EFFECT OF CHRONIC RENAL INSUFFICIENCY ON HEPATIC AND RENAL UDP-GLUCURONYLTRANSFERASES IN RATS

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ABSTRACT:

Significant evidence exists regarding altered CYP450 enzymes in chronic renal insufficiency (CRI), although none exists for the phase II enzymes. The objective of this study was to investigate the effect of CRI on hepatic and renal UDP-glucuronosyltransferase (UGT) enzymes. Three groups of rats were included: CRI induced by the 5/6th nephrectomy model, control, and control pair-fed (CPF) rats. UGT activities were determined in liver and kidney microsomes by the 3- and 17-glucuronidation of β-estradiol (E2-3G and E2-17G), glucuronidation of 4-methylumbelliferone (4-MUG), and 3-glucuronidation of morphine (M3G). UGT isoforms responsible for these catalytic activities were screened using recombinant rat UGT1A1, UGT1A2, UGT1A3, UGT1A7, UGT2B2, UGT2B3, and UGT2B8. UGT protein levels were examined by Western blot analysis using polyclonal antibodies. There was no significant difference between CRI and CPF rats in hepatic and/or renal E2-3G (UGT1A1), E2-17G (UGT2B3), 4-MUG (UGT1A6), and M3G (UGT2B1) formation. Formation of E2-17G and 4-MUG in the liver and E2-3G and 4-MUG in the kidney was significantly reduced (p < 0.05) in CPF and CRI rats compared with control rats. The down-regulated glucuronidation activities were accompanied by corresponding reductions in protein content of specific UGT isoforms. These results suggest that CRI does not seem to influence the protein levels or catalytic activity of most of the major hepatic or renal UGT enzymes. The observed down-regulation of hepatic and renal UGTs in CRI and CPF rats could be caused by restricted food intake in these groups of rats.

UDP-glucuronosyltransferase (UGT) enzymes are a major class of phase II enzymes that catalyze the conjugation of glucuronic acid to many drugs and certain endogenous compounds (Monteith et al., 1990; Tephly and Burchell, 1990; Miners and Mackenzie, 1991; Jansen et al., 1992). The water-soluble glucuronides are readily eliminated from the body via biliary and renal excretion (Fisher et al., 2001). UGT enzymes are a family of membrane-bound enzymes mainly located in the rough endoplasmic reticulum (ER) of mammalian liver and various extrahepatic tissues, including the gastrointestinal tract, the kidney, and olfactory epithelium (Tukey and Strasser, 2000). Drug glucuronidation is influenced by many factors, including age, disease states, smoking, diet, drug therapy, etc. (Miners and Mackenzie, 1991).

Chronic renal insufficiency (CRI) is a condition in which there is a progressive loss of renal function. Many diseases can cause CRI, including diabetes, hypertension, polycystic kidney disease, rapidly progressive glomerulonephritis, renal disposition diseases, and renal vascular disease. Other risk factors include a family history of kidney diseases, increasing age, pregnancy, and medications. In patients with CRI, many types of renal functions are affected, including glomerular blood flow and filtration, tubular secretion, reabsorption, and renal parenchymal mass. The kidney is one of the major organs responsible for the elimination of drugs and their metabolites from the body. Therefore, it is not surprising that in CRI, the kinetics of drugs that are primarily eliminated by the kidney could be significantly modified. Hepatic function has often been assumed to be unchanged in patients with CRI compared with patients with normal renal function. However, there is significant evidence that the disposition of highly metabolized drugs could also be altered in CRI (Gibson, 1986; Touchett and Slaughter, 1991). For example, the β-blocking agent bufuralol is extensively metabolized by oxidation and conjugation in the body. After an oral dose, there was a 5-fold increase in the AUC value of patients with severe renal failure compared with healthy volunteers.

