Down-Regulation of Hepatic CYP3A in Chronic Renal Insufficiency

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Received February 20, 2003; accepted June 9, 2003

Purpose. The objective of this study was to investigate the mechanisms underlying the decrease in hepatic clearance of some drugs metabolized by CYP450 enzymes in chronic renal insufficiency (CRI).

Methods. CRI was induced in male Sprague-Dawley rats (n = 7) by the remnant kidney model (RKM); control animals (C) (n = 12) underwent sham surgery, of which n = 6 rats were pair-fed (CPF) with CRI rats and others (n = 6) had free access to food. Serum creatinine (S_{cr}) and urea nitrogen (SUN) were monitored every 2 weeks. On day 36, livers were isolated, and microsomes were prepared. Catalytic activities were measured through O-demethylation (CYP2D) and N-demethylation of dextromethorphan (CYP3A) and O-deethylation of 7-ethoxyresorufin (CYP1A2). CYP450 protein and mRNA levels were also measured.

Results. Compared with CPF, S_{cr} and SUN levels in CRI rats were increased twofold ($p < 0.01$) and 2.5-fold ($p < 0.01$), respectively. No effect on CYP1A2 and CYP2D activities, mRNA, or protein levels was observed between the groups. There was a reduction (41.8 ± 20%, $p < 0.01$) in CYP3A activity, mRNA ($p < 0.05$), and protein levels ($p < 0.05$) in CRI rats compared to CPF.

Conclusions. CRI induced by RKM does not have an effect on hepatic CYP1A2 and CYP2D enzymes but does reduce CYP3A activity, probably through down-regulation of CYP3A2.

KEY WORDS: remnant kidney model; chronic renal insufficiency; cytochrome P450; hepatic metabolism.

INTRODUCTION

Chronic renal insufficiency (CRI) is the ninth leading cause of death in the United States and one of the costliest illnesses in the country. The pharmacokinetics of drugs that are primarily eliminated by the renal route are known to be significantly perturbed in CRI. The importance of renal function in drug clearance is well recognized, and dosage adjustments are frequently necessary for these drugs, particularly if they have a narrow therapeutic index. Hepatic elimination of drugs, on the other hand, has generally been assumed to be unchanged in patients with CRI when compared to individuals with normal renal function. However, there is significant evidence accumulating that suggests that reduction in hepatic clearance of drugs can occur as a result of renal failure (1). Clinical studies of drugs primarily eliminated by hepatic metabolism in CRI patients have shown a five- to sixfold increase in AUC values, resulting from corresponding decreases in their metabolic clearances (1–4). This phenomenon may lead to greater systemic availability for orally administrated drugs that exhibit high extraction ratios. This phenomenon is even more common in drugs that are substrates of cytochrome P450 (CYP450) enzymes (1,5).

Inhibition of hepatic metabolism in CRI patients is highly clinically relevant. For example, bufuralol exhibited approximately 5-fold increased concentrations in CRI patients, resulting in increased incidences of adverse effects, prolonged β blockade, and greater variability (2). In another study, accumulation of erythromycin as a result of altered hepatic clearance led to ototoxicity in some CRI patients (4). Despite the clinical relevance and prevalence of this problem, the underlying mechanisms are still unknown. In the present study, we have attempted to explain the possible mechanisms for the effect of CRI on hepatic metabolism using an animal model for renal insufficiency, the remnant kidney model (RKM). Among various experimental models such as puromycin aminonucleoside nephrosis, Heymann nephritis, uranyl nitrate–induced renal interstitial fibrosis, or adriamycin-induced glomerulopathy; RKM is the most widely used model to study CRI. It differs from other models in that the renal insufficiency is not induced chemically but by surgical removal of kidney mass, which progressively induces focal and segmental glomerulosclerosis (6). Furthermore, histopathologic changes in nephrons resulting from this model closely resemble nephritic syndrome in humans. Previously published animal studies utilizing the RKM as a means to induce renal insufficiency have shown reduction in hepatic CYP450 content and catalytic activities (7–10). However, only recently have attempts been made to study the mechanisms underlying this phenomenon. The primary goal of the present study was to systemically evaluate the effect of chronic renal insufficiency on CYP450 activities using isoform-specific probes.

The specific aims were (a) to set up and validate an animal model by inducing CRI using the RKM (6); (b) to study the effect of CRI on hepatic CYP2D, CYP3A, and CYP1A2 functional activities; and (c) to investigate the effect of CRI on mRNA and protein expression of CYP450 enzymes using reverse transcriptase–polymerase chain reaction (RT-PCR) and Western blot analysis, respectively.

MATERIALS AND METHODS

Chemicals

Dextromethorphan (DM), dextrorphan (DR), levallorphan, 3-methoxy morphinan (MM), 7-ethoxyresorufin (ER), resorufin (R), and β-nicotinamide adenine dinucleotide reduced form (β-NADPH) were obtained from Sigma Chemical Co. (St. Louis, MO). Ether used for anesthesia was obtained from Fisher Scientific (Pittsburgh, PA). All reagents used for the HPLC analysis were analytic grade and were obtained from VWR Scientific Products (Bridgeport, NJ).

