Drug Metabolism in Chronic Renal Failure

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Abstract: Pharmacokinetic studies conducted in patients with CRF demonstrate that the nonrenal clearance of multiple drugs is reduced. Although the mechanism by which this occurs is unclear, several studies have shown that CRF affects the metabolism of drugs by inhibiting key enzymatic systems in the liver, intestine and kidney. The down-regulation of selected isoforms of the hepatic cytochrome P450 (CYP450) has been reported secondary to a decrease in gene expression. This is associated with major reductions in metabolism of drugs mediated by CYP450. The main hypothesis to explain the decrease in liver CYP450 activity in CRF appears to be the accumulation of circulating factors which can modulate CYP450 activity. Liver phase II metabolic reactions are also reduced in CRF. On the other hand, intestinal drug disposition is affected in CRF. Increased bioavailability of several drugs has been reported in CRF, reflecting decrease in either intestinal first-pass metabolism or extrusion of drugs (mediated by P-glycoprotein). Indeed, intestinal CYP450 is also down-regulated secondary to reduced gene expression, whereas, decreased intestinal P-glycoprotein activity has been described. Finally, although the kidneys play a major role in the excretion of drugs, it has the capacity to metabolize endogenous and exogenous compounds. CRF will lead to a decrease in the ability of the kidney to metabolize drugs, but the repercussions on the systemic clearance of drugs is still poorly defined, except for selected xenobiotics. In conclusion, reduced drug metabolism should be taken into account when evaluating the pharmacokinetics of drugs in patients with CRF.

Key Words: Chronic renal failure, cytochrome P450, gene expression, drug metabolism, intestine, liver, serum mediators, P-glycoprotein.

1. INTRODUCTION

The prevalence of chronic renal failure (CRF), predominantly due to aging, diabetes, hypertension and renal vascular disease, has increased steadily during the last decades [1, 2]. The cost of care for these patients has also risen progressively in part because of excessive or inappropriate drug use [3, 4]. Some studies have revealed that patients with CRF require an average of more than seven drugs to manage the underlying renal disease and their comorbid states [3]. Furthermore 40% of patients with creatinine clearance of less than 40 mL/min receive drug dosages higher (range 1.07-6.45) than required [5]. Both, excessive use of drugs and especially inappropriate dosage increase the risk of adverse effects, including the risk of nephrotoxicity, and increase the cost of care [3, 4, 6]. For instance, Johnson and Bootman have estimated that $US76.6 billion are spent every year to manage drug-related morbidity and mortality [7]. Thus, appropriate dosage of drugs in CRF is a crucial consideration in avoiding adverse effects, minimizing the time and cost of management of adverse events, and ensuring optimal patient outcome.

CRF interferes with the elimination of many drugs because of the reduction in glomerular filtration rate (GFR), and tubular secretion [8]. Dose adjustment of drugs excreted by the kidney is made according to the GFR. However despite dosage adjustment, patients with CRF still present a great number of adverse effects [6, 8]. Part of this phenomenon is related to the fact that CRF also affects the nonrenal route of elimination of drugs, *i.e.* hepatic, intestinal and renal metabolism of drugs is decreased in CRF [6, 9-14]. It could also be explained by alteration in non-metabolic elimination of drugs by the liver and the intestine, *i.e.* drug elimination mediated by P-glycoprotein.

The aim of this article is to review drug metabolic disturbances in renal failure, emphasizing on drug metabolizing enzymes, particularly cytochrome P450 (CYP450). The repercussions of renal failure on intestinal and liver P-glycoprotein will also be reviewed.

2. LIVER DRUG METABOLISM

2.1. Human Studies

Many investigators have shown, that in patients with renal failure there is a decrease in the metabolic clearance of numerous drugs. Several reviews have been published on the subject [3, 4, 6, 8, 9, 13]. Some of these drugs are presented in table **1**. The vast majority is metabolized in the liver, suggesting that renal failure impedes hepatic biotransformation of drugs. Several of the substrates in table **1** are eliminated catalyzed by the CYP450, suggesting that hepatic CYP450 is altered in CRF [9, 15, 16].

