Effects of Chronic Renal Failure on Kidney Drug Transporters and Cytochrome P450 in Rats

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Received February 24, 2011; accepted April 27, 2011

ABSTRACT:

Chronic renal failure (CRF) leads to decreased drug renal clearance due to a reduction in the glomerular filtration rate. However, little is known about how renal failure affects renal metabolism and elimination of drugs. Because both depend on the activity of uptake and efflux by renal transporters as well as enzymes in tubular cells, the purpose of this study was to investigate the effects of CRF on the expression and activity of select renal drug transporters and cytochrome P450. Two groups of rats were studied: control and CRF (induced by 5/6 nephrectomy). Compared with control rats, we observed reductions in the expression of both protein and mRNA of Cyp1a, sodium-dependent phosphate transport protein 1, organic anion transporter (Oat) 1, 2, and 3, OatK1/K2, organic anion-transporting polypeptide (Oatp) 1 and 4c1, P-glycoprotein, and urate transporter 1, whereas an induction in the protein and mRNA expression of Mrp2, 3, and 4 and Oatp2 and 3 was observed. Cyp3a expression remained unchanged. Similar results were obtained by incubating a human proximal tubule cell line (human kidney-2) with sera from CRF rats, suggesting the presence of uremic modulators. Finally, the renal elimination of [14C]benzylpenicillin was decreased in CRF rats, compared with controls, as shown by a 4- and 9-fold accumulation, respectively, of these drugs in kidneys of rats in CRF. Our results demonstrate that CRF affects the expression and activity of several kidney drug transporters leading to the intrarenal accumulation of drugs and reduced renal clearance that could, at least partially, explain the tubular toxicity of many drugs.

Introduction

The kidneys play a major role in the elimination of endogenous and exogenous compounds from the bloodstream by the following three processes: glomerular filtration, tubular secretion, and tubular reabsorption. It has been known for decades that chronic renal failure (CRF) reduces the renal clearance of filtered drugs and endogenous substances through a decrease in the glomerular filtration rate. However, little is known about how renal failure affects renal secretion and reabsorption in the proximal tubule. Both these processes are dependent on the activity of uptake and efflux renal transporters expressed in tubular cells (Inui et al., 2000; Russel et al., 2002; Sekine et al., 2006; El-Sheikh et al., 2008; Feng et al., 2010; Giacomini et al., 2010; Masereeuw and Russel, 2010; Nagai and Takano, 2010).

Tubular transporters are members of two families: ATP-binding cassette transporters and solute carriers. The former include multidrug-resistance related proteins (Mrp) and P-glycoprotein (P-gp), which depend on ATP phosphorylation. They are efflux transporters expressed either on the apical brush-border membrane where they excrete drugs in the urine or on the basolateral membrane where they participate in the reabsorption of compounds from the urine. On the other hand, solute carrier transporters include the sodium-dependent phosphate transport protein 1 (Npt1), organic anion transporters (Oat), organic anion transporting polypeptides (Oatp), and urate transporters (URAT) and are mostly, but not exclusively, uptake transporters located on either the apical or basolateral membrane where they participate in the uptake of compounds from the bloodstream. On the other hand, solute carrier transporters include the sodium-dependent phosphate transport protein 1 (Npt1), organic anion transporters (Oat), organic anion transporting polypeptides (Oatp), and urate transporters (URAT) and are mostly, but not exclusively, uptake transporters located on either the apical or basolateral membrane where they participate in the uptake of compounds from the bloodstream or from the urine into the tubular cell. The most important drug transporters are illustrated in Fig. 1 (Hsiang et al., 1999; Russel et al., 2002; Masereeuw and Russel, 2010).

