Downregulation of Intestinal Cytochrome P450 in Chronic Renal Failure

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Abstract. Chronic renal failure (CRF) is associated with a decrease in intestinal drug metabolism. The mechanisms remain poorly understood, but one hypothesis involves a reduction in cytochrome P450 levels. This study aimed to investigate the effects of CRF on intestinal cytochrome P450. Two groups of rats were defined, i.e., rats with CRF (induced by 5/6 nephrectomy) and control pair-fed rats. Total cytochrome P450 levels and protein and mRNA expression of cytochrome P450 isoforms, as well as in vitro N-demethylation of erythromycin (a probe for CYP3A activity) and 7-ethoxyresorufin O-deethylase activity (a probe for CYP1A1), were assessed in intestinal microsomes. Body weights were similar in the two groups. Creatinine clearance was reduced by 77% (P < 0.001) in CRF rats, compared with control pair-fed animals. Total intestinal cytochrome P450 activity was reduced by 32% (P < 0.001) in CRF rats. CYP1A1 and CYP3A2 protein expression was considerably reduced (>40%, P < 0.001) in rats with CRF. CYP2B1, CYP2C6, and CYP2C11 levels were the same in the two groups. RT-PCR assays revealed marked downregulation of CYP1A1 and CYP3A2 gene expression in CRF rats (P < 0.001). Although intestinal cytochrome P450 levels were reduced in CRF, induction by dexamethasone was present. N-Demethylation of erythromycin and 7-ethoxyresorufin O-deethylase activity were decreased by 25% (P < 0.05) in CRF rats, compared with control rats. In conclusion, CRF in rats is associated with decreases in intestinal cytochrome P450 activity (mainly CYP1A1 and CYP3A2) secondary to reduced gene expression.

Reductions in renal function alter the disposition of many drugs, mainly by decreasing the elimination of those excreted via the kidney (1,2). However, drug metabolism is also altered among patients with chronic renal failure (CRF) (3). Several studies have demonstrated that the metabolic clearance of various substrates is reduced among patients with CRF (1,3,4). Indeed, in rats with experimental renal failure, CRF inhibits liver cytochrome P450, as well as other cytosolic enzymes (5–8). We recently demonstrated that, in rats, CRF induces marked decreases in liver total cytochrome P450 activity secondary to reduced protein expression of selected cytochrome P450 isoforms, namely CYP2C11, CYP3A1, and CYP3A2 (9). The mechanism underlying this downregulation involves reductions in the levels of mRNA encoding these proteins (9). The effects on drug metabolism by the liver are important, because we observed a 50% reduction in erythromycin biotransformation mediated by the CYP3A family. We also demonstrated that the in vivo metabolism of some drugs (namely aminopyrine and erythromycin) was reduced in rats with CRF (10).

Although the liver plays a major role in drug metabolism, enzymes that contribute to the metabolism of drugs are also present at other sites, particularly in the intestine (11,12). The predominant biotransformation system in the intestine involves cytochrome P450 (13). The intestine contains several isoforms of the CYP1A, CYP2B, CYP2C, CYP2D, and CYP3A families (12–14). The ability of these cytochrome P450 isoforms to metabolize drugs is as important as that of enzymes in the liver. More importantly, small-intestinal cytochrome P450 provides the principal initial source of biotransformation for orally ingested xenobiotics. Small-intestinal cytochrome P450 provides the principal initial source of biotransformation for orally ingested xenobiotics. Small-intestinal cytochrome P450 is implicated in the phenomenon of first-pass metabolism, which prevents the absorption of drugs (15). Therefore, any modification of intestinal cytochrome P450 could have important effects on the bioavailability of xenobiotics (15).

No study has directly evaluated the effects of CRF on intestinal metabolism of drugs. However, several investigators have demonstrated that, among patients with CRF, the bioavailability of many drugs is increased (1). Because some of these drugs are subject to intestinal first-pass metabolism, the possibility that CRF could reduce the ability of the intestine to metabolize drugs cannot be excluded; this could explain, at least in part, the accumulation of drugs during CRF.

The objectives of this study were to determine the effects of CRF on intestinal cytochrome P450 and to define the mechanisms leading to its downregulation. For this purpose, we measured, in rats with CRF and control pair-fed rats, (1) total...
intestinal cytochrome P450 levels, (2) levels of the main cytochrome P450 isoforms involved in drug metabolism (i.e., CYP1A1, CYP2B1, CYP2C6, CYP2C11, and CYP3A2), as well as some of their specific metabolic activities, and (3) levels of the mRNA encoding some of these specific isoforms. Finally, the effects of CRF on intestinal cytochrome P450 were also studied in rats treated with a known inducer of cytochrome P450, namely dexamethasone (16).