Animals

Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA, USA) (n = 19), weighing 150 to 200 g,

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ABBREVIATIONS: DM, dextromethorphan; DR, dextrorphan; ER, 7-ethoxy resorufin; R, resorufin; RKM, remnant kidney model; HPLC, high performance liquid chromatography; CRI, chronic renal insufficiency.

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were housed in the animal care facility in Virginia Commonwealth University (VCU), Richmond, VA. The animals were allowed to acclimatize for 7 days before any surgical procedures. The protocol was approved by the VCU Institutional Animal Care and Use Committee (IACUC), and the procedures were conducted according to their guidelines.

Induction of CRI by Remnant Kidney Model

Studies were performed in three groups of animals: control (C) (n = 6), control pair-fed (CPF) (n = 6), and CRI (n = 7). CRI was induced by a two-stage five-sixths nephrectomy method in which two-thirds of the left kidney was excised, followed by complete right nephrectomy after 7 days. All the surgical procedures were done under ether anesthesia. The control group of animals (C and CPF) underwent sham operations in which animals were subjected to same surgical procedures except the removal of kidney mass. After the surgery, all the animals were maintained on a powdered commercial diet (Harlan Laboratory Chow, Indianapolis, IN, USA) and water *ad libitum*. Food intake by CRI animals was monitored every day, and the same amount of food was fed to weight-matched CPF animals. The animals in the C group had free access to food and water. Body weight of animals was monitored every day for the entire duration of the study.

Blood (0.5 ml) was drawn every 2 weeks from each rat through the right external jugular vein under light ether anesthesia. It was allowed to coagulate for 20 min and then centrifuged at 8000 *g* to collect serum as the supernatant. Induction of renal insufficiency was monitored by measuring serum creatinine (S_{cr}) and serum urea nitrogen (SUN) on the day of the sampling. SUN was measured using a commercially available kit from Sigma Diagnostics (St. Louis, MO), and S_{cr} was measured using Beckman Creatinine Analyzer (Beckman Instruments, Fullerton, CA, USA).

On day 36, after the second surgery, the animals were sacrificed by exsanguination under ether anesthesia, and the liver was removed and stored at −80°C until preparation of microsomes. Microsomes were prepared from the liver tissues by differential centrifugation. All the samples were maintained at 4°C during microsomal preparation. The microsomes were stored at −80°C in Tris 0.1M (pH 7.4) buffer containing 20% glycerol and 10 mM ethylenediamine tetraacetate (EDTA) until enzyme analysis. Total microsomal protein concentration was determined using Bicinchoninic Acid Protein Assay Kit from Pierce Chemical Co. (Rockford, IL).

Determination of CYP2D, CYP3A, and CYP1A2 Catalytic Activity

CYP2D and CYP3A catalytic activity was measured in the same incubation mixtures using dextromethorphan O-demethylation (DOD) and N-demethylation (DND), respectively. The corresponding metabolites, dextrorphan (CYP2D) and 3-methoxymorphinan (CYP3A), formed after microsomal incubations were analyzed using a previously published method (11). These two metabolites have recently been established as simultaneous probes for both enzyme activities (12,13). A typical microsomal incubation included microsomal protein, phosphate buffer (0.1 M, pH 7.4), and substrate (DM). The reaction was initiated by addition of β-NADPH (1 mM). The reaction was terminated by addition of 70% perchloric acid. The samples were centrifuged at 6000 *g* for 10 min, and the supernatant was used for analysis of the metabolites. CYP1A2 catalytic activity was measured through 7-ethoxyresorufin O-deethylation (EROD) to form resorufin. The rate of formation of resorufin was monitored spectrofluorometrically at excitation and emission wavelengths of 570 nm and 597 nm, respectively. The microsomal incubation procedure for EROD assay was similar to DOD/DND assay except that frozen acetonitrile was used to terminate the reaction. The microsomal incubation conditions such as microsomal protein concentration and time period of incubation were optimized. Michaelis-Menten parameters were estimated for each metabolic pathway using nonlinear regression method by WINNONLIN® software (V2.11, Pharsight Corp., CA).