ABBREVIATIONS: UGT, UDP-glucuronosyltransferase; ER, endoplasmic reticulum; CRI, chronic renal insufficiency; AUC, area under the curve; 4-MU, 4-methylumbelliferone; UDPGA, UDP-glucuronic acid; HPLC, high-performance liquid chromatography; CPF, control pair-fed; E2-3G, β-estradiol 3-glucuronide; E2-17G, β-estradiol 17-glucuronide; ODS, octadeylsilane; 4-MUG, 4-methylumbelliferone glucuronide; M3G, morphine 3-glucuronide; LOD, limit of detection; LOQ, limit of quantification.
(Balant et al., 1980). Another example is the anthelmintic drug zidovudine, which is primarily eliminated in the liver by glucuronidation. Singhla et al. (1989) demonstrated that after a single oral dose of zidovudine, the mean AUC values were significantly higher in the renal failure group (11.7 μmol·L⁻¹·h) than in the normal population (5.2 μmol·L⁻¹·h). Because the increase in AUC can be fully explained by the decrease in renal clearance (Cl₀ren), it is possible that hepatic glucuronidation of zidovudine may be decreased in renal failure. Because a number of extensively glucuronidated drugs have a narrow therapeutic window (e.g., zidovudine, morphine), altered glucuronidation is likely to have important clinical consequences (Miners and Mackenzie, 1991). In rats induced with renal failure using the nephrectomy model, Ali et al. (1979) showed that the rate of glucuronidation of phenolphthalein but not 4-nitrophenol by liver was significantly (p < 0.05) reduced. However, the specific UGT isoforms affected by renal dysfunction and the exact mechanism underlying this phenomenon have not been systematically studied.

The UGTs are classified into two families, UGT1 and UGT2, in both rats and humans (Tukey and Strassburg, 2000). In the rat UGT1 family, UGT1A1 and UGT1A6 are two important isoforms responsible for the glucuronidation of endogenous compounds (bilirubin, estradiol, etc.) and xenobiotic small planar phenols (4-methylumbelliferone, 4-nitrophenol), respectively. Of the rat UGT2 family, UGT2B1 is an important isoform involved in the glucuronidation of steroids (Narayanan et al., 2000). Most UGT isoforms are not only expressed in the liver but also appear to have a distinct pattern of extrahepatic expression, particularly in the kidney, gastrointestinal tract, lung, etc. (Fisher et al., 2001). Kidneys from different species have shown glucurononyltransferase activities toward various compounds (Anders, 1980; Coughtrie et al., 1987; Peters and Jansent, 1988; Le Guellec et al., 1995). Therefore, the kidney may not only be a major excretory organ but also might play an important role in the glucuronidation of drugs. Therefore, it is especially necessary to examine whether renal glucurononyltransferase activities are influenced in subjects with renal dysfunction.

The specific aims of this study were to determine the possible effect of CRI on hepatic and renal UGTs and to elucidate a possible mechanism to explain this phenomenon if observed. These aims were carried out by first characterizing glucuronidation activities of recombinant rat UGTs (UGT1A1, UGT1A2, UGT1A3, UGT1A7, UGT2B3, and UGT2B8) toward estradiol, 4-methylumbelliflere (4-MU), and morphine. This effect was further investigated by determining hepatic and renal UGT catalytic activity and protein content in rats surgically induced with CRI as well as control and control pair-fed animals.

**Materials and Methods**

**Materials.** 4-MU, β-estradiol, morphine, their glucuronides, and UDP-glucuronic acid (UDPGA) were purchased from Sigma Chemical (St. Louis, MO). All reagents used for HPLC analysis were HPLC grade and purchased from Fisher Scientific Products (Pittsburgh, PA). Rat recombinant UGT enzymes and antibodies were prepared as previously described (Ritter et al., 1999; Miles et al., 2005).

**Induction of Chronic Renal Insufficiency by the 5th Nephrectomy Method (Remnant Kidney Model).** Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) (n = 13), weighing 150 to 200 g, were housed in the animal care facility in Virginia Commonwealth University, Richmond, VA, and allowed to acclimate for 7 days before surgical procedures. The surgical protocol was approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee.