Materials and Methods

Experimental Models

Male Sprague-Dawley rats (Charles River, Saint-Charles, Canada), weighing 200 to 300 g, were housed in the Research Center animal care facility and were maintained on a diet of Purina rat pellets and water ad libitum. An acclimatization period of at least 3 d was allowed before any experimental work was undertaken. All experiments were conducted according to the Canadian Council on Animal Care guidelines for the care and use of laboratory animals.

Studies were performed with two groups of 20 animals each, i.e., CRF and control pair-fed rats. To further evaluate the effects of CRF on the regulation of intestinal cytochrome P450, two other groups (n = 6 in each group) were studied, i.e., CRF rats and CRF rats treated with dexamethasone, which is potent inducer of intestinal cytochrome P450 (16).

CRF was induced by two-stage 5/6 nephrectomy, as described previously (9). Rats in the control group underwent two sham laparotomies (on days 1 and 7). After surgery, CRF animals were fed Purina rat chow and water ad libitum. Control pair-fed rats were fed the amount of rat chow that had been ingested by the CRF rats on the previous day. Body weights were measured every other day for the duration of the study. On day 41 after nephrectomy, the rats were housed in metabolic cages and urine was collected for 24 h, for determination of creatinine clearance. Rats were euthanized by decapitation 42 d after nephrectomy. Blood was collected for the measurement of serum creatinine and urea levels. Cytochrome P450 induction was achieved with intraperitoneal injections of dexamethasone (100 mg/kg daily) on days 38 to 41 (16).

Preparation of Intestinal Microsomes

Intestinal microsomes were prepared from enterocytes obtained by elution, using the previously published methods (17,18). Briefly, two-thirds of the small intestine (starting beyond the pyloric valve) was used. The segment was rinsed with solution A (1.5 mM KCl, 96 mM NaCl, 27 mM sodium citrate, 8 mM KH2PO4, 5.6 mM Na2HPO4, pH 7.4), filled with solution B [phosphate-buffered saline, pH 7.2, containing 1.5 mM ethylenediaminetetraacetate (EDTA), disodium salt, 3 U/ml heparin, sodium salt, and 0.5 mM dithiothreitol], immersed in a solution of phosphate-buffered saline (pH 7.2) containing 20% glycerol, and shaken for 15 min. The solution B and suspended cells were drained into a centrifuge tube. This process was repeated seven times to collect separated samples of eluted cells, which were washed with solution C (5 mM histidine, 250 mM sucrose, 0.5 mM EDTA, disodium salt, pH 7.0). The pellet of enterocytes was lysed by sonication and centrifuged for preparation of microsomes via differential centrifugation. The pellet containing the microsomes was stored at −80°C, in 0.1 M Tris (pH 7.4), 20% glycerol, 10 mM EDTA, until analysis.

Determination of Total Cytochrome P450 Levels

Microsomal protein contents were determined by using the method described by Lowry et al. (19). Total cytochrome P450 levels were determined from the difference spectrum of the reduced protein, according to a previously reported method (20).

Western Blot Analysis

The major cytochrome P450 isoforms implicated in the metabolism of drugs were assessed by Western blot analysis, as described previously (9). Eighty micrograms of protein were subjected to electrophoresis in a 7.5% polyacrylamide gel containing 0.1% sodium dodecyl sulfate (21,22). CYP1A1, CYP2B1, CYP2C6, CYP2C11, and CYP3A2 were detected by using polyclonal goat anti-rat CYP1A1, CYP2B1, CYP2C6, CYP2C11, and CYP3A2, respectively (Gentest Corp., Woburn, MA). Immune complexes were revealed with secondary antibody (swine anti-goat IgG; Biosource International, Camarillo, CA) coupled to peroxidase and the luminol derivative Lumilight Western blotting substrate (Roche Diagnostics, Laval, Canada). Immune reaction intensity was determined via computer-assisted densitometric analysis of exposed Biomax MR film (Eastman Kodak, Rochester, NY).

