DMD Fast Forward. Published on October 16, 2007 as doi:10.1124/dmd.107.018192
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Effects of chronic renal failure on liver drug transporters

Judith Naud M Sc, Josée Michaud M Sc, Francois A. Leblond Ph.D, Stéphane Lefrancois PhD, Alain Bonnardeaux MD, PhD, and Vincent Pichette MD Ph.D

Service de néphrologie et Centre de recherche Guy-Bernier, Hôpital Maisonneuve-Rosemont, Faculté de Médecine, Université de Montréal, Québec, Canada (J.N., J.M., F.A.L., S.L., A.B., V.P.)

Département de pharmacologie, Faculté de Médecine, Université de Montréal, Québec, Canada (J.N., J.M., V.P.)

DMD 18192
Hepatic drug transporters in renal failure

Correspondence should be addressed to: Vincent Pichette MD Ph.D.

Centre de recherche de l'hôpital Maisonneuve-Rosemont

5415 boul. de l'Assomption, Montréal, Québec, Canada

H1T 2M4

Tel: (514) 252-3489

Fax: (514) 255-3026

E-mail: vpichette.hmr@ssss.gouv.qc.ca

Number of text pages: 14

Number of tables: 2

Number of figures: 4

Number of references: 25

Number of words in Abstract: 230 words

Number of words in Introduction: 550 words

Number of word in Discussion: 1050 words

List of non-standard abbreviations: CMPF: 3-carboxy-4-methyl-5-propyl-2-furan-propanoic acid, Cl_b: biliary clearance, Cl_u: urinary clearance, C_b: biliary concentration, $C_{p,ss}$: concentration in the plasma at steady state, C_u: urinary concentration, CRF: chronic renal failure, CTL: control, GFR: glomerular filtration rate, IL-1: interleukin-1, IL-6: interleukin-6, MRP2: multi-drug-

resistance-related-protein type 2, Oatp2: organic-anion-transporting-polypeptide type 2, Oatp3: organic–anion-transporting-polypeptide type 3, P450: cytochrome P450, PBS: Phosphate Buffered Saline, PCR: polymerase chain reaction, Pgp: P-glycoprotein, PMSF: Phenylmethylsulfonylfluoride, PTH: parathyroid hormone, qPCR: quantitative PCR, TNF-α: Tumor-necrosis-factor-α, V_b: biliary volume, V_u: urinary volume

ABSTRACT.

Chronic renal failure (CRF) is associated with a decrease in liver drug metabolism, particularly mediated by the cytochrome P450. CRF also impedes intestinal drug transporters (mainly P-glycoprotein [Pgp] and multidrug resistance protein [MRP]). However, very few studies have evaluated the effects of CRF on liver drug transport. The present study aimed to investigate the repercussions of CRF on liver drug transporters involved in hepatic uptake (organic anion-transporting-polypeptide [Oatp2]) and in drug extrusion (Pgp and MRP2). Two groups of rats were studied: control and CRF. Oatp2, Pgp and MRP2 protein expressions and mRNA levels, as well as some of their metabolic activity were assessed. The effects of CRF serum on drug transporters were also evaluated in cultured hepatocytes. Compared to control, creatinine clearance was reduced by 70% ($p<0.01$) in rats with CRF. Protein expression and mRNA levels of Pgp were increased by 25% and 40% ($p<0.01$), respectively, in liver from rats with CRF. MRP2 protein expression was identical in both groups, while its mRNA levels were increased by 35% ($p<0.01$) in CRF rats. Finally, Oatp2 protein expression was reduced by 35% while its mRNA levels remained unchanged. Similar results were obtained when hepatocytes were incubated with uremic serum. In conclusion, CRF is associated with a decrease in liver transporters involved in drug absorption and an increase in those involved in drug extrusion. Uremic mediators appear to be responsible for these modifications.

INTRODUCTION

Several studies have shown that the metabolic clearance of various substrates is reduced in patients with CRF (Touchette and Slaughter, 1991; Talbert, 1994; Matzke and Frye, 1997). The decrease in drug biotransformation is mainly secondary to alterations in drug metabolism by the liver (Touchette and Slaughter, 1991). Indeed, in rats with experimental renal failure, studies have shown that CRF induces a marked decrease in liver cytochrome P450 (P450) activity secondary to reduced protein and gene expression of selective P450 isoforms, namely CYP2C11, 3A1 and 3A2 (Leblond et al., 2001). This down-regulation is produced by uremic factors, namely parathyroid hormone (PTH).