Recently, we have demonstrated that CRF can affect the expression of intestinal P-gp and Mrp2 and hepatic P-gp and Oatp2 in rats, thus

ABBREVIATIONS: CRF, chronic renal failure; CTL, control; P450, cytochrome P450; Mrp, multidrug resistance-related protein; Npt1, sodium-dependent phosphate transport protein 1; Oat, organic anion transporter; Oatp, organic anion-transporting polypeptide; Papp, apparent permeability; PCR, polymerase chain reaction; P-gp, P-glycoprotein; PMSF, phenylmethylsulfonyl fluoride; URAT, urate transporter; HK-2, human kidney-2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
increasing the bioavailability and decreasing the biliary elimination of various drugs (Naud et al., 2007, 2008). We have also demonstrated that CRF can decrease extrarenal elimination of drugs by decreasing intestinal and hepatic drug metabolism by cytochrome P450 (P450) and phase II drug metabolism (Leblond et al., 2000, 2001, 2002; Simard et al., 2008). Finally, we have shown a nearly 100% decrease in the urinary clearance of rhodamine 123, a P-gp substrate, in nephrectomized rats (Naud et al., 2007, 2008). Thus, it is possible that CRF could affect drug transport and metabolism in the diseased kidney.

To date, very few studies have been published concerning the effects of CRF on renal drug transport. Ji et al. (2002) demonstrated a decrease in the protein expression of the organic cation transporter 2 2 weeks after rats were submitted to 5/6 nephrectomy, whereas Oat1 and Oat3 expression remained unchanged. In addition, Laouari et al. (2001) showed an increase in both the protein and mRNA expression of Mrp2 6 weeks post-5/6 nephrectomy, whereas P-gp expression remained stable.

The objective of this study was to investigate the effects of CRF on the expression and activity of the major renal drug transporters and cytochrome P450. For this purpose, we have compared renal protein and mRNA expression in CRF and control rats of 1) Cyp1a, Cyp3a, Npt1, Oat1, 2, and 3, Oatp1, 2, 3, and 4c1, Mrp2, 3, and 4, P-gp, and URAT1 and 2) the in vivo renal accumulation of [3H]digoxin and [14C]benzylpenicillin. Furthermore, we have studied the effects of uremic serum on the expression of drug transporters and cytochromes P450 in a proximal tubule cell line as well as on the activity of P-gp in the same cell line.

Materials and Methods

Experimental Model. Male Sprague-Dawley rats (Charles River, Portage, MI), weighing 176 to 225 g, were housed in the Research Center animal care facility and maintained on Harlan Teklad rodent diet (Harlan Teklad Global Diets, Montreal, QC, Canada) and water ad libitum. Animals were allowed an acclimatization period of at least 7 days before the first nephrectomy was performed. All the experiments were conducted according to the Canadian Council on Animal Care guidelines for the care and use of laboratory animals and under the supervision of our local animal care committee.

Experimental Protocol. Studies were performed in two groups of animals: control pair-fed and CRF. The expression levels of protein and mRNA of kidney drug transporters were measured in 12 rats per group. Drug transporter activity was measured in vivo through the renal accumulation of [3H]digoxin and [14C]benzylpenicillin in at least six rats per group per experiment.

Chronic renal failure was induced by two-stage 5/6 nephrectomy as described previously (Leblond et al., 2001). Every animal had free access to water, but to limit the effects of CRF-induced malnutrition, control pair-fed rats were fed the same amount of chow that CRF rats ate on the previous day. We have previously demonstrated that the calorie reduction in control animals induced by pair feeding did not influence the expression of drug-metabolizing enzymes (Leblond et al., 2001) and drug transporters (our unpublished data). At day 41 after nephrectomy, the rats were housed in metabolic cages, and urine was collected for a 24-h period to determine the clearance of creatinine. Rats were sacrificed by decapitation 42 days after nephrectomy for organ and blood collection, or underwent a radiolabeled drug accumulation study. After the animals were sacrificed, the remnant kidney of CRF rats and the left kidney of CTL rats were immediately excised, rinsed in ice-cold saline, and flash frozen in liquid nitrogen. Samples were stored at −80°C until membrane preparation or mRNA extraction was performed. Blood was collected for the measurement of serum creatinine and urea and for bioassays with proximal tubule cells.