RNA Isolation and RT-PCR

After elution of intestinal cells, pools of enterocytes were suspended in solution C, and cell concentrations were evaluated with a hemacytometer. Aliquots of 50 × 106 cells (approximately one-sixth of the total pool) were removed from the pool and pelleted. The dry pellet was flash-frozen in liquid nitrogen and maintained at −80°C until RNA extraction. Total RNA was extracted with a RNeasy kit (Qiagen, Mississauga, Canada). RNA concentrations were determined by measurement of absorbance at 260 nm. RNA coding for CYP1A1, CYP2C11, and CYP3A2 was evaluated by quantitative RT-PCR analysis.

One microgram of total RNA was used to prepare cDNA by reverse transcription, using an Omniscript reverse transcription kit (Qiagen) and random primers from Invitrogen (Burlington, Canada). Quantitative PCR was performed by using Taq DNA polymerase (Qiagen) and aQuantumRNA Classic 18S kit (Ambion, Austin, TX), which enabled comparison of control and CRF samples via normalization to 18S rRNA contents.

Specific primer sets were designed for each isoform with the aid of the Jellyfish and BLASTN 2.2.1 computer programs (23). Primers were selected to discriminate between isoforms (e.g., CYP3A2 and CYP3A1) and between cDNA and genomic DNA. Figure 1 presents the sequences and positions of primers in the respective gene of each isoform, according to the sequences available in GenBank. PCR products obtained with these primers were sequenced with the fmol DNA cycle sequencing system (Promega, Madison, WI), to confirm the specificity of the primers. All primers were obtained from Sigma Chemical Co. (St. Louis, MO). The PCR were performed with a GeneAmp PCR system 2400 (Perkin-Elmer, Norwalk, CT), with 15-s incubation at 94°C, 15-s incubation at 55°C, and 30-s incubation at 72°C. PCR products were fractionated on a 1.5% agarose gel and detected with ethidium bromide staining. Quantification of band intensities was performed with an Alpha Imager 2200 (Alpha Innotech Corp., San Leandro, CA).

In Vitro Metabolism of Erythromycin

To evaluate the metabolic activity of CYP3A in intestinal microsomes, erythromycin N-demethylation was assessed with 1.0 mg of rat intestinal microsomes (from either control pair-fed or CRF animals), as described elsewhere (9).
Intestinal 7-Ethoxyresorufin O-Deethylase Activity

To evaluate the catalytic activity of CYP1A1, 7-ethoxyresorufin O-deethylase (EROD) activity was determined as described by Paine et al. (24). Briefly, 7-ethoxyresorufin (25 µM) was incubated with 0.5 mg of rat intestinal microsomes (from either control pair-fed or CRF animals) in the presence of a NADPH-generating system consisting of: 25 mM NADP, 0.25 M glucose-6-phosphate, and 25 U/ml glucose-6-phosphate dehydrogenase. The fluorescence of resorufin was measured by spectrofluorometry (excitation, 530 nm; emission, 585 nm).

Blood and Urine Chemical Analyses

Blood urea and creatinine levels and urinary creatinine levels were determined with an Hitachi 717 autoanalyzer (Boehringer Mannheim Canada, Laval, Canada).

Statistical Analyses

The results are expressed as mean ± SD. Differences between groups were assessed by using the unpaired t test or ANOVA. Significant ANOVA results were followed by Fisher’s least-significant difference, multiple-comparison procedure. The threshold for significance was $P < 0.05$.

Results

Biochemical Parameters and Body Weights for Control Pair-Fed and CRF Rats

Table 1 presents the biochemical parameters and body weight of the two groups of animals studied. Compared with control pair-fed animals, CRF rats exhibited higher plasma creatinine and urea levels and lower creatinine clearance values (reduced by 77%; $P = 3 \times 10^{-5}$). There was no difference in body weights between control pair-fed and CRF rats.

Intestinal Total Cytochrome P450 Levels in Control Pair-Fed and CRF Rats

Total cytochrome P450 levels were significantly reduced (by 32%) for CRF rats, compared with the control group ($P = 3 \times 10^{-5}$) (Table 1). Cytochrome P450 levels were positively correlated with creatinine clearance ($r^2 = 0.9$, $P < 0.001$). A similar correlation was observed between cytochrome P450 levels and blood urea and creatinine levels.