Western Blot Analysis

Changes in protein content were assessed in all three groups of animals for the following CYP450 enzymes: CYP2D1, CYP3A1, CYP3A2, and CYP1A2. Microsomal protein (30 μg) was electrophoresed on 12% polyacrylamide gel containing 0.1% SDS. The separated proteins were electrotransferred onto nitrocellulose membrane. Nonspecific sites on the membrane were blocked by incubating in blocking solution containing 5% nonfat dry milk and Tween 20 in Tris-buffered saline. CYP1A2 was detected using polyclonal goat anti–rat CYP1A2 antibodies (Gentest Corporation, Woburn, MA). CYP3A1 and CYP3A2 were detected using polyclonal rabbit anti–rat CYP3A1 and CYP3A2 antibodies (Research Diagnostics Inc, Flanders, NJ), respectively. CYP2D1 was detected using rabbit anti–rat CYP2D1 polyclonal antibody (Chemicon International, Inc., Temecula, CA). The antigen–antibody complexes were then incubated with secondary antibodies (mouse anti–goat IgG from Sigma Chemical Co., St. Louis, MO, and goat anti–rabbit IgG from Chemicon International, Inc.) coupled with horseradish peroxidase. The membranes were washed and detected by a femtoLucent™ detection system from Chemicon International, Inc. The optical densities of Western blots were determined using Scion Image software® (Scion Corp., MD) and compared between groups after normalizing with β-actin.

Table I. Primers Used for RT-PCR Analysis of mRNA Expression of CYP450 Isoforms in Liver Tissues Isolated from Rats of C, CPF, and CRI Groups

CYP450 enzyme	Forward primer	Reverse primer	Fragment size	Ref.
CYP2D1	ATCGCTGGACTTCTCGCTAC	GTCTTCTGACCTTGGAAGAC	658	24
CYP3A1	GGAAATTCGATGTGGAGTGC	AGGTTTGCCTTTCTCTTGCC	326	29
CYP3A2	TACTACAAGGGCTTAGGGTG	CTTGCCTGTCTCCGCCTCTT	376	29
CYP1A2	CAGTCACAACAGCCATCTTC	CCACTGCTTCTCATCATGGT	302	30

Table II. Amplification Conditions Used in RT-PCR Analysis of CYP450 Isoforms mRNA Expression in Liver Tissues Isolated from Animals of C, CPF, and CRI Groups

CYP450 enzyme	Cycles			No. of cycles	Ref.
	Denaturation	Annealing	Extension		
CYP2D1	94°C, 1 min	56°C, 1 min	72°C, 1 min	30	24
CYP3A1	94°C, 1 min	52°C, 1 min	72°C, 1 min	26	29
CYP3A2	94°C, 1 min	52°C, 1 min	72°C, 1 min	26	29
CYP1A2	93°C, 1 min	55°C, 1 min	72°C, 1 min	26	30

RT-PCR analysis of CYP450 mRNA

Total RNA was isolated from liver tissues by using Triazol reagent (Life Technologies Inc., Rockville, MD). The RNA concentration and purity were determined spectrophotometrically. The primers and thermal cycle programs used for CYP2D1, CYP3A1, CYP3A2, and CYP1A2 are described in Tables I and II, respectively, the number of cycles being optimized for our laboratory conditions. The primers for CYP450 mRNA and β-actin were obtained from Sigma Genosys (St. Louis, MO) and Clontech (Palo Alto, CA). The products of RT-PCR were subjected to electrophoresis in 2% agarose, ethidium bromide containing gel. Relative mRNA levels were determined as a ratio of CYP450 expression with respect to the internal housekeeping gene β-actin. The optical densities of the PCR products were determined using Scion Image Software (Scion Inc.)

Statistical Analyses

All results were reported as mean ± standard deviation. The statistical analyses were performed using the JMP® statistical software V3.2.6 (SAS Institute, Cary, NC). One-way analysis of variance was used to evaluate whether there were differences among the groups. Dunnetts comparison of means relative to control was carried out to determine evidences of inhibition in CRI and CPF groups as compared to Control group.

RESULTS

Induction of CRI in Rats

CRI was evident in the nephrectomized rats as observed by the elevated S_{cr} and SUN levels as shown in Figs 1a and 1b, respectively. Compared with C and CPF, S_{cr} levels in CRI rats were increased by 98% ($p < 0.01$). There was a 2.5-fold increase ($p < 0.01$) in SUN levels in CRI rats compared with C and CPF groups. The changes in body weight and food intake by all three groups of animals are shown in Figs. 2 and 3, respectively. At the end of week 5 of the study, food intake