Preparation of Crude Kidney Membranes. Rat kidney biopsies (200 mg) were homogenized in 1 ml of homogenizing buffer (250 mM sucrose, 10 mM HEPES, and 10 mM Tris-HCl, pH 7.4) containing 0.1 mM phenylmethylsulfonyl fluoride (PMSF) using a Potter-Elvehjem tissue grinder (Wheaton Scientific Products, Millville, NJ). Differential centrifugation was used to obtain a crude membrane fraction (Chow et al., 2010). In brief, the kidney homogenates were centrifuged at 9000g for 10 min at 4°C. The supernatant was then spun at 33,000g for 60 min at 4°C, and the resulting pellet was resuspended in phosphate-buffered saline containing 0.1 mM PMSF. Samples were then sonicated on ice for 10 s to ensure homogeneity. Protein concentration was determined using the method of Lowry et al. (1951), using bovine serum albumin as a reference protein. Aliquots were stored at −80°C until use in Western blot analysis.

Western Blot Analysis. Major renal drug transporters were assessed by Western blot analysis following a previously described protocol (Leblond et al., 2002; Naud et al., 2007, 2008). Protein expression in control animals was arbitrarily defined as 100%.

P-gp and Mrp2 were detected using monoclonal antibodies from ID Labs (London, ON, Canada), C219 and M, III-6, respectively. Npt1, Oat2, Oat3, OatK1/K2, Oatp2, and Oatp3 were detected using antibodies from Alpha Diagnostic International (San Antonio, TX). Anti-Mrp3 was from Sigma-Aldrich (St. Louis, MO), anti-Mrp4 was from Novus Biological (Littleton, CO), anti-Oat1 and anti-URAT1 were from Abbiotec (San Diego, CA), anti-Oatp1 was from Millipore Corporation (Billerica, MA), and anti-OatpC1 was from Santa Cruz Biotechnology (Santa Cruz, CA). Cyp1a and Cyp3a were
detected using primary antibodies from US Biological (Swampscott, MA) and Daichi Pure Chemicals (Tokyo, Japan), respectively. Villin-1, a protein specific to tubular brush-border cells (Cartier et al., 1993), was used as a loading control, was detected using rabbit anti-Villin-1 from Cell Signaling Technology (Beverly, MA). The antibodies used recognized rat and human proteins.

mRNA Analysis. Total RNA was extracted from frozen tissue using TRIzol reagent (Invitrogen, Burlington, ON, Canada) according to the manufacturer’s protocol. RNA concentration was determined by measuring absorbance at a wavelength of 260 nm. One microgram of total RNA was used to prepare cDNA by reverse transcription using the SuperScript VILO cDNA Synthesis kit from Invitrogen. The mRNA encoding for P-gp (mrp1a), Mrp2, Oatp2, and villin-1 was measured by quantitative real-time PCR as described previously (Naud et al., 2007, 2008). Other genes were measured using appropriate TaqMan gene expression assays from Applied Biosystems (Carlsbad, CA). Table 1 shows the TaqMan gene expression assays used for the quantification of mRNA for each transporter. PCR products were analyzed using the ΔΔC_{T} method (Livak and Schmittgen, 2001) using villin-1 as a standard gene.

In Vivo Intrarenal Accumulation of Radiolabeled Drugs. The intrarenal accumulation of radiolabeled drugs was used to determine the activity of the kidney drug transporters. Two different drugs were tested. [14C]Benzenepenicillin is an antibiotic and a known substrate for Oat3 and Mrp3 (Windsass et al., 2007; Choi et al., 2009). [3H]Digoxin is a cardiac glycoside and a known substrate for Oatp4c1 and P-gp (Mikkaich et al., 2004; Yamaguchi et al., 2010). Digoxin (0.5 mg/kg) was administered via intraperitoneal injection, and benzenepenicillin (5 mg/kg CTL, 1 mg/kg CRF) was administered via intramuscular injection. Both were injected with an approximate 1:1500 hot/cold ratio so the milligram amount of radioactive compound wouldn’t factor into the final dosage calculation. To evaluate renal accumulation, similar plasma concentrations of drugs need to be attained, and therefore CRF rats received a lower dose of benzepenicillin because of its reduced clearance and short-term accumulation in CRF. For digoxin, there is no short-term accumulation in patients with CRF, thus patients with CRF receive the same loading dose as patients with normal renal function with an adjustment in their maintenance dose (Jelliffe, 1968, 1969; Gault et al., 1980). Because this study with rats requires a single loading dose, no digoxin dose adjustment was necessary to obtain plasma concentrations similar to those observed in CTL rats. Rats were anesthetized using isoflurane 2 h after injection, and a cardiac puncture was performed to recover the maximal amount of blood in heparinized tubes to obtain plasma. Remnant left kidneys were harvested, rinsed in ice-cold phosphate-buffered saline, weighed, minced, and placed in 2 ml of Solvable solution (PerkinElmer Life and Analytical Sciences, Waltham, MA) at 56°C overnight. Dissolved kidney volumes were noted, and radioactivity was counted on a Wallac 1409 beta counter (PerkinElmer Life and Analytical Sciences) in 1 ml of dissolved kidney after addition of 4 ml of ScintiSafe Plus Scintillator (Fisher, Ottawa, ON, Canada). Plasma radioactivity was counted in 2 ml of plasma with 4 ml of Scintillator. The results were calculated as a ratio between the radioactivity count (counts per minute) for 1 g of kidney and the radioactivity count for 1 ml of plasma. The mean for the CTL groups was arbitrarily defined as 100%.