Protein Expression of Intestinal Cytochrome P450 Isoforms in Control Pair-Fed and CRF Rats

The levels of CYP1A1 and CYP3A2 were reduced by 43% ($P = 8 \times 10^{-6}$) and 71% ($P = 2 \times 10^{-8}$), respectively, in
Table 1. Characteristics of control pair-fed and CRF rats

<table>
<thead>
<tr>
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<th>Control (n = 20)</th>
<th>CRF (n = 20)</th>
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<tbody>
<tr>
<td>Body weight (g) b</td>
<td>308 ± 21</td>
<td>299 ± 46</td>
</tr>
<tr>
<td>Serum creatinine level (µM)b</td>
<td>54 ± 5</td>
<td>211 ± 117²</td>
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<tr>
<td>Creatinine clearance (µl/100 g body wt per min)b</td>
<td>377 ± 38</td>
<td>85 ± 42²</td>
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<tr>
<td>Serum urea level (mM)b</td>
<td>6.7 ± 2.1</td>
<td>48.0 ± 38.2²</td>
</tr>
<tr>
<td>Intestinal total cytochrome P450 level (nmol/mg protein)d</td>
<td>0.13 ± 0.01</td>
<td>0.09 ± 0.03²</td>
</tr>
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a Data are mean ± SD. CRF, chronic renal failure.

b Measurements were made 41 d after the first operation.

P < 0.001, compared with control pair-fed animals.

d Cytochrome 450 levels were measured in microsomes prepared on day 42.

CRF rats, compared with control pair-fed animals (Figure 2). However, the levels of CYP2B1, CYP2C6, and CYP2C11 were not modified in CRF rats, compared with control rats.

mRNA Encoding Intestinal Cytochrome P450 Isoforms in Control Pair-Fed and CRF Rats

To evaluate whether intestinal cytochrome P450 isoforms in CRF were downregulated secondary to a decrease in their synthesis or an increase in their degradation, mRNA encoding the different isoforms was evaluated by RT-PCR. Significant decreases in mRNA encoding the CYP1A1 and CYP3A2 isoforms (32 and 36%, respectively; P = 3 × 10⁻⁶) were observed for CRF rats, compared with control pair-fed animals (Figure 3). Therefore, the decrease in protein expression of different cytochrome P450 isoforms that is observed during CRF is secondary to reduced gene expression.

In Vitro Metabolism of Erythromycin and EROD Activity in Control Pair-Fed and CRF Rats

To determine the effects of cytochrome P450 reductions during CRF on the metabolism of drugs, in vitro erythromycin N-demethylation and EROD activities were assessed in intestinal microsomes. These enzymatic reactions are mediated primarily by the CYP3A and CYP1A families, respectively. The N-demethylation of erythromycin was decreased by >25% in rats with CRF, compared with control pair-fed animals (P = 0.02) (Figure 4A). EROD activity was decreased by 25% in CRF rats, compared with control pair-fed animals (P = 0.009) (Figure 4B).

Induction of Cytochrome P450 in CRF Rats with Dexamethasone

Although our results demonstrated that the decrease in intestinal cytochrome P450 in CRF was secondary to reduced gene expression, we were also interested in determining whether cytochrome P450 was still inducible, despite its inhibition by CRF. We studied the effects of dexamethasone (a potent inducer of the CYP3A family) on CYP3A2 protein expression and mRNA levels in CRF rats (Figure 5). In CRF rats, CYP3A2 levels were greatly enhanced by dexamethasone.
sone, secondary to upregulation of the mRNA encoding this protein.

Discussion

This study demonstrates that, in rats, CRF produces a marked decrease in intestinal total cytochrome P450 levels secondary to reduced protein expression of selected cytochrome P450 isoforms, namely CYP1A1 and CYP3A2. The principal mechanism underlying this downregulation involves reduction of the levels of mRNA encoding these proteins. The effects on drug metabolism by the intestine are important, because we observed a reduction in erythromycin biotransformation (mediated by the CYP3A family) and EROD activity (mediated by the CYP1A family). Although intestinal cytochrome P450 levels were decreased during CRF, dexamethasone regulation was still present, as demonstrated by the induction of CYP3A by dexamethasone.

Renal failure has generally been thought to decrease only the renal clearance of drugs (25). However, several studies have demonstrated that animals with CRF also exhibit decreased hepatic drug metabolism mediated by cytochrome P450 (5–9,26,27). Only selected cytochrome P450 isoforms, i.e., CYP2C6, CYP2C11, CYP3A1, and CYP3A2, are decreased in the liver (9,27). We recently demonstrated that, in rats with CRF, CYP2C11, CYP3A1, and CYP3A2 mRNA levels in the liver were significantly reduced. Our results also demonstrated that there was an association between lower mRNA and protein levels for those cytochrome P450 isoforms, suggesting reduced gene expression (9).