Another major route for drug elimination is drug extrusion by transporters. These membranous proteins are located in several epithelia such as the kidney, the liver and the intestine. They are implicated not only in renal, biliary or intestinal drug extrusion but also in their uptake especially in enterocytes and hepatocytes. Recently, we have demonstrated that CRF could impede several drug transporters in the intestine (Naud et al., 2007). Indeed, P-glycoprotein (Pgp) (also known as mdr1a, *abcb1* gene family) and multi-drug-resistance-related protein (MRP2) (also known as cMOAT, *abcc2* gene family) are decreased significantly in the intestine of CRF rats compared to control animals. Organic-anion-transporting-polypeptide (Oatp3) (gene *Slc21a7*) was not modified by uremia. These findings could explain, in part, why patients with CRF have an increase in bioavailability of some drugs (Naud et al., 2007).

Several drug transporters are present in the hepatocytes. Uptake transporters are mainly

located on the basolateral membrane of the hepatocytes and mediate drug uptake from the blood circulation to the hepatocytes. Excretion transporters are mainly located on the apical membrane of the hepatocytes and mediate excretion of the drugs from the hepatocytes to the biliary canaliculi. Most common uptake transporters include organic-anion-transporting-polypeptides (Oatp), organic-cation-transporters (OCT), organic-anion-transporters (OAT) and sodium-dependant hepatocyte bile-salt uptake system (NTCP) whilst most common excretion transporters include hepatocellular bile-salt export pump (BSEP), breast-cancer resistance protein (BCRP), MRP and Pgp (Meier and Stieger, 2002; Takikawa, 2002; Kullak-Ublick et al., 2004).

Very few studies have evaluated the repercussions of CRF on liver drug transporters and conflicting results have been published. Laouari *et al*. have reported that in CRF rats, MRP2 was increased while Pgp was unchanged compared to control animals (Laouari et al., 2001). However the severity of renal failure was quite low. This could have confounded the results, since it is well established that there is a strong correlation between the severity of CRF and the modulation of metabolizing enzymes (both in the liver and intestine) as well as in drug transporters (in the intestine) (Pichette and Leblond, 2003; Naud et al., 2007). Furthermore, they did not study the uptake transporters. On the other hand, Sun *et al.* have demonstrated that some uremic toxins present in the serum of CRF patients decrease the uptake of erythromycin in hepatocytes, suggesting an inhibition of the organic-anion-transporting-polypeptide (Oatp2) (Sun et al., 2004)

The objectives of this study were to determine the effects of CRF on hepatic drug transporters. For this purpose, we measured in control and CRF rats 1) liver protein and gene expression of Pgp (mdr1a, *abcb1*), MRP2 (cMOAT, *abcc2*) and Oatp2 (gene *Slc21a5*) and 2) *in vivo* activity of Pgp. Furthermore we also evaluated the effect of uremic serum on the drug transporters in cultured hepatocytes.

MATERIALS AND METHODS

Experimental model.

Male Sprague-Dawley rats (Charles River, Saint-Charles, PQ), weighing 200 to 300 g were housed in the Research Center animal care facility and maintained on Harlan Teklad rodent diet (Harlan Teklad Global Diets, Montreal, Canada) and water *ad libitum*. An acclimatization period of at least 3 days was allowed to the animals before any experimental work was undertaken. All the experiments were conducted according to the Canadian Council on Animal Care guidelines for 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 35 animals each: control paired-fed and CRF. Hepatic drug transporters' protein expression and mRNA levels were measured in 28 rats per group. Pgp activity was measured *in vivo* using clearance of rhodamine 123, a Pgp substrate, in 7 rats per group.

Chronic renal failure was induced by two-stage five-sixth nephrectomy as previously described (Leblond et al., 2001). Control pair-fed rats were fed the same amount as CRF rats ate on the previous day and water *ad libitum*, in order to control for the effect of CRF-induced malnutrition. At day 41 after the nephrectomy, the rats were housed in metabolic cages and urine was collected for 24 hours to determine the clearance of creatinine. Rats were sacrificed by decapitation 42 days after nephrectomy. Blood was collected for the measurement of serum creatinine and urea.

Preparation of liver homogenates

Rat liver was immediately excised following sacrifice, rinced in ice-cold saline and flash frozen in liquid nitrogen. Samples were stored at -80°C until homogenate preparation. Briefly, 200 mg of liver were homogenized in 1 ml 0.9% NaCl, 0.1 mM PMSF using a Potter-Elvehjem tissue grinder (Wheaton Science Products, Millville, NJ). Samples were then sonicated on ice for 10 seconds filtered across a 5µm membrane (PALL, St-Laurent, Québec, Canada) and aliquots were stored at -80 °C up to analysis.

Hepatocyte isolation and culture.

The hepatocytes were isolated from male Sprague Dawley rats weighing 200-250 g and cultured as previously described (Guevin et al., 2002; Michaud et al., 2005). Hepatocytes were incubated for 48 hours in culture medium containing 10 % rat control or CRF serum. Hepatocytes were harvested by scraping in phosphate-buffered-saline (PBS) with 0.1mM PMSF for protein analysis and in RLT Buffer (Qiagen, Mississauga, Ontario, Canada) for mRNA analysis.