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Table 1. Effect of Chronic Renal Failure on Nonrenal Clearance (% Change from Normal Clearance)

Acyclovir (62)	Cimetidine (62)	Moxalactam (63)
Aztreonam (33)	Ciprofloxacin (33)	Minoxidil (46)
Captopril (50)	Codeine (17)	Nicardipine (37)
Cefmenoxime (45)	Fluconazole (50)	Procainamide (61)
Cefonicid (60)	Imipenem (85)	Verapamil (54)
Cefsulodin (52)	Metclopramide (66)	

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We are not aware of human studies demonstrating a reduction in liver cytochrome P450 isoforms in CRF. However, several methods have been described to assess, *in vivo*, the CYP activity in humans and the most widely used is the administration of probe drugs that are selectively metabolized by specific CYP450 isoforms [17]. Kevorkian *et al.* reported that the use of dextromethorphan or sparteine, to assess CYP2D6 activity, was possible in patients with CRF [18]. The results of their study revealed that there was a decrease in the metabolic clearance of sparteine, suggesting a decrease in CYP2D6 activity in CRF patients [18]. Other authors have also suggested that CYP2D6 is compromised in parallel with the deterioration of renal function in CRF patients [19].

There are few studies on liver phase II reactions in CRF. Sulfation appears to be normal, whereas acetylation (isoniazide) and glucuronidation (fluconazole) have been reported to be reduced [20-22] (see section 2.2.2).

Although the studies cited above strongly suggest that renal failure reduces the metabolism of drugs in CRF patients, it is still difficult to predict exactly how renal failure will influence the disposition of a specific drug. Part of this problem comes from the fact that most clinical studies were made in conditions where concurrent medications, age and smoking habits (all factors known to strongly influence CYP450) were not necessarily controlled. Furthermore, the severity of the renal failure should be taken into account in interpreting pharmacokinetics studies in renal failure because some investigators have reported a correlation between the decrease in GFR and the decrease in nonrenal clearance [6, 23-25]. In addition to the severity, the duration of the renal failure seems to influence the hepatic metabolism. For instance, the nonrenal clearance of imipenem was 50 ml/min in patients with CRF, 95 ml/min in patients with acute renal failure (ARF) and 130 ml/min in control patients [26].

In summary, renal failure in human is associated with a decrease in the metabolism of several drugs that are preferentially biotransformed by the liver. This decrease in biotransformation appears to be related to a reduction in the activity of hepatic CYP450, and the importance of this phenomenon seems to be related to the severity and the duration of renal failure.

2.2. Animal Studies

2.2.1. Cytochrome P450

2.2.1.1. In Vitro Metabolism

Several studies have looked at the repercussions of experimental renal failure on microsomial and cytosolic enzyme content of the liver [27-34]. Most of the studies have focused on the CYP450 since it is the major enzymatic system involved in drug metabolism. The results of these studies show that in male rats with CRF, total hepatic CYP450 content decreases between 19 to 47%. Moreover, significant reductions in enzymatic reactions normally carried by the CYP450 have been reported *in vitro*: N-demethylation of erythromycin, aminopyrine and ethylmorphine, O-demethylation of codeine and hydroxylation of aniline. There is a correlation between the decrease in total CYP450 activity and the severity of renal failure in rats [30-31, 33, 34]. We also reported a correlation between the reduction in creatinine clearance and the decrease in *in vitro* metabolism of erythromycin [33, 34].

2.2.1.2. CYP450 Protein Expression

In male adult rat, hepatic cytochrome CYP450 is composed of several isoforms and those involved in drug metabolism processes include CYP1A2, CYP2C11, CYP2D, CYP2E1 and CYP3A1/3A2 [35]. Knowledge of which isoforms are decreased in CRF is critical in order to predict which drugs are at risk for accumulation. Although several studies have focused on the repercussions of CRF on total hepatic CYP450 content, only few investigators have studied specific CYP450 isoforms. Uchida *et al.* reported a reduction in the levels of hepatic CYP2C6, CYP2C11 and CYP3A2 and a slight increase in CYP1A2 in male adult rats with CRF [31]. In our laboratory, we described a significant reduction in CYP2C11, CYP3A1 and CYP3A2 in male adult rats with CRF, while no isoform induction was noted (Fig. (**1**)) [33, 34]. Furthermore, the levels of CYP450 protein were inversely correlated with the degree of renal failure, assessed by the creatinine clearance [33, 34]. Interestingly, CYP3A1 and 3A2 in the rat correspond to CYP3A4 in humans. Since this isoform is responsible for the metabolism of multiple drugs commonly used in CRF patients, patients with CRF could be at risk for drug accumulation and toxicity.