Although the liver plays a major role in drug metabolism, the intestine is also an important organ for the biotransformation of drugs (11,12). The effects of CRF on intestinal metabolism are unknown. However, several pharmacokinetic studies have revealed that the bioavailabilities of several drugs are reduced in CRF, suggesting a decrease in intestinal first-pass metabolism (1).

Our results demonstrate that, although there are major reductions in total intestinal cytochrome P450 levels in CRF rats, only selected isoforms (namely CYP1A1 and CYP3A2) are reduced (Figure 2). The metabolic consequences are important, because we observed a 30% decrease in the in vitro substrate metabolism mediated by these two isoforms (Figure 4). Knowledge of which isoforms are reduced in CRF is critical for prediction of which drugs might accumulate when used during CRF. Interestingly, CYP1A1 and CYP3A1/3A2 in rats correspond to CYP1A1 and CYP3A4 in human subjects. Because these isoforms are responsible for the metabolism of several drugs that are commonly used during CRF, patients could be at risk for drug accumulation and toxicity. Indeed, these results could explain why the bioavailabilities of erythromycin and tacrolimus (both metabolized by the CYP3A isoform) are reduced in CRF rats.
family in the intestine) are increased by >30% among patients with CRF and in rats with experimental renal failure (28,29). Furthermore, recent data suggested that patients with end-stage renal disease exhibit absolute reductions in baseline and induced (by rifampicin) CYP3A activity (as assessed with the erythromycin breath test) (30).

A number of studies indicated that intestinal function could be altered in CRF. Several enzymatic reactions performed by the intestinal mucosa were demonstrated to be decreased in rats with CRF. For example, sucrase and maltase activities were significantly reduced, whereas the activities of other dipeptidases were unchanged (31). More recently, Vaziri et al. (32) reported that enteric xanthine oxidase activity was decreased in animals with CRF. The results of the study presented here strongly support the hypothesis that CRF is associated with perturbations in intestinal function.

Our results demonstrated that there was an association between lower mRNA and protein levels for CYP1A1 and CYP3A2 isoforms (Figure 3). This finding suggests reduced gene expression. The mechanisms responsible for the selective decreases in the expression of some intestinal genes in CRF are not known. Further studies will require evaluation of the effects of uremia on the function of cytochrome 450 promoters. However, a number of studies have indicated that animals with CRF exhibit impaired protein synthesis, as a result of reduced mRNA levels, in rats (33,34). For example, hepatic lipase and insulin-like growth factor 1 receptor mRNA levels are decreased in hepatocytes and skeletal muscle, respectively, in rats with CRF (33,34). CRF is associated with sustained elevations in calcium levels in many cell types, including hepatocytes and skeletal muscle, and these high intracellular calcium levels seem to represent a major factor underlying cell dysfunction and reduced protein synthesis (35,36). However, the effects of intracellular calcium levels on the regulation of cytochrome P450 remain poorly defined (37,38). Whether increased intracellular calcium levels are implicated in the downregulation of intestinal cytochrome P450 in CRF remains to be studied.

In this study, downregulation of cytochrome P450 in CRF was overcome by dexamethasone, which is a potent inducer of CYP3A isoforms (Figure 5). This finding suggests that, although intestinal cytochrome P450 levels are decreased in CRF, dexamethasone regulation is preserved. The clinical significance of this finding remains to be defined. However, it could be anticipated that, in CRF, intestinal cytochrome P450 could be modulated by known cytochrome P450 inducers (e.g., steroids or phenobarbital).

There seems to be a correlation between the decrease in drug metabolism and the severity of renal failure among human subjects (39). Interestingly, in rats, we observed significant correlations of the decrease in renal function with the reduction in intestinal cytochrome P450 levels, as well as with the reductions in cytochrome P450 activity (erythromycin N-demethylation and EROD activities). These results suggest that, as CRF worsens, patients are at risk of drug accumulation and toxicity secondary to reductions in metabolism.

In conclusion, CRF is associated with decreases in intestinal levels of cytochrome P450 isoforms (mainly CYP1A1 and 3A2), secondary to reduced mRNA levels, in rats. Drug metabolism, as assessed with the N-demethylation of erythromycin and EROD activity, is also depressed in CRF rats. Intestinal cytochrome P450 downregulation is correlated with the degree of renal failure. This decrease could explain the reductions in intestinal drug metabolism observed among patients with CRF, because these isoforms (especially CYP1A1 and CYP3A2) correspond to human CYP1A and CYP3A4, which are responsible for the metabolism of several drugs commonly used to treat patients with CRF.

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