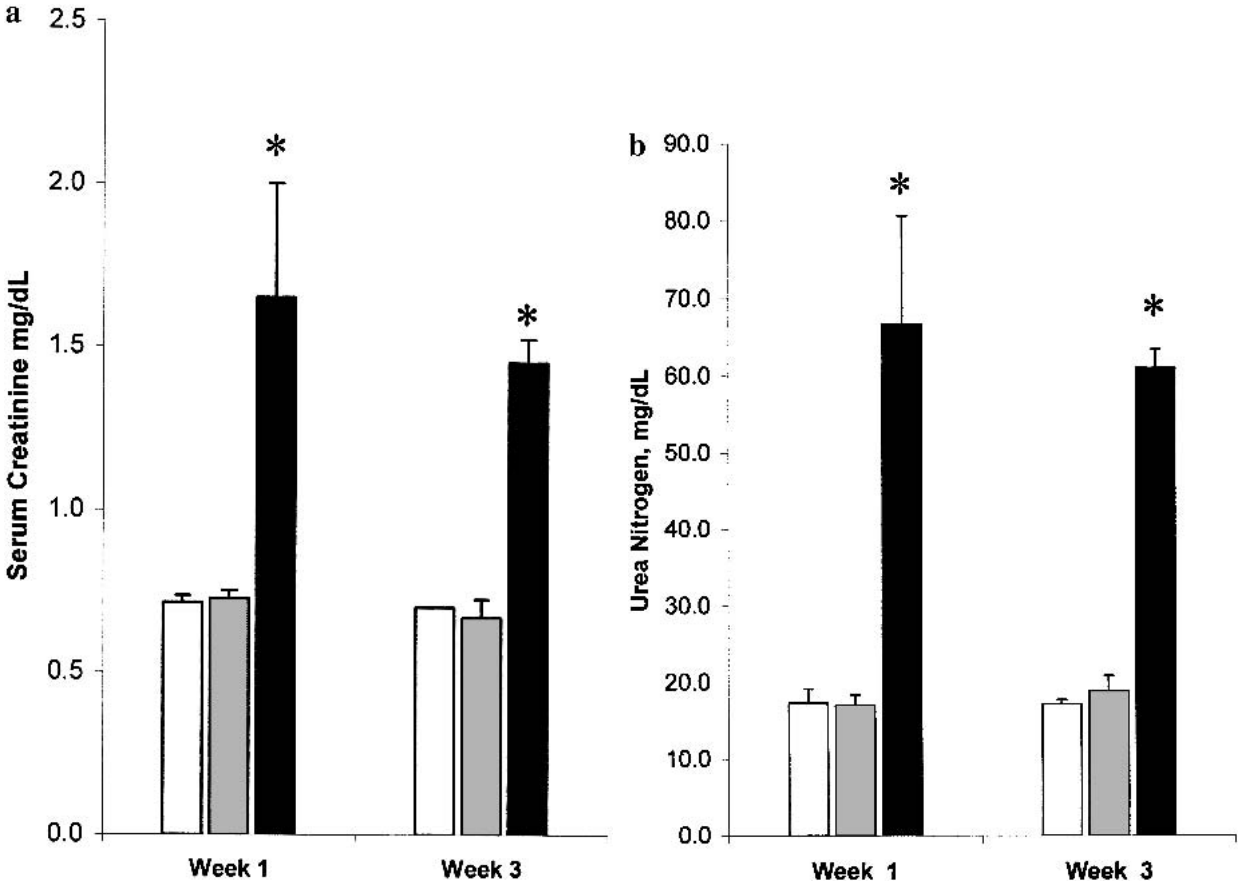

Fig. 1. Plasma serum urea nitrogen (a) and serum creatinine levels (b) in control (open bars) (n = 6), control pair fed (CPF; gray bars) (n = 6), and chronic renal insufficiency–induced (CRI; solid bars) (n = 7) rats in weeks 1 and 3 after the second surgery. Rats from CPF group are sham-operated rats that received the same amount of food as the CRI-induced animals. The details of the assay are explained in the Methods section. The data are expressed as mean ± SD in each group. *$p < 0.01$.

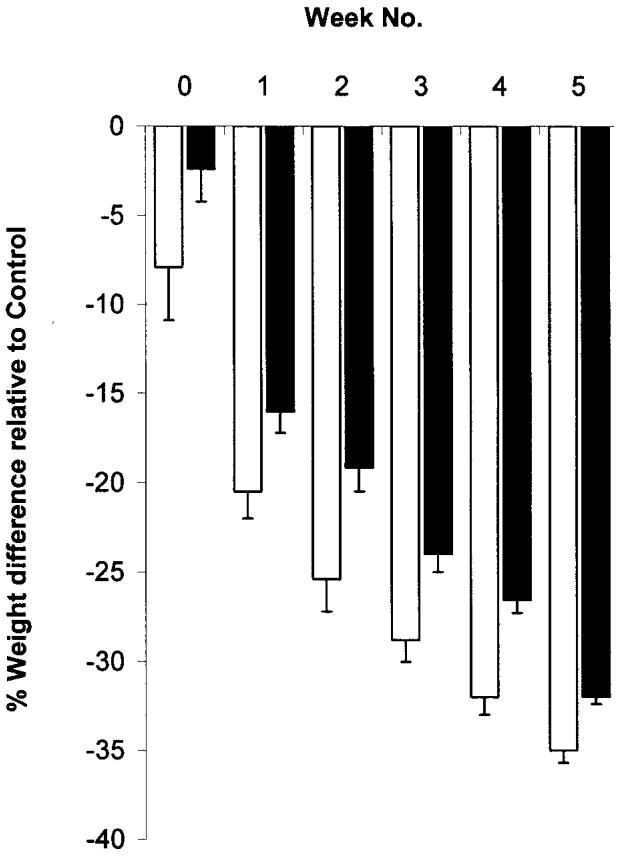

Fig. 2. Changes in body weight in control pair-fed (CPF; open bars) (n = 6) and chronic renal insufficiency–induced rats (CRI; solid bars) (n = 7) each week after the second surgery. The details of the surgery involved in the remnant kidney model are explained in Methods section. Food intake was monitored for CRI-induced rats on daily basis, and the same amount of food was fed to sham-operated rats (CPF). The data shown for each week are averages of daily body weight measurements. The results are expressed as mean percentage difference in body weight in CPF and CRI groups relative to control. The data are mean ± SD for each week.