**Effects of Uremic Serum on Kidney Drug Transporters.** To determine whether uremic serum could affect the expression and function of kidney drug transporters, two sets of experiments were conducted using human kidney-2 (HK-2) cells, a proximal tubule cell line. The first studied the effects of uremic serum on drug transporters’ protein expression, whereas the second studied the effects of uremic serum on P-gp activity.

**Incubation of HK-2 Cells with Uremic Serum.** HK-2 cells were purchased from American Type Culture Collection (Manassas, VA) and seeded into 25-mm² uncoated polystyrene flasks (Sarstedt, St. Leonard, QC, Canada) in Dulbecco’s modified Eagle’s Medium (Invitrogen) containing 10% fetal bovine serum (PAA Laboratories, Inc., Etobicoke, ON, Canada). The cells were subcultured 4 days later into two 75-mm² uncoated polystyrene flasks and expanded by 1:3 subcultures every 3 to 4 days. For an experiment, culture medium was replaced by Dulbecco’s modified Eagle’s medium containing 10% CTL or CRF rat serum (one serum per flask). After 48 h of culture, the cells were trypsinized, pelleted, and homogenized in phosphate-buffered saline containing 0.1 mM PMSF. Protein concentrations were determined using the Micro BCA Protein Assay kit from Thermo Fisher Scientific (Waltham, MA). One hundred micrograms was loaded for Western blot analysis of each studied transporter, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a loading control. It was detected using rabbit anti-GAPDH from Abcam Inc. (Cambridge, MA).

**Rhodamine 123 Transport on HK-2 Cells.** The effects of uremic serum on P-gp function was evaluated in HK-2 cells using the trans-epithelial transport of rhodamine 123 across HK-2 monolayers. Cells were subcultured into 12-well, 0.4-μm pore size polycarbonate transwell filters (PET Transwell chambers (Becton Dickinson, Mississauga, ON, Canada) and allowed up to 7 days to reach confluence and form a polarized monolayer. Forty-eight hours before the transport experiment, culture medium was replaced by culture medium containing 10% CTL or CRF rat serum. The transport experiment was conducted as reported previously with Caco-2 cells (Naud et al., 2007).

**Other Assays.** Blood and urine chemistries were determined with an Architect C1600 clinical analyzer (Abbott Laboratories, Saint-Laurent, QC, Canada).

**Statistical Analysis.** Results are expressed as mean ± S.D. Differences between groups were assessed using an unpaired Student’s t test or an analysis of variance test. Significant analysis of variance was followed by a Scheffe post hoc comparison of groups. The threshold of significance was p < 0.05.

**Results**

**Biochemical Parameters and Body Weight in Control and CRF Rats.** Table 2 presents the biochemical parameters and body weights of both groups of rats. Compared with control animals, CRF rats had higher levels of serum creatinine and urea and lower creatinine clearance (reduced by 80%, p < 0.001). There was no difference in body weight between control and CRF rats.