CRI was induced in rats by the method published previously by our laboratory (Rege et al., 2003). Animals were randomly divided into three groups: control (Control) (n = 4), control pair-fed (CPF) (n = 5), and CRI (n = 4). CRI was induced by the 5th nephrectomy method in which two-thirds of left kidney and the complete right kidney were excised. CPF animals were subjected to a sham operation in which animals underwent the same surgical procedures without the removal of kidney mass. They were fed the same amount of food as the weight-matched CRI animals based on the food intake of the CRI animals from the previous day. Rats in the control group were allowed free access to food and were not subjected to a surgical procedure. Five weeks after the surgeries, animals were sacrificed, and the liver and kidney were removed and stored at −70°C until preparation of microsomes. Microsomes were prepared from liver and kidney tissue by the differential centrifugation method as described previously by Sarkar et al. (1992). Microsomes were suspended in a buffer containing 10 mM Tris acetate (pH 7.4), 20% (v/v) glycerol, and 1 mM ethylenediamine tetraacetate (EDTA) and stored at −70°C. Total microsomal protein concentrations were determined using the Bio-Rad Protein Assay kit (Bio-Rad, Hercules, CA).

**UGT Catalytic Activity. Incubation Conditions.** UGT1A1, UGT1A6, and UGT2B1 catalytic activities were evaluated in microsomal incubations using estradiol (King et al., 1996), 4-MU (Falany and Tephly, 1983), and morphine (Pritchard et al., 1993) as probe substrates, respectively. To investigate the specificity of these probe substrates, estradiol, 4-MU, and morphine glucuronidation activity of recombinant rat UGT1A1, UGT1A2, UGT1A3, UGT1A7, UGT2B2, UGT2B3, and UGT2B8 enzymes expressed in HepG2 cells were also measured. The concentration of microsomal protein UDPGA and incubation time were optimized in preliminary experiments (data not shown) to ensure glucuronidation experiments were carried out under initial rate conditions. The ER luminal localization of the active site of UGT proteins results in the phenomenon of microsomal latency, which can be overcome by adding detergents, albumin, or lipids to disrupt the membrane (Fisher et al., 2001). Triton X-100 was used in these experiments to remove the latency and observe optimal enzyme efficiency (data not shown). Substrate concentrations were determined based on the kinetic profiles of enzymatic reactions (data not shown).

The enzyme activity assay mixture (250 μl) contained 50 mM Tris–HCl (pH 7.4), MgCl₂ (10 mM for the estradiol assay, 5 mM for the 4-MU and the morphine assays), microsomal protein or cell lysate expressed with rat UGT isoform (1.0 mg/ml for estradiol and morphine assays, 0.4 mg/ml for 4-MU assay), 0.02% Triton X-100, 3 mM UDPGA, and substrate (150 μM estradiol, 1000 μM 4-MU, or 20 μM morphine). After preincubation at 37°C for 3 min, the reaction was initiated by addition of UDPGA and was incubated at 37°C for 30 min (estradiol and morphine) or 15 min (4-MU). Then the reaction was terminated by addition of 5 μl of 70% perchloric acid.

**Analytical Method.** The proteins were removed by centrifugation at 14,000 rpm for 5 min, and a portion of the supernatant (100 μl for estradiol assay and 20 μl for 4-MU and morphine assay) was injected into a HPLC system for analysis.

**Estradiol Assay.** Estradiol (E2) and its metabolites, estradiol 3- (E2-3G) and 17-glucuronide (E2-17G), were separated on a HPLC system previously described by BD Gentest (Woburn, MA) with slight modification. The HPLC system was equipped with a Waters Alliance 2690 separation module and column oven (45°C), a 5-μm, 4.6 × 250-mm ODS column (Beckman, CA) and a Brownlee RP-18 guard column (Perkin Elmer, CT). Mobile phase A was 10% methanol. Mobile phase B was 100% acetonitrile. Mobile phase C was 30% acetonitrile and 1 mM perchloric acid. Initial mobile phase composition was 85% mobile phase A, 0% mobile phase B, and 15% mobile phase C. Elution of E2, E2-3G, and E2-17G was carried out by an increase in mobile phase B to 85% over 15 min, whereas mobile phase C remained constant during the run. The flow rate was 1.0 ml/min. UV detection was at 280 nm for estradiol and its metabolites.