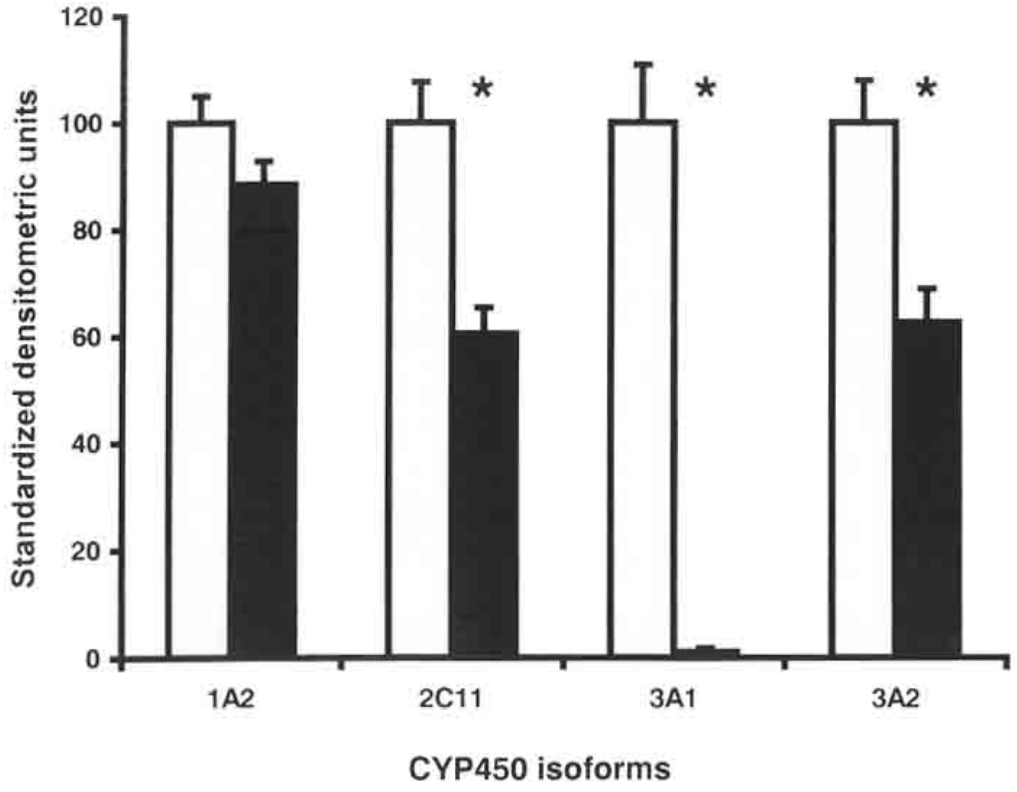

Fig. (1). *Protein expression of liver CYP450 isoforms in control paired-fed* (□) *and CRF rats* (■). Protein bands are expressed in densitometry units (%). The densitometry units of control paired-fed rats were arbitrarily defined as 100%. Data are the mean ± S.E. of six rats in each group.

*$P < 0.001$ compared with control paired-fed rats.

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2.2.1.3. In Vivo Metabolism

In vitro studies clearly demonstrated that CRF is associated with a down-regulation of liver CYP450. However, the consequences of liver CYP450 decrease induced by CRF on the *in vivo* drug metabolizing capacity remain poorly documented. In animals, very few data are available on the repercussions of CRF on systemic drug metabolism by the liver. A major problem with drug disposition studies in rats is that the blood samples used for pharmacokinetic analysis can lead to significant blood loss and hypovolemia. Uchida *et al.* studied the changes in trimethadione (TMO) metabolism using the microdialysis method (avoiding excessive blood samples) in CRF rats [31]. They found that the N-demethylation of TMO was reduced by 25 % in CRF. However, since TMO N-demethylation is catalyzed by several CYP450 isoforms, a reduction in its metabolism does not indicate which specific isoform is reduced [36]. On the other hand, Tvedegaard *et al.* found no modification in the antipyrine clearance in rabbits with CRF [37].