**Protein Expression of Kidney Drug Transporters and Cytochrome P450 in CRF Rats.** Figure 2 presents the measured protein expression of select drug transporters and cytochrome P450 from a crude kidney membrane preparation from control and CRF rats. The level of expression of Mrp2, 3, and 4 and of Oatp2 and 3 were significantly increased by more than 2-fold in CRF rat kidneys compared with control rat kidneys as shown by Western blot. On the other

### TABLE 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>TaqMan Gene Expression Assay</th>
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<tr>
<td>Cyp1a1</td>
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<tr>
<td>Cyp3a1</td>
<td>Rn01412395_g1</td>
</tr>
<tr>
<td>Mrp3</td>
<td>Rn00587866_m1</td>
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<tr>
<td>Mrp4</td>
<td>Rn01465702_m1</td>
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<td>Rn00590562_m1</td>
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<tr>
<td>URAT1</td>
<td>Rn01479631_m1</td>
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</table>

### TABLE 2

Characteristics of control and CRF rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 20)</th>
<th>CRF (n = 20)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>342.7 ± 30.9</td>
<td>327.4 ± 55.1</td>
<td>0.29</td>
</tr>
<tr>
<td>Serum creatinine (μmol/l)</td>
<td>62.5 ± 4.0</td>
<td>229.5 ± 86.2</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Creatinine clearance (μl/min/100 g of b.wt)</td>
<td>313.1 ± 49.9</td>
<td>63.7 ± 35.4</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Serum urea (mmol/l)</td>
<td>4.5 ± 1.0</td>
<td>38.2 ± 19.0</td>
<td></td>
</tr>
</tbody>
</table>
hand, the expression of Cyp1a, Npt1, Oat1, Oatp1, Oatp4c1, P-gp, and URAT1 was significantly reduced from 50 to 85% in CRF rats compared with controls. The only measured protein that remained stable was Cyp3a. We found significant correlations \( p < 0.05 \) between protein expression and creatinine clearance for Cyp1a \( r = 0.787 \), Mrp2 \( r = -0.662 \), Mrp3 \( r = -0.719 \), Mrp4 \( r = -0.626 \), Npt1 \( r = 0.687 \), Oat1 \( r = 0.716 \), Oat2 \( r = 0.720 \), Oat3 \( r = 0.723 \), OatK1/K2 \( r = 0.861 \), Oatp1 \( r = 0.717 \), Oatp2 \( r = -0.586 \), Oatp3 \( r = -0.684 \), Oatp4c1 \( r = 0.757 \), P-gp \( r = 0.811 \), and URAT1 \( r = 0.603 \).

### mRNA Expression of Kidney Drug Transporters and Cytochrome P450 in CRF Rats

Figure 3 presents the results of the analysis of mRNA coding for kidney transporters and cytochrome P450. There was a significant increase in the mRNA expression of Mrp2, 3, and 4 \( (40, 92, \text{ and } 72\%, \text{ respectively}) \) and of Oatp2 and 3 \( (125 \text{ and } 180\%, \text{ respectively}) \) in CRF rats compared with controls. On the other hand, there were significant decreases in the mRNA expression of Cyp1a \( (95\%) \), Npt1 \( (45\%) \), Oat1 \( (75\%) \), Oat2 \( (95\%) \), Oat3 \( (70\%) \), Oatp1 \( (90\%) \), Oatp4c1 \( (90\%) \), Oatp5 \( (95\%) \), P-gp \( (50\%) \), and URAT1 \( (75\%) \) in CRF rat kidneys compared with controls. Only Cyp3a mRNA remained stable between the groups.

### In Vivo Accumulation of Radiolabeled Drugs in the Kidney of Rats with Surgically Induced Chronic Renal Failure

Two hours after administration, plasma radioactivity of digoxin was similar in the control and CRF rats, whereas the plasma radioactivity of benzylpenicillin was slightly elevated by 17% in CRF rats compared with control animals (Fig. 4). There was a 9-fold greater accumulation of \[^{14}C\]benzylpenicillin and a 4-fold accumulation of \[^{3}H\]digoxin in the kidney of CRF rats, compared with control rat kidneys. These data were obtained via a ratio of radioactivity for 1 g of kidney/1 ml of plasma.