**4-Methylumbelliflere (4-MU) Assay.** The glucuronide metabolite of 4-MU was determined by dilution of the supernatant 5-fold before directly injecting into the HPLC column. 4-MU and 4-MU glucuronide (4-MUG) were analyzed according to the method published by Narayanan et al. (2000) with slight modification. The separation was achieved on a 5-μm, 250 × 3.0-mm Luna-CN column (Phenomenex, CA) with a Brownlee CN guard column (Perkin Elmer). The mobile phase consisted of acetonitrile (solvent A) and 20 mM ammonium phosphate buffer (pH 4.5) (solvent B). 4-MU and 4-MUG
were resolved using the following gradient: \( t = 0 \) min, A% = 90; \( t = 0.5 \), A% = 70%; \( t = 4.5 \), A% = 90; total run time = 10 min. The flow rate was held at 0.5 ml/min. 4-MU and 4-MUG were detected by fluorescence detection with excitation wavelengths at 290 nm and emission wavelengths at 400 nm.

**Morphine Assay.** Morphine and morphine 3-glucuronide (M3G) were separated based on a previously published method (Innocenti et al., 2001), on a 5-\( \mu \)m, 250 \( \times \) 4.6-mm ODS column and a guard column. The mobile phase consisted of 73.5% of 10 mM sodium phosphate in 1 mM sodium dodecyl sulfate (pH 2.1) and 26.5% of acetonitrile. The flow rate was 1.0 ml/min. Morphine and M3G were detected by fluorescence detection with the excitation wavelength at 210 nm and the emission wavelength at 340 nm.

Estradiol, 4-MU, and morphine metabolites were quantified by standard curves of glucuronides. All the analytical methods were validated according to Food and Drug Administration’s Guidelines for Bioanalytical Method Validation and were well within the acceptance criteria (+15%). The limit of detection (LOD) and quantification (LOQ) were determined to be 0.29 and 0.98 \( \mu \)M for E2-3G, respectively. The LOD and LOQ were determined to be 0.13 and 0.45 \( \mu \)M for E2-17G, respectively. The lowest calibration standards of 4-MUG and M3G were determined to be the experimental LOQ, which was 5 \( \mu \)M.

**Western Blot Analysis.** The protein content of UGT1A1, UGT1A6, UGT1A7, total UGT1A, and UGT2B were assessed in liver and kidney microsomes using polyclonal antibodies specific for rat UGT1A1, human UGT1A6, rat UGT1A7, rat UGT1A, and UGT2B common region. Microsomal protein (15 \( \mu \)g) was loaded onto an 8.5% SDS-polyacrylamide gel electrophoresis gel and resolved under constant voltage (70 V) for 2.5 h at room temperature. The resolved protein samples and molecular weight marker were transferred to a nitrocellulose membrane (Bio-Rad). The nitrocellulose membranes were blocked with 5% nonfat dry milk in Tris-buffered saline-Tween buffer and immunoblotted with primary antibody for specific UGT isoform and secondary antibody coupled with horseradish peroxidase (sheep anti-mouse IgG from J.K.R.). The membranes were detected by Western Lightning Chemiluminescence Reagent Plus (PerkinElmer Life and Analytical Sciences, Inc. MA). The optical density of Western blot bands was determined using ImageQuant 5.2 software (Molecular Dynamics, CA).

**Statistical Analysis.** All results were reported as mean ± standard deviation (S.D.). One-way analysis of variance was used to evaluate differences between the groups at an alpha level of 0.05. If the differences were significant, then a comparison of means between all groups was carried out using Tukey-Kramer’s test. The statistical analysis was performed using JMP statistical software v4.2.6 (SAS Institute, Cary, NC). Although data has not been included for the purposes of brevity, Michaelis-Menten parameters were estimated for each metabolic pathway using nonlinear regression method.

**Results**

**Biomedical Parameters and Body Weight in CPF and CRI Rats.** Based on the previously published data by our laboratory (Rege et al., 2003), serum creatinine and urea nitrogen levels were increased by 98% \(( p < 0.01)\) and 250% \(( p < 0.01)\), respectively, in the CRI animals compared with the CPF and control groups. At the end of week 5 of the study, food intake and body weight of the rats in the CRI groups were reduced by 37% \(( p < 0.01)\) and 35% \(( p < 0.01)\), respectively, compared with control rats. Rats in the CPF group had similar decrease in body weight as CRI rats while being fed similar amounts of food.