We used breath tests as probes for evaluating *in vivo* liver metabolism in CRF rats. Breath tests have been developed as methods to evaluate the catalytic activity of CYP450 isoenzymes by measuring the rate of demethylation of a drug [38]. The formaldehyde generated by CYP450 mediated demethylation reactions is rapidly oxidized and excreted as carbon dioxide in the breath. The rate of production of $^{14}CO_2$ from a suitable radiolabelled substrate reflects the *in vivo* rate of its demethylation and thus the catalytic activity of either a subset or a specific cytochrome P450 depending on the studied substrate. Various substrates have been used in breath tests to evaluate CYP450 activity *in vivo* in rats and humans. The aminopyrine breath test has often been used to evaluate liver metabolic function [39, 40]. *In vitro* and *in vivo* studies suggest that aminopyrine breath tests can be used to evaluate the activity of CYP2C11 in the rat [41, 42] although other CYP450 isoenzymes also contribute to its demethylation, including CYP1A2, 2A2, 2B, and 2D1. Caffeine and erythromycin breath tests have also been used to measure the liver catalytic activity of CYP1A2 and CYP3A2 isoenzymes [42-45].

In our study, the aminopyrine breath test was used to evaluate the activity of CYP2C11; caffeine and erythromycin breath tests were used to measure the catalytic activity of CYP1A2 and CYP3A2 isoenzymes, respectively. We reported that aminopyrine and erythromycin breath tests were reduced by 35 % in CRF rats, while the caffeine breath test remained unchanged (Fig. (**2**)) [32]. We also found a correlation between the reduction in creatinine clearance and the decrease in the *in vivo* metabolism of drugs. These results demonstrate that in rats, CRF is associated with a reduction in the metabolism of drugs *in vivo*, secondary to a decrease in selective liver CYP450 isoforms, namely CYP2C11 and CYP3A1/3A2. Whether reduction in extrahepatic metabolism of drugs (e.g. intestinal), that could further participate in the reduction of drug metabolism found in this study, remains to be defined.

2.2.1.4. CYP450 Gene Expression

The reduction in liver CYP450 in CRF could be secondary to a decrease in synthesis or an increase in degradation. Our group studied, in the male adult rat, the repercussions of CRF on the CYP450 gene expression in the liver [33, 34]. The results demonstrated that there was an association between lower levels of mRNAs and protein expression for several isoforms of hepatic cytochrome P450, namely CYP2C11, 3A1 and 3A2 [33, 34]. These results strongly suggest that CRF leads to a reduction in gene expression of liver CYP450 isoforms. However, the mechanisms underlying the diminution of liver CYP2C11, 3A1 and 3A2 gene expression in CRF are not known. Caloric restriction, as seen in CRF rats, down-regulates hepatic genes of drug metabolizing enzymes in the mouse and in the rat [46, 47]. However, in our control paired-fed rats we did not observe