### Protein Expression of Renal Drug Transporters and Cytochrome P450 in HK-2 Cells Incubated with the Serum of CRF Rats

Protein expression of select drug transporters and cytochrome P450 was measured in HK-2 cells, a proximal tubule cell line, incubated for 48 h with serum from control or CRF animals (Fig. 5). There was a significant decrease in the protein expression of Cyp1a \( (45\%) \), Npt1 \( (30\%) \), Oat3 \( (30\%) \), Oatp1 \( (25\%) \), and P-gp \( (35\%) \) when incubated with CRF serum compared with control serum. On the other hand, the expression of Mrp2, Mrp4, and Oatp2 was significantly increased by 45, 35, and 30%, respectively. Only the expression of Cyp3a remained unaltered. The expression of the reporter protein GAPDH was unchanged between both groups. These results were similar in directional change to those obtained in vivo in CRF rats.

### Apical-to-Basolateral Transport of Rhodamine 123 in HK-2 Cells Incubated with the Serum of CRF Rats

The effect of uremic serum on P-gp function was evaluated in HK-2 cells using the transepithelial transport of rhodamine 123 across HK-2 monolayers (Fig. 6). We found a nearly 50% increase in the apical-to-basolateral apparent permeability \( (P_{app}) \) of rhodamine 123 across HK-2 cells preincubated with uremic sera compared with control. Moreover, the addition of P-gp inhibitor cyclosporine did not affect the \( P_{app} \) of rhodamine 123 in cells incubated with uremic sera but caused a great increase in permeability in HK-2 cells preincubated with control sera.

### Discussion

The purpose of this study was to evaluate the effect of chronic renal failure on the expression and activity of select drug transporters and cytochrome P450 in the rat kidney. We have demonstrated that CRF affects drug transporters in different manners. The protein and mRNA expression of many transporters were significantly reduced (Npt1, Oat1, Oat2, Oat3, OatK1/K2, Oatp1, Oatp4c1, P-gp, and URAT1), whereas those of Mrp2, Mrp3, Mrp4, Oatp2, and Oatp3 were significantly up-regulated. In addition, the protein and mRNA expression of Cyp1a were significantly reduced, whereas those of Cyp3a remained unchanged.

These results differ from those previously published by Ji et al. (2002), who had shown no changes in the expression of Oat1 and Oat3 2 weeks after nephrectomy. We hypothesize that 2 weeks was too short to fully develop the long-term effects of renal failure. Our results also confirm and differ from those obtained by Lauuari et al. (2001), who had demonstrated an increase in the protein and mRNA expression of Mrp2 3 and 6 weeks after nephrectomy, but no change in the expressions of P-gp at 3 weeks, and an increase in P-gp mRNA after 6 weeks. We confirmed the results obtained for Mrp2 expression, but we have demonstrated a 50% decrease in P-gp protein and mRNA expressions 6 weeks after nephrectomy. The reasons for this discrepancy remain unclear.

We found significant correlations between the clearance of creatinine and the protein expression of transporters and Cyp1a, indicating that changes in transporter expression are directly related to the
severity of renal failure. We also found significant correlations between the protein and mRNA expression of transporters and Cyp1a (data not shown), suggesting that changes in protein expression are the result of a decrease in mRNA levels. In addition, we have demonstrated that the modifications in drug transporter expression and activity occurred in response to one or many factors present in the sera from uremic rats, as demonstrated by the changes in protein expression of transporters and P450 enzymes in HK-2 cells, which are similar to those observed in vivo, although they are of smaller magnitude, probably due to the shorter length of exposure to uremic toxins.

