 1984; Uchida et al., 1995). In the present study, we also found a 40% reduction in total CYP450, confirming our previous work (Leblond et al., 1999). Important reductions in enzymatic reactions normally catalyzed by the liver CYP450 have also been reported in vitro: N-demethylation of aminopyrine and ethy morphine, O-demethylation of codeine, and hydroxylation of aniline (Gibson, 1986).

Few studies have examined specific CYP450 isoforms in CRF (Uchida et al., 1995). Knowledge of which isoform is reduced by CRF is critical to determine which drugs are at risk for accumulation when used in CRF. Uchida et al. (1995) reported a reduction in the levels of hepatic CYP2C6, CYP2C11, and CYP3A2 and a slight increase in CYP1A2 in rats with CRF. Our results demonstrate that CYP2C11, CYP3A1, and CYP3A2 are significantly reduced, whereas CYP1A2 is normal.

Our results and those obtained by other investigators clearly demonstrated that CRF is associated with a down-regulation of liver CYP450. However, the consequences of liver CYP450 decrease induced by CRF on the in vivo drug-metabolizing capacity remain poorly documented. In humans, several investigators have demonstrated that in patients with renal failure there is a decrease in the metabolic clearance of many drugs (ranging from 17 to 85%) (Touchette and Slaughter, 1991; Talbert, 1994; Matzke and Frye, 1997). A majority of these drugs are thought to be metabolized in the liver by the CYP450. Although reductions in in vitro liver drug metabolism have been reported in rats with experimental kidney failure (Gibson, 1986), few data are available on the repercussions of CRF on systemic drug metabolism by the liver. A major problem with drug disposition studies in rats is that the blood samples used for pharmacokinetic analysis can lead to significant blood loss and hypovolemia. Uchida et al. (1995) studied the changes in trimethadione (TMO) metabolism using the microdialysis method (avoiding excessive blood samples) in CRF rats. They found that the N-demethylation of TMO was reduced by 25% in CRF. However, because TMO N-demethylation is catalyzed by several CYP450 isoforms, a reduction in its metabolism does not indicate which specific isoform is reduced (Nakamura et al., 1994). On the other hand, Tvedegaard et al. (1985) found no modification in the antipyrine clearance in rabbits with CRF.

In the present study we used breath tests as probes for evaluating in vivo liver metabolism. Breath tests have been developed as methods for evaluating the catalytic activity of CYP450 isoenzymes by measuring the rate of demethylation of a drug (Tanaka and Breimer, 1997). The formaldehyde generated by a CYP450-mediated demethylation reaction is rapidly oxidized and excreted as carbon dioxide in the breath. The rate of production of $^{14}$CO$_2$ from a suitable radiolabeled substrate reflects the in vivo rate of its demethylation and thus the catalytic activity of either a subset or a specific cytochrome P450, depending on the substrate studied. Various substrates have been used in breath tests to evaluate CYP450 activity in vivo in rats and humans. The aminopyrine breath test has often been used to evaluate liver metabolic function (Villeneuve et al., 1983; Perri et al., 1994). In vitro
and in vivo studies suggest that aminopyrine breath tests can be used to evaluate the activity of CYP2C11 in the rat (Imaoka et al., 1988; Bastien and Villeneuve, 1998), although other CYP enzymes also contribute to its demethylation, including CYP1A2, 2A2, 2B, and 2D1. The caffeine and erythromycin breath tests have been used to measure the liver catalytic activity of CYP1A2 and CYP3A2 isoenzymes (Watkins et al., 1989; Schaad et al., 1995; Bastien and Villeneuve, 1998).

In the present study, aminopyrine and erythromycin breath tests were reduced by 35% in CRF rats, whereas the caffeine breath test remained unchanged. These results demonstrate that in rats, CRF is associated with a reduction in the in vivo metabolism of drugs secondary to a decrease in selective liver CYP450 isoforms, namely CYP2C11 and CYP3A1/3A2. Whether reduction in extrahepatic metabolism of drugs (e.g., intestinal) that could further participate in the reduction of drug metabolism found in this study remains to be defined.

The mechanisms leading to CYP450 down-regulation in CRF remains unknown. In rat with acute renal failure, several studies have shown an increase in the bioavailability of propranolol (Terao and Shen, 1983, 1984; Katayama et al., 1984). Interestingly, Terao and Shen (1985) reported a reduced extraction of propranolol by perfused rat liver in the presence of uremic blood, suggesting that the reduction in propranolol extraction is due to an inhibitory factor in the uremic blood. Whether circulating factors present in CRF could also result in a decrease of liver CYP450 remains to be defined. Interestingly, our results show that there is a correlation between the severity of CRF and the decrease in liver CYP450 isoforms, as well as with the breath tests. One could hypothesize that as CRF progresses, circulating factors are accumulated, leading to a decrease in CYP450.

In conclusion, CRF induces, in the rat, a decrease in liver total CYP450 content secondary to a selective reduction in the protein expression of some CYP450 isoforms, and in the elimination of drugs metabolized by these isoforms. This study emphasizes the fact that reduced liver drug metabolism should be taken into account when evaluating the pharmacokinetics of drugs in severe CRF.

References