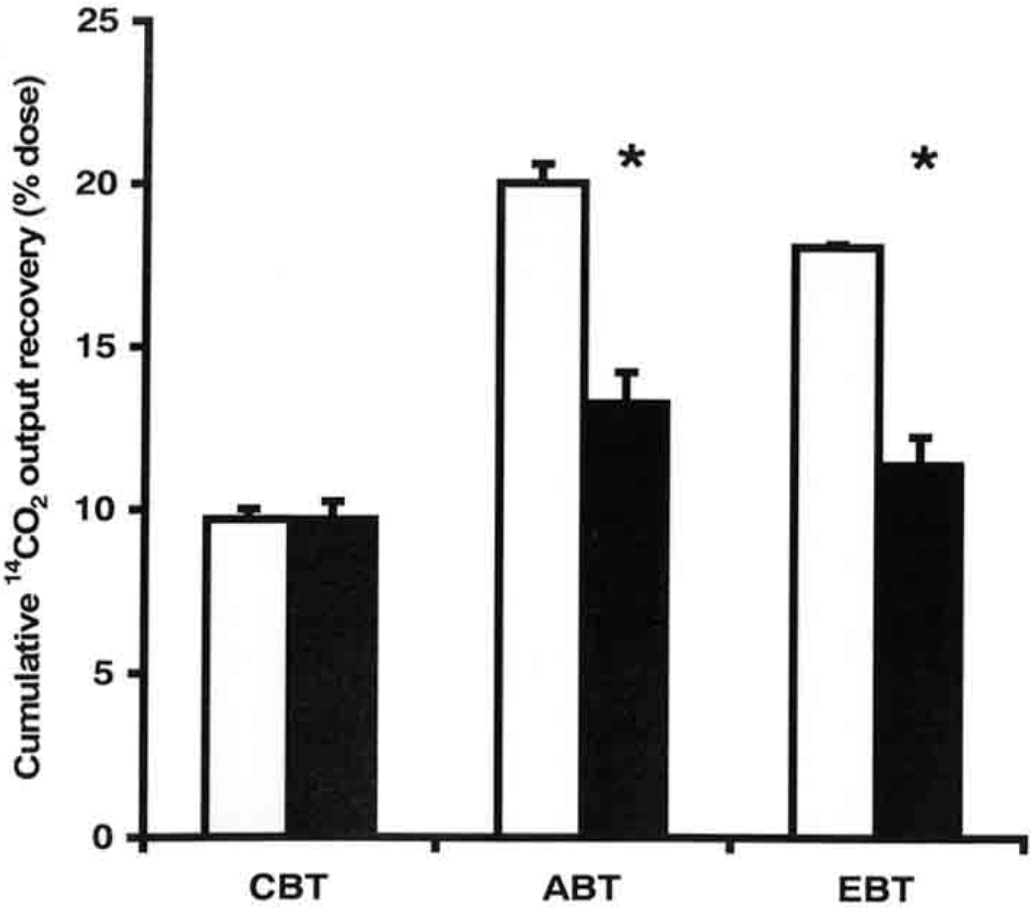

Fig. (2). *Breath tests with erythromycin (EBT), aminopyrine (ABT), and caffeine (CBT) in control paired-fed* (□) *and CRF rats* (■). Values represent the 2-h cumulative $^{14}CO_2$ output expressed as a percentage of the injected dose. Data are the mean ± S.E. of six rats in each group.

*$P < 0.001$ compared with control paired-fed rats.

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any modification in cytochrome CYP450 levels despite a similar weight loss in controls and CRF rats [33, 34].

2.2.1.5. Mechanisms Implicated in Liver CYP450 Activity and Expression Down-Regulation

The mechanism underlying the down-regulation of liver CYP450 in CRF remains poorly understood. Leber and Schutterle have shown that in CRF, the reduction of liver CYP450 induced by CRF could be reversed in part by l-aminolevulinic acid but these results have not been confirmed [27]. It appears excluded that chronic protein malnutrition, as seen in CRF, could affect CYP450 synthesis in the liver [33, 34].

An attractive hypothesis to explain CYP450 activity and expression down regulation is the presence of endogenous inhibitors in the blood of uremic animals that may modulate the activity of CYP450. Terao and Chen have shown, in single-pass rat liver perfusion studies, that the extraction of L-propranolol was significantly lower in rats with acute renal failure compared to control rats [48]. When livers of rats with acute renal failure were perfused with blood from control rats, the extraction of L-propranolol was similar to that of control rats. However, when livers of control rats were perfused with blood from animals with renal insufficiency, there was a significant decrease in L-propranolol extraction. Although this study was done in rats using an acute renal failure model and reported no data on drug metabolizing enzyme, it provided the first evidence for the presence of an inhibitory factor in the uremic blood that could modify the biotransformation of drugs [48].