Several uremic toxins accumulate in renal failure. They are breakdown products of metabolic pathways that are no longer being eliminated by the kidneys. Those that were most studied in relation to drug transport are indoxyl sulfate, para-aminohippuric acid, and 3-carboxy-4-methyl-5-propyl-2-furan-propanoic acid. They are known substrates and inhibitors of organic anion transporters including Oat1, Oat3, and Mrps (Leier et al., 2000; Deguchi et al., 2002, 2004, 2005; Tsutsumi et al., 2002; Sun et al., 2006; Enomoto and Niwa, 2007) and even play a role in the progression of renal failure as demonstrated by the induction of renal damage by indoxyl sulfate (Niwa and Ise, 1994; Niwa et al., 1994, 1997; Sun et al., 2006). The down-regulation of uptake transporters Oat1, Oat2, and Oat3 and of bidirectional transporters OatK1/K2, Oatp1, Oatp4c1, and URAT1 combined with the up-regulation of efflux transporters Mrp2, Mrp3, and Mrp4 and of bidirectional transporters Oatp2 and Oatp3, all of which are anion transporters, could thus be an adaptive and protective response against uremic toxin accumulation and renal toxicity. Still, these changes do not seem to be sufficient to prevent the accumulation of organic anions as demonstrated by the 9-fold accumulation of \(^{14}C\)-benzylpenicillin, a substrate of OATs and Mrps, in the kidneys of animals suffering from CRF compared with controls and thus could probably be insufficient to prevent the progression of renal failure by uremic toxin toxicity. Competitive inhibition of the transporters by uremic toxins could explain this phenomenon.

Although most efflux transporter expression was increased in the current study, P-gp expression and its in vitro activity were significantly reduced by chronic renal failure and uremic sera. P-gp differs from most other studied transporters as it transports mostly cationic or neutral substrates (Masereeuw et al., 2000). Thus, we have evaluated the in vivo accumulation of the P-gp substrate \(^{3}H\)digoxin. Digoxin is also a substrate of the uptake transporter Oatp4c1 (Mikkaichi et al., 2004). Although plasma concentrations of digoxin were similar between the control and CRF animals, there was a significant 4-fold increase in the kidneys of CRF rats compared with controls.

**Fig. 3.** mRNA expression of select kidney drug transporters and cytochrome P450 in CRF rats. mRNA encoding renal drug transporters and P450 enzymes in control and CRF rats were measured by quantitative real-time PCR. mRNA levels are expressed in relative quantities using the \(\Delta\Delta C_T\) method (Livak and Schmittgen, 2001) with villin-1 as a reference gene. The mean relative quantity of control rats was arbitrarily defined as 100% (dotted line). The graphic shows the mean expression in CRF rats expressed as percentage of controls ± S.D. of 12 rats in each group. *<p<0.05; **<p<0.01; ***<p<0.001 compared with control rats.

**Fig. 4.** In vivo accumulation of radiolabeled drugs in the kidney of rats with surgically induced chronic renal failure. The kidney/plasma radioactivity (counts per minute) ratio for 1 g of kidney/1 ml of plasma in CTL (open bars) and CRF (filled bars) rats, after a 2-h latency period after drug injection, is shown. CRF rats received one-fifth of the CTL rats’ dose of benzylpenicillin but had a 17% (<p<0.05) higher plasma radioactivity of benzylpenicillin, whereas, with equal dosing between the groups, the digoxin plasma radioactivity was similar in the CTL and CRF rats although it had a tendency to be approximately 15% increased in CRF, but this result did not reach statistical significance (data not shown). The mean ratio for CTL animals was arbitrarily defined as 100%. Data are expressed as mean ± S.D. of at least six rats in each group. **<p<0.01; ***<p<0.001 compared with CTL rats.
increase in the intrarenal accumulation of digoxin in CRF kidneys. This indicates a reduced secretion of digoxin most likely due to the reduced expression of its efflux transporter P-gp. This result could be reproduced in vitro by the incubation of a HK-2 monolayer with sera from uremic rats where we demonstrated a significant increase in the \( P_{\text{app}} \) of rhodamine 123 from the apical to the basolateral side of the monolayer. This argues in favor of a decrease in the transport activity of P-gp, facilitating the migration of rhodamine 123 toward the basolateral side. The addition of the P-gp inhibitor cyclosporine to both sides of the monolayer caused an increase in the \( P_{\text{app}} \) of rhodamine in the CTL group, demonstrating that rhodamine 123 transport is P-gp-dependent, but induces no significant change in the CRF group, indicating a very weak P-gp activity even before the addition of cyclosporine. This could explain the nearly 100% reduction in rhodamine 123 urinary clearance that we reported previously (Naud et al., 2008) and strongly suggests that a factor present in uremic serum has a modulatory effect on P-gp protein expression and activity, similar to what we previously showed in the intestine and liver (Naud et al., 2007, 2008).