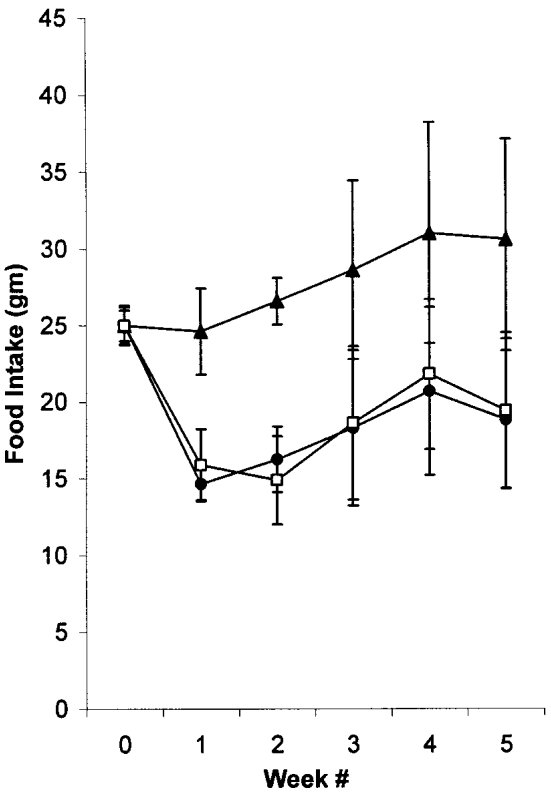

Fig. 3. Amount of food intake by rats from control (▲) (n = 6), control pair-fed (□) (n = 6), and chronic renal insufficiency–induced (●) (n = 7) groups each week after the second surgery. The details of the animal groups and surgery involved in the remnant kidney model are explained in the methods section. The data are expressed as mean ± SD food consumed each week.

and body weight of animals in CRI and CPF groups were reduced by 37% ($p < 0.01$) and 35% ($p < 0.01$), respectively.

Effect of CRI on CYP450 Catalytic Activity

Microsomal incubations carried out in triplicate in liver microsomes from each animal using 25 μM dextromethorphan (for CYP2D and CYP3A) and 5 μM 7-ethoxyresorufin (for CYP1A2) showed measurable activities in all groups of animals . There was no difference in CYP1A2, CYP2D and CYP3A catalytic activities between C and CPF animals as shown in Fig. 4. Compared to CPF animals, CYP3A activity was reduced by 41.8 ± 20% ($p < 0.05$) in the CRI group.

Effect of CRI on CYP450 Protein Expression

The results of Western blot analysis of all four CYP450 enzymes are shown in Fig. 5. After normalizing with β-actin, there was no significant difference in protein expression of CYP2D1, CYP3A1, and CYP1A2 in the CRI group compared to the CPF rats. However, there was a significant reduction in CYP3A2 protein expression (41 ± 24.1% decrease, $p < 0.05$) in CYP3A2 protein expression in the CRI as compared to the CPF group.

Effect of CRI on CYP450 mRNA Expression

The results obtained from RT-PCR analysis were similar to those of Western blot analysis. Fig. 6 illustrates the mRNA expression of CYP450 isoforms in all three groups of animals. There was no significant difference in CYP2D1, CYP3A1, and CYP1A2 mRNA expressions among the various groups of animals. However, compared to CPF, there was a fourfold decrease ($p < 0.01$) in CYP3A2 mRNA expression for CRI group after normalization with β-actin.

DISCUSSION

This study has presented clear evidence that CRI induced by the five-sixths nephrectomy method in rats results in decreased CYP3A catalytic activity. The mechanism underlying the reduction of catalytic activity might be explained by the corresponding decrease in mRNA expression of the specific CYP3A isoform (i.e., CYP3A2) primarily responsible for the catalytic turnover of the probe substrate. In the present study we have also demonstrated that the remnant kidney model (RKM) is capable of inducing chronic renal insufficiency in rats. RKM involving the five-sixths nephrectomy method is the most widely used model for CRI (14–16). The reduction of kidney mass to one sixth of its original size re-