**Glucuronidation by Recombinant Rat UGT Enzymes.** UGT1A1 and UGT1A7 isoforms were involved in the glucuronidation of estradiol at 3-position as shown in Fig. 1A. UGT1A1 had approximately 2-fold higher estradiol 3-glucuronidation activity (213.08 pmol/min/mg protein) than UGT1A7 (101.62 pmol/min/mg protein). UGT1A2, UGT2B2, and UGT2B3 showed glucuronidation activity toward estradiol at 17-position as shown in Fig. 1B. Estradiol was glucuronidated at the 17-position with highest activity by UGT2B3 (695.29 pmol/min/mg protein) and to lesser extent by UGT1A2 (249.49 pmol/min/mg protein) and UGT2B2 (65.04 pmol/min/mg protein). All UGT1A isoforms tested showed glucuronidation activity toward 4-MU as shown in Fig. 1C. 4-MU was glucuronidated largely by UGT1A7 (1857.39 pmol/min/mg protein) and to a much lesser extent by UGT1A1 (103.63 pmol/min/mg protein), UGT1A2 (299.71 pmol/min/mg protein), and UGT1A3 (11.17 pmol/min/mg protein). Morphine 3-glucuronidation was below the limit of detection and not measurable with any of the recombinant hepatic UGT enzymes investigated (data not shown).

**Effect of CRI on Renal and Hepatic UGT Catalytic Activities.** The rates of formation of estradiol, 4-MU, and morphine glucuronide metabolites in liver and kidney microsomes from CRI, CPF, and control rats are shown in Table 1. The formation of E2-17G and M3G was not detectable in the kidney microsomes from any animal. E2-3G glucuronide was below the limits of quantitation in the CPF and CRI animals. There were no significant differences in glucuronidation activity of liver or kidney microsomes toward estradiol, 4-MU, or morphine between the CPF and CRI groups. Although not statistically significant, hepatic estradiol 3-glucuronidation was increased by 24% \(( p = 0.09)\) in the CRI group compared with CPF group (Table 1).
hepatic E2-3G formation was similar between CPF (185.13 ± 31.35 pmol/min/mg) and the control group (174.67 ± 28.22 pmol/min/mg). Compared with the control rats, hepatic glucuronidation of 4-MU and estradiol at the 17-position and renal glucuronidation of 4-MU and estradiol at the 3-position was significantly (p < 0.05) down-regulated in both the CPF and CRI groups. Hepatic M3G formation, although not statistically significant, tended to be lower in the control group compared with the CPF (p = 0.06) and CRI (p = 0.07) groups.

**Effect of CRI on Hepatic and Renal UGT Protein Expression.** Western blot analysis of total UGT2B and 1A protein levels is shown in Fig. 2. Rat livers had much higher levels of UGT2B protein than UGT1A protein, and these levels were much higher in the liver compared with kidney (Fig. 2). The immunoblot bands showed that UGT2B and UGT1A protein expression in the livers was not obviously altered in the CPF and CRI groups compared with the control group. The intensity of the UGT2B and UGT1A immunoblots appeared to be lower in the CPF and CRI kidneys compared with the levels in the control animals. There was no significant difference in either hepatic or renal UGT1A1, UGTA16, and UGT1A7 protein content between the CPF and CRI groups (Fig. 3). UGT1A6 was significantly (p < 0.05) down-regulated in the liver as well as the kidney from CPF and CRI animals compared with the control group. In the kidney, UGT1A1 and UGT1A6, but not UGT1A7, expression decreased in the CPF and CRI groups compared with the control rats. The decreases in protein expression for renal UGT1A1 and UGT1A6 significantly correlated with decreases in renal E2-3G formation (r² = 0.88, p < 0.0001) (Fig. 4B) and renal 4-MUG formation (r² = 0.77, p < 0.0001) (Fig. 4D). The protein content of UGT1A1 and UGT1A6 exhibited weak correlation with E2-3G formation (r² = 0.28, p = 0.06) (Fig. 4A) and moderately correlated with 4-MUG formation (r² = 0.43, p = 0.02) (Fig. 4C) in rat liver. There was no correlation found between the amount of UGT1A7 and E2-3G protein or 4-MUG formation, either in the liver or the kidney (not shown).