We also measured the protein and mRNA expression of Cyp1a and Cyp3a in the kidneys of control and CRF rats. They are drug-metabolizing enzymes with wide substrate variability. CYP3A is implicated in the biotransformation of nearly 60% of drugs metabolized by P450 enzymes in humans, such as acetaminophen, cyclosporine, erythromycin, etc. (Guengerich, 2003), whereas Cyp1a is mostly implicated in the biotransformation of environmental pollutants such as polycyclic aromatic hydrocarbons and tobacco smoke or substances such as caffeine and tamoxifen (Guengerich, 2003). In the kidney, Cyp1a and Cyp3a are expressed in the tubular cells, where they can activate or deactivate their substrates before urinary secretion. We have demonstrated a significant decrease in the expression of Cyp1a in CRF that could cause intrarenal toxicity of Cyp1a-active substrates, if they were to accumulate in the kidney because of reduced drug transporters. On the other hand, Cyp3a expression seems unaffected by CRF that would allow normal activation or deactivation of substrates within the renal tubular cells. Toxicity could occur if activated metabolites were to remain trapped inside the tubular cells because of reduced drug transport.

In conclusion, this study demonstrates that CRF significantly alters the expression and activity of select renal drug transporters that could contribute to reduced renal clearance as well as renal drug accumulation and nephrotoxicity. The modifications in drug transporters

![Fig. 5. Protein expression of select renal drug transporters and cytochrome P450 in HK-2 cells incubated with the serum of CRF rats. Protein bands are expressed in densitometry units. The densitometry units measured for transporters and P450 enzymes were standardized by dividing them by the value obtained for GAPDH. The standardized densitometry units of control rats were arbitrarily defined as 100% (dotted line). The graphic shows the mean expression in CRF rats expressed as percentage of controls ± S.D. of 10 different sera in each group. * \( p < 0.05 \); ** \( p < 0.01 \); *** \( p < 0.001 \) compared with control rats.]

![Fig. 6. Apical-to-basolateral transport of rhodamine in HK-2 cells incubated with the serum of CRF or CTL rats. \( P_{\text{app}} \) of rhodamine across a HK-2 cell monolayer preincubated with sera from control (open bars) and CRF (filled bars) rats, in the absence or presence of the P-gp inhibitor cyclosporine, is shown. \( P_{\text{app}} \) was determined using the equation \( P_{\text{app}} = (dC/dt)/(AC_0) \), where \( dC/dt \) represents the rate at which the substrate appears in the receiving (basolateral) compartment, \( A \) is the monolayer surface, and \( C_0 \) is the initial substrate concentration in the donor (apical) compartment. The mean \( P_{\text{app}} \) of controls without cyclosporine was arbitrarily defined as 100%. Data are presented as the mean compared with controls ± S.D. of eight different sera in each group. NS, no significant difference.]

\[ \text{Protein expression of select renal drug transporters and cytochrome P450 in HK-2 cells incubated with the serum of CRF rats. Protein bands are expressed in densitometry units. The densitometry units measured for transporters and P450 enzymes were standardized by dividing them by the value obtained for GAPDH. The standardized densitometry units of control rats were arbitrarily defined as 100% (dotted line). The graphic shows the mean expression in CRF rats expressed as percentage of controls ± S.D. of 10 different sera in each group. * \( p < 0.05 \); ** \( p < 0.01 \); *** \( p < 0.001 \) compared with control rats.} \]
induced by uremic serum were similar to that produced by CRF in our animal model, suggesting that uremic toxins such as indoxyl sulfate, para-aminomipippuric acid, and 3-carboxy-4-methyl-5-propyl-2-furanopropanic acid, which are known to modulate drug transporters (Leier et al., 2000; Deguchi et al., 2002, 2004, 2005; Tsutsumi et al., 2002; Sun et al., 2006; Enomoto and Niwa, 2007), could be responsible for the alterations in renal transporters in vivo.

Authorship Contributions

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References