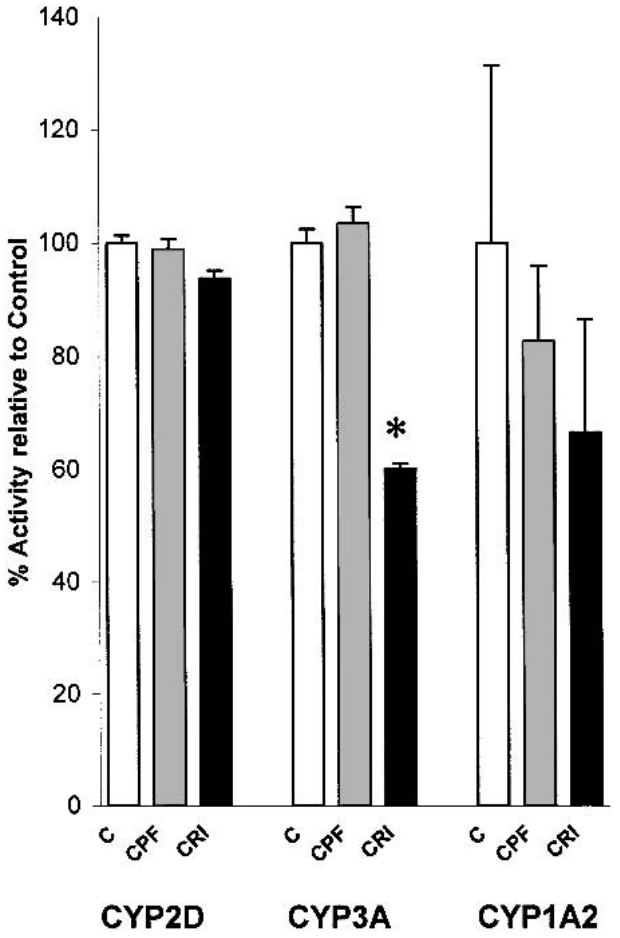

Fig. 4. Percentage CYP2D, CYP3A, and CYP1A2 catalytic activity in livers isolated from control (C) (n = 6), control pair-fed (CPF) (n = 6), and chronic renal insufficiency–induced (CRI) (n = 7) rats. The details of the surgery and groups are explained in the Methods section. CYP2D and CYP3A catalytic activities were monitored through O-demethylation and N-demethylation of dextromethorphan, respectively. Microsomal incubation mixture included phosphate buffer (0.1 M, pH 7.4), dextromethorphan (25 μM), microsomal protein (0.5 mg/ml), and β-NADPH (1 mM). The reaction was stopped by addition of 70% perchloric acid. The samples were processed and analyzed by HPLC with fluorescence detection at excitation and emission wavelengths of 278 nm and 312 nm, respectively. CYP1A2 catalytic activity was monitored through O-deethylation of 7-ethoxyresorufin spectrofluorometrically at excitation and emission wavelengths of 570 nm and 597 nm, respectively. The microsomal incubation conditions were similar except 5 μM 7-ethoxyresorufin was used. The results are presented as percentage catalytic activity in each group relative to control. Data are mean ± SD; *$p < 0.05$.