The presence of a circulating factor in uremia that inhibits liver metabolism has also been implicated in CRF patients. Ahmed *et al.* studied the pharmacokinetics of nicardipine in three groups, control subjects, patients with CRF and patients undergoing hemodialysis [49]. Plasma clearance of nicardipine was reduced in patients with CRF (6.5 ± 2.6 ml/min/kg) compared with control subjects (10.4 ± 3.1 ml/min/kg) and patients undergoing hemodialysis (12.5 ± 4.6 ml/min/kg). The fact that the clearance of nicardipine was identical in control subjects and patients undergoing hemodialysis suggest that the inhibitors can be removed by dialysis. Further supporting the presence of serum inhibitors, it has been shown that incubation of microsomes, prepared from healthy human livers, with serum of patients with CRF is associated with a decrease in the metabolism of midazolam and tolbutamide, reflecting CYP3A4 and CYP2C9 activities [50].

In order to confirm the presence of serum mediators affecting activity and/or expression of the isoforms of hepatic CYP450, we recently conducted a study where we incubated normal rat hepatocytes with serum from CRF and control male adult rats [51]. This study revealed, that in normal hepatocytes incubated for 24 hours with serum (concentration of 10%) from rats with CRF, total CYP450 level decreased by 35% compared to serum from control animals [51]. We also showed that protein expression of several CYP450 isoforms (CYP2C6, 2C11, 3A1 and 3A2) were decreased by more than 35% in normal hepatocytes incubated with serum from CRF rats (Fig. (**3**)). The decrease in protein expression of CYP450 isoforms mediated by serum from rats with CRF was secondary to reduced gene expression (Fig. (**4**)). Although the mechanisms responsible for the diminished hepatic gene expression in CRF are not known, the present study suggests that uremic mediator(s) may affect CYP450 promoters.

A number of studies indicate that animals with CRF display impaired protein synthesis, by reduced gene expression in the liver, the skeletal muscle, and the cardiomyocytes [52-54]. For instance, the mRNA of hepatic lipase and insulin-like growth factor 1 receptor are decreased in hepatocytes and skeletal muscle of rats with CRF, respectively [52-54]. CRF is associated with sustained elevations in calcium in many cell types, including the hepatocytes. This high intracellular calcium seems to be a major factor underlying cell reduced protein synthesis [55, 56]. It has been suggested that the increase in basal $[Ca^{2+}]_i$ of hepatocytes was secondary to parathyroid hormone (PTH) elevation that accompanies CRF [57]. The predominant pathway for the PTH-induced increase in $[Ca^{2+}]_i$ is the stimulation of a G protein-adenylate cyclase-cAMP system, which leads to stimulation of a calcium transport system [57]. On the other hand, cAMP has been shown to down-regulate CYP450 [58]. We recently demonstrated that the molecular weight of the mediator(s) present in uremic serum is between 10 and 30 kDa. Since rat PTH molecular weight is around 10 kDa , it could be a potential mediator of hepatic CYP450 down-regulation in CRF [51].

Other potential uremic serum mediators implicated in the down-regulation of liver CYP450 are cytokines, since their molecular weight averages 20 kDa. Several studies have demonstrated that CRF is associated with a chronic activation of inflammatory response and patients with CRF present an increase in plasma levels of many cytokines [59-63]. On the other hand, cytokines are able to down-regulate hepatic CYP450 *in vitro* and *in vivo* [64-66].

In summary, animal studies demonstrate that CRF is associated with a decrease in the expression of liver CYP450 isoforms secondary to reduced mRNA levels. Drug metabolism activity, assessed by several oxidative reactions, normally carried out by the CYP450 is also depressed in rats with CRF. Hepatic CYP450 down-regulation is correlated with the degree of renal failure. We may speculate that this down-regulation could explain the reduction in drug metabolism observed in patients with CRF, since rat CYP3A1 and 3A2 correspond to CYP3A4 in humans which is responsible for the metabolism of many drugs commonly used in patients with CRF. The main hypothesis for decreased CYP450 activity and expression appears to be the presence of uremic factors that accumulate in CRF.

2.2.2. Phase II Reactions

Phase II reactions in CRF have not been studied as extensively as phase I reactions. Several human studies have shown that conjugation reactions can be altered in CRF. Singlas *et al.* studied the disposition of zidovudine in patients with CRF, drug eliminated by glucuronidation (75%), and renal excretion (25%), and demonstrated that zidovudine AUC was significantly higher in patients with CRF than in patients without renal failure [20]. Since the increase in AUC could not be explained solely by the

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