**Discussion**

This study has demonstrated that CRI had no significant effect on either UGT catalytic activities or protein content in rat liver or kidney. CRI was successfully induced in rats as confirmed by elevated serum creatinine and serum urea nitrogen levels in the CRI animals compared with the CPF and control groups. Furthermore, some degree of malnutrition was observed in the CRI rats based on reduced food intake and body weight compared with normal rats. Because malnutrition itself is known to alter drug metabolism (Krishnaswamy and Naidu, 1977; Mandl et al., 1995; Walter-Sack and Klotz, 1996), CPF rats were included. The CPF rats lost a similar amount of body weight as the CRI rats, which suggests that the reduced body weight of the CRI rats was caused by decreased food intake.

Recently, more evidence is emerging that not only renal elimination but also hepatic clearance of drugs could be impaired in patients with renal diseases (Gibson, 1986; Touchette and Slaughter, 1991). One possible mechanism underlying this phenomenon could be a change in hepatic intrinsic clearance in CRI. CRI induced in rats has been shown to down-regulate specific CYP450 enzymes in the liver (Leblond et al., 2000, 2001; Rege et al., 2003). In the present study, we examined the effect of CRI on UGT enzymes in rat liver and kidney using estradiol, 4-MU, and morphine as probe substrates for UGT1A1, UGT1A6, and UGT2B1, respectively. UGT1A1 plays an important physiological role in glucuronidation of endogenous substrates, such as bilirubin and estrogens, as well as flavonoids (Senafi et al., 1994). Rat and human UGT1A1 have been shown to be functionally identical (King et al., 1996). UGT1A6 is a major human UGT1A enzyme that is predominantly expressed in the liver but also found in the kidney and other extrahepatic tissues (Ritter, 2000). UGT2B1 is the corresponding isoform for UGT2B7 in rats (Tukey and Strassburg, 2000). UGT2B7 is a major human UGT2B isoform that is highly expressed in the liver/extrahepatic tissues and glucuronidates various compounds, including androgens, estrogens, catechol estrogens, and opioid compounds (Turgeon et al., 2001). UGT2B1 is the corresponding isoform for UGT2B7 in rats (Tukey and Strassburg, 2000).

**Estradiol Metabolism.** Estradiol is glucuronidated at the 3-position by UGT1A1 and at the 17-position by several UGT2B enzymes (Lepine et al., 2004). By screening a panel of recombinant rat UGT
enzymes, we found that in addition to UGT1A1, UGT1A7 was active in E2-3G formation (Fig. 1A). UGT2B3 is the major isoform responsible for E2-17G formation, although UGT2B2 and UGT1A2 also had a minor role (Fig. 1B). In the present study, we used estradiol 3-glucuronidation as a probe for UGT1A1 and E2-17G formation as a surrogate probe for UGT2B in the same sample (Table 1).

There was no difference in hepatic estradiol 17-glucuronidation or 3-glucuronidation between the CPF and CRI groups, which indicates that CRI did not influence hepatic UGT1A1 and UGT2B activity. Hepatic E2-3G formation tended to be higher in the CPF and CRI groups, perhaps to compensate for the decreased E2-17G formation. These results also indicate that estradiol is glucuronidated at the 3- and 17-positions by different enzyme systems in the liver, which might be regulated separately in the liver of CPF and CRI rats.

Renal E2-3G formation, on the other hand, was significantly downregulated in CPF and CRI rats compared with control rats (only...
measurable in normal control rats). The lack of detectable E2-17G metabolites in the renal tissue of all animals suggests that UGT2B3 and UGT1A2 are not expressed in the rat kidney.

The Western blot results revealed that the expression of UGT1A1 and UGT1A7 proteins (Fig. 3), similar to the catalytic activity, were not affected by CRI in either the liver or the kidney. The down-regulated UGT1A1 but not UGT1A7 protein expression suggests that the reduced E2-3G formation in the kidney of CPF and CRI rats was due to decreased UGT1A1 protein amount, which was further verified by the high correlation between the UGT1A1 protein level and E2-3G formation in the kidney (Fig. 4). Because the isoform-specific antibody for rat UGT2B was not available, only total UGT2B protein content was measured using the antibody for the UGT2B common region. The total UGT1A protein content was determined as well. The band density suggests that the protein amount of UGT2B in rat liver could potentially be higher than that of UGT1A. The Western blot results also revealed that UGT2B2s could be expressed at a lower level in the liver than in the kidney (Fig. 2), which is consistent with the relative mRNA expression level of UGT2B2s in rat liver and kidney reported by Shelby et al. (2002). This observation is also supported by E2-17G formation, suggesting UGT2B is the main enzyme responsible for this metabolite. The role of other UGT enzymes cannot be ruled out however. Because the CPG group did not undergo renal surgery and the common treatment effect between the two groups was reduced food intake, it seems that food restriction may affect hepatic and renal glucuronidation of estradiol.