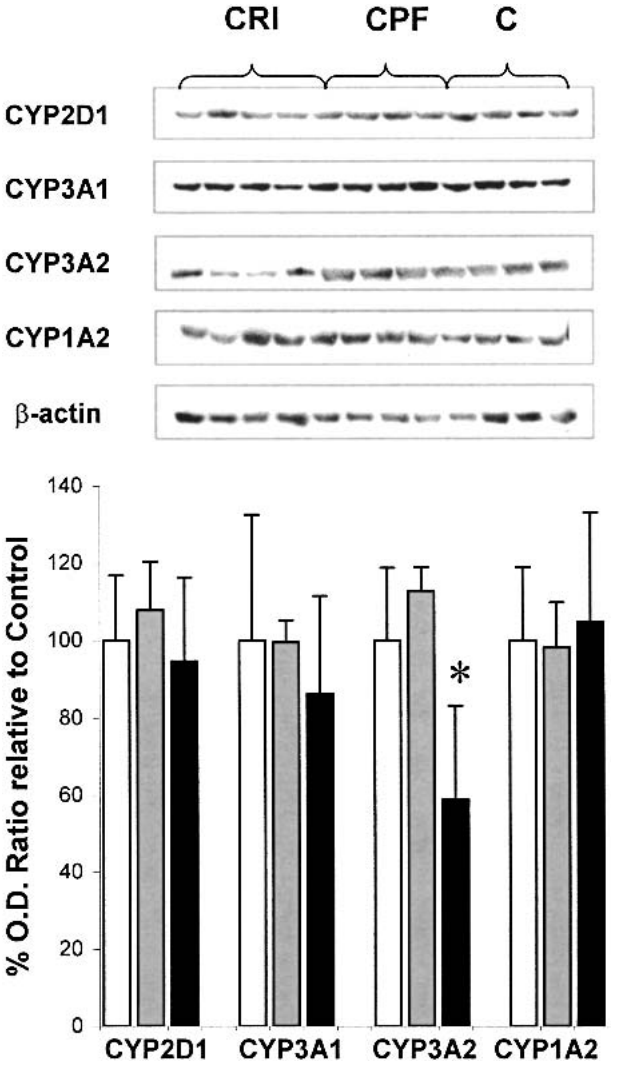

Fig. 5. Expression of CYP2D1, CYP3A1, CYP3A2, and CYP1A2 protein in control (C) (n = 4), control pair-fed (CPF) (n = 3), and chronic renal insufficiency–induced (CRI) (n = 5) rats. Thirty micrograms of microsomal protein was subjected to Western blot analysis with respective polyclonal antibodies as described in the Methods section. Housekeeping gene β-actin was used as an internal standard. Relative levels of CYP450 proteins and β-actin were quantified using densitometry. The results are expressed as percentage optical density ratios (CYP450 : β-actin) for control (open bars), CPF (gray bars), and CRI (solid bars) groups. The data are mean ± SD for each group. *$p < 0.05$.

sults in greater workload for remaining nephrons, which then results in the progressive renal insufficiency. Uremia in this model emerges from series of events: hypertrophy of the remnant kidney, increased intraglomerular pressure and hyperfiltration, increased renal blood flow, proteinurea, intracapillary coagulation, and interstitital fibrosis, which ultimately leads to focal and segmental glomerulosclerosis (6). The series of events following the surgical removal of kidney mass closely resembles nephrotic syndrome in humans. In the present study, it appears that induction of renal insufficiency in rats by RKM tended to induce some degree of undernutrition as observed by the decrease in food intake (Fig. 3). We observed about 37% reduction in food intake in CRI rats as compared to control rats, which resulted in a 35% reduction in their body weight (Fig. 2). These findings support previously published data, which showed that induction of CRI by subtotal nephrectomy in rats caused a decrease in food intake and body weight as compared to control rats (17,18). A similar reduction in body weight was observed in the pair-fed group because these animals were receiving the same amount of food as the CRI group. It was necessary to include the CPF group to clearly delineate the effects of undernutrition on CYP450 as compared to CRI group, particularly because undernutrition itself is known to modulate hepatic CYP450 activities in rats (19).

Induction of CRI in rats was confirmed by elevated S_{cr}

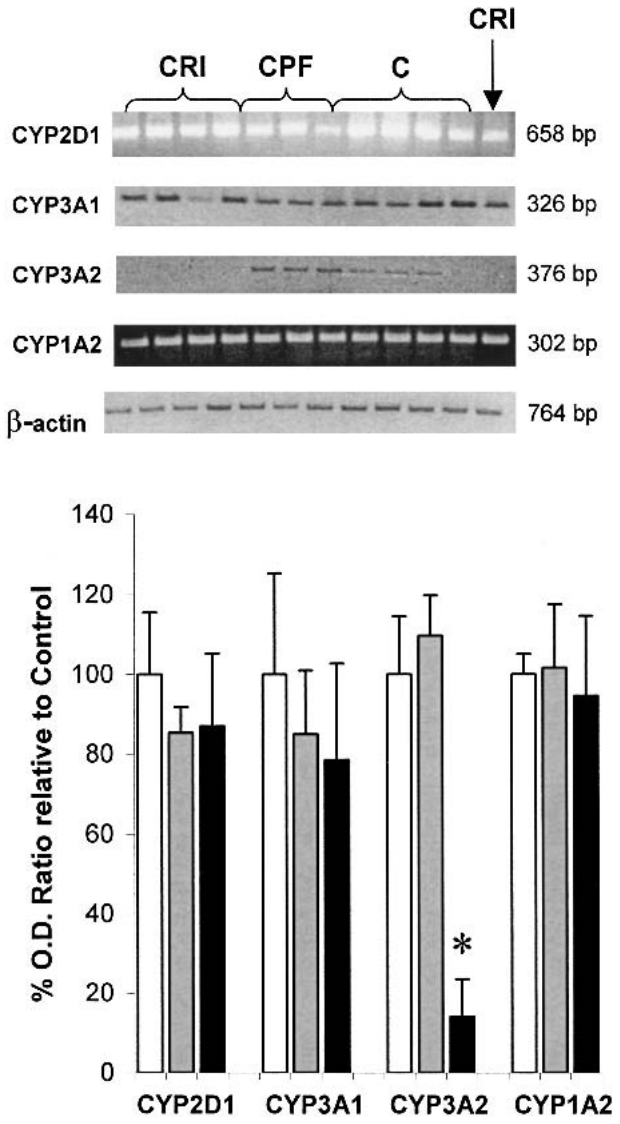

Fig. 6. Expressions of CYP2D1, CYP3A1, CYP3A2, and CYP1A2 mRNA in control (C) (n = 4), control pair-fed (CPF) (n = 3), and chronic renal insufficiency–induced (CRI) (n = 5) rats. Total RNA was isolated, and RT-PCR was conducted as described in the Methods section. Housekeeping gene β-actin was used as internal standard. Relative levels of PCR products of CYP450 isoforms and β-actin were quantified using densitometry. Results are expressed as percentage optical density ratios (CYP450 mRNA: β–actin) for control (open bars), CPF (gray bars), and CRI (solid bars) groups. The data are mean ± SD *p < 0.01.

and SUN levels by 98% and 250% respectively as compared to the CPF group (Fig. 1). Previously published data (6) demonstrated a steady rise in S_{cr} and SUN levels in CRI-induced rats for the first 5 weeks, which appears to level off after 5 weeks, The S_{cr} and SUN levels observed in our study were comparable to those observed in the previously reported data (6). Therefore, a duration of 5 weeks was selected for the induction of CRI in the rats.

Several studies have shown that animals with experimental renal failure exhibit decreased hepatic drug metabolism (7,8,20). Most of these studies have used nonspecific substrates that have overlapping CYP450 catalytic activity, e.g., N-demethylation of aminopyrine, O-demethylation of codeine, and hydroxylation of aniline. However, recent studies (9,21) have examined the effect of CRI on specific CYP450 isoforms but have not assessed the catalytic activity of important CYP450 enzymes such as CYP2D. In the present study we have investigated the effect of CRI on catalytic activities of CYP450 using specific probe substrates to CYP3A, CYP2D, and CYP1A2 isoforms, i.e., dextromethorphan and 7-ethoxyresorufin. Dextromethorphan is widely used as a probe substrate to characterize CYP2D and CYP3A catalytic activities in human as well as rat liver microsomes (13,22). A significant reduction (~42%) in CYP3A catalytic activity was observed in CRI rats as compared to CPF; however, there was no change in CYP2D catalytic activities among the groups (Fig. 4). In this study we have also evaluated the changes in CYP1A2 catalytic activity caused by CRI, using 7-ethoxyresorufin as a probe substrate. Results of these experiments showed no change in CYP1A2 activity between CRI and CPF rats. LeBlond *et al.* (10) have used the caffeine breath test as a marker of CYP1A2 activity, which has some limitations. The rate of exhaled radiolabeled carbon dioxide is used to measure catalytic activity, which is not a direct measure of CYP450 activity. Determination of the rate of metabolite formation is a more accurate measure of enzyme activity. Furthermore, there are some concerns raised regarding the loss of exhaled carbon dioxide in the exhalate chamber, e.g., in the animal fur, etc.

Contrary to previous reports of changes in CYP450 activities as a result of caloric deficiency (19), we did observed reduction in only specific but not all CYP450 activities despite similar weight loss in CPF group. This observation suggests that the effect observed in the CRI rats is secondary to the renal insufficiency and not to the malnutrition arising from the compromised renal function.

Although multiple isoforms of CYP2D exist in the rat (23), it has been shown that CYP2D1 in rat liver is equivalent of human CYP2D6 (24). The expression and protein levels of the CYP2D1 isoform, similar to the catalytic activity, were not affected by CRI. This observation contradicts the reports of changes in bufuralol (a CYP2D6 substrate) clearance in CRI patients. The reason for this anomaly could be explained with reference to the report by Terao and Shen (25), which suggested that the reduced hepatic extraction of l-propranolol in the presence of uremic blood could be caused by the presence of an inhibitory factor in the uremic blood. A variety of endogenous substances are found to accumulate in uremic blood, such as creatinine, urea, aliphatic and aromatic amines, indolic derivatives, phenolic substances, amino acids, etc. (26,27). Therefore, it is likely that multiple mechanisms might be responsible for the reduction in hepatic CYP450 activity.

The changes in CYP450 catalytic activity in CRI rats were supported by similar results in RT-PCR and Western blot analysis (Figs. 5 and 6). We found significant reductions in CYP3A2 mRNA and protein levels, but there was no change in CYP2D1, CYP1A2, and CYP3A1 in CRI. It appears that the reduction in CYP3A activity caused by CRI induced by the RKM model can be attributed to the decreased protein levels following down-regulation of mRNA. Although there is no direct evidence, one can hypothesize that one or more of the endogenous substances could interfere with CYP3A2 gene regulation. Furthermore, it is also known that CYP3A1 and CYP3A2 in rat correspond to human CYP3A4 (28). Therefore it can be speculated that the

observed reduction in hepatic clearance of drugs cleared by CYP3A4 (e.g., erythromycin) (4) in renal failure patients could possibly be a result of down-regulation of the P450 expression, which needs to be confirmed by additional studies. Because CYP3A4 is a major oxidative enzyme responsible for metabolism of several drugs in humans, significant reduction in hepatic CYP3A4 activity in renal failure, secondary to its reduced protein expression, poses a serious risk of drug accumulation and toxicity, emphasizing the need to study changes in human CYP3A4 in CRI.

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