4-MU Metabolism. To examine UGT1A6 catalytic activity, glucuronidation of 4-MU, a probe substrate for human or rat UGT1A6 (Narayan et al., 2000; Hanikoa et al., 2001), was determined (Table 1). When comparing the CRI group to the CPG group, CRI itself did not have an effect on 4-MU glucuronidation in either the liver or the kidney. Interestingly, food restriction seemed to reduce both the hepatic and renal formation of 4-MUG (40–54% decrease, p < 0.05) in CPF and CRI groups compared with the control group. 4-MU glucuronidation activity was also determined using the same set of recombinant rat UGT enzymes (Fig. 1C). UGT1A7 turned out to have high 4-MU glucuronidation activity. The Western blot results showed that the protein content of UGT1A6 and UGT1A7 was not different between the CPF and CRI groups, which is similar to the activity results. Protein expression of UGT1A6, but not UGT1A7, was down-regulated in the liver and kidney of CRI and CPG groups compared with the control group. It suggests that although 4-MU is glucuronidated by both UGT1A6 (Iwano et al., 1999) and UGT1A7 (Fig. 1C), only UGT1A6 contributes to the down-regulation of 4-MU glucuronidation by food restriction. This was further confirmed by a significant correlation between 4-MU glucuronidation activity and UGT1A6, but not UGT1A7, protein expression (Fig. 4). The formation of 4-MUG was better correlated with UGT1A6 protein levels in the kidney than in the liver, suggesting that UGT1A6 is the primary isoform responsible for 4-MUG formation in the kidney; however, other isoforms cannot be ruled out.

Morphine Metabolism. Morphine is a known substrate for both rat UGT2B1 and human UGT2B7 (King et al., 2000). Morphine is metabolized predominantly to two glucuronide isomers: M3G and M6G (Ishii et al., 1994). Only M3G is efficiently formed in both humans and rats (King et al., 1996). In this study, the formation of M3G was determined (Table 1). There was no measurable M3G formation in kidney microsomal incubations, which is consistent with the results reported by Brunelle and Verbeck (1996). CRI did not affect hepatic glucuronidation of morphine at the 3-position, whereas the formation of M3G was decreased by ~24% in the CPF (p = 0.06) and the CRI (p = 0.07) groups compared with the control group. Although not statistically significant, the trend suggests that food restriction is associated with reduced M3G formation. Morphine is known to be glucuronidated by rat UGT1A1 and UGT2B1 at low and high rates, respectively (Ishii et al., 1994; King et al., 1997). In our study, none of the recombinant rat UGT enzymes, including UGT1A1, showed measurable glucuronidation activity toward morphine. Although the role of UGT2B1 in M3G formation could not be confirmed in this study, the role of UGT2B is implied from the observation that protein expression of UGT2B enzymes, although similar to morphine glucuronidation activity, was nonstatistically changed in the liver of CPF and CRI rats compared with control rats.

In summary, our results suggest that neither hepatic nor renal UGT enzymes were affected directly by renal insufficiency. However, food restriction seemed to be responsible for reduction in the rate of formation of hepatic E2-17G (UGT2B3) and 4-MUG (UGT1A6), along with renal E2-3G (UGT1A1) and 4-MUG. The down-regulated glucuronidation activities were accompanied by corresponding reductions in protein content of specific UGT isoforms. Because food restriction was found to selectively down-regulate hepatic and/or renal UGT enzyme protein repression, it can be speculated that the modified hepatic drug glucuronidation in some CRI patients could be due to the malnutrition arising from compromised renal function. These observations need to be verified in a well controlled study.

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ARE HEPATIC AND RENAL UGTS AFFECTED IN RATS BY CRI?