Samples were stored at –80°C up to analysis.

***In vivo* excretion of rhodamine.**

In order to evaluate the *in vivo* activity of Pgp in the liver of CRF and control rats, biliary transport of rhodamine 123 was evaluated on anesthesized rats using an adaptation of the protocol published by Yumoto *et al.* (Yumoto et al., 1999). Briefly, the right femoral vein was catheterized with polyethylene tubing and a 1 mL bolus of saline (0.9 % NaCl) was administered followed by perfusion at 2 mL/h for a 30 minute stabilization period. Meanwhile, the left femoral artery, the bile duct and the urinary bladder were canulated for blood, bile and urine collection. After the stabilization period, a bolus of 4.36 mL/kg rhodamine 123 (Sigma, Oakville, Ontario, Canada) was injected, at a concentration of 100 µM in PBS, followed by perfusion at 2 mL/h for a 50 minute stabilization period at the end of which collection began. Total bile and urine were collected every 10 minutes for 40 minutes and 200 µL of blood was collected halfway through each collection period under constant rhodamine 123 perfusion. A 2.7 mL/kg bolus of cyclosporine A (4.16 mM), a Pgp inhibitor, was then administered followed by perfusion (2 mL/h) of rhodamine 123 (100 µM) and cyclosporine A (80 µM) in PBS for a 20 minute stabilization period. Finally, bile, urine and blood were collected for 50 minutes under constant perfusion. At the end of the protocol, animals were sacrificed and the liver was excised, flash frozen in liquid nitrogen and kept at -80 °C until analysis.

Western blot analysis.

Three major hepatic transporters were assessed by Western blot analysis following a previously described protocol (Leblond et al., 2002; Naud et al., 2007) Pgp, MRP2 and Oatp2. Each blot was repeated three times and results were pooled to obtain reported values. Protein expression in control animals was arbitrarily defined as 100%.

Pgp and MRP2 were detected using monoclonal antibodies from ID Labs (London, Ontario, Canada): C219 and M_2 III-6 respectively. Oatp2 was detected using a rabbit anti-rat Oatp2 antibody from US Biological (Swampscott, MA). GAPDH, used as a loading control, was detected using rabbit anti-GAPDH from Abcam (Cambridge, MA). In preliminary experiments, we have demonstrated that GAPDH expression is not modulated by CRF compared to controls (data not shown).

mRNA analysis.

At the time of sacrifice, liver was rinsed in ice-cold saline and flash frozen in liquid nitrogen. Samples were kept at –80°C until RNA extraction. Total RNA was extracted from frozen tissue by the RNeasy mini-kit (QIAGEN). RNA concentrations were determined by measuring absorbance at a wavelength of 260nm. One µg of total RNA was used to prepare cDNA by reverse transcription using Omniscript RT kit from QIAGEN and random primer from Invitrogen (Burlington, Ontario, Canada). The mRNA encoding for Pgp (mdr1a), MRP2, Oatp2 and GAPDH were measured by quantitative Real-Time PCR (qPCR) using Platinum SybrGreen PCR Supermix UDG from Invitrogen and appropriate primers on a Icycler thermocycler (BioRad, Mississauga, Ontario, Canada) with 30-sec incubation at 94°C, 30-sec incubation at

60°C and 30-sec incubation at 72°C. Table I shows the sequences of primers used for the quantification of mRNA for each transporter. PCR products were analysed using the $\Delta\Delta C_T$ method (Livak and Schmittgen, 2001) using GAPDH as standard gene.

Rhodamine 123 extraction and HPLC analysis

Rhodamine was isolated by solid phase extraction using Sep-Pack Vac 1cc C18 Cartridges (Waters, Milford, MA). Briefly, columns were primed with 100% methanol, washed with water and acidified samples (20 µL phosphoric acid/mL sample) were loaded. Columns were then washed with 5% methanol and the samples were eluted in 100% methanol. Samples were evaporated under nitrogen stream and resuspended to their initial volume in mobile phase (acetic acid 1% and acetonitrile, 60:40, % v/v) for HPLC analysis. HPLC was conducted as described by Yumoto *et al.* (Yumoto et al., 1999) on a Beckman System Gold HPLC (Beckman Coulter, Mississauga, Ontario, Canada) with a Jasco FP-920 fluorescence detector (Jasco, Tokyo, Japan) at 485 nm excitation and 546 nm emission wavelengths on a Gemini 5µ C18 110A column (Phenomenex, Torrance, CA). Sample concentration was obtained by interpolation on a standard curve made with pure rhodamine 123 treated by the same procedure.

Rhodamine excretion analysis

Biliary clearance was determined using the equation $Cl_b = (C_b \times V_b)/(C_{p,ss} \times \text{time})$ where C_b is the rhodamine concentration in the bile, V_b is the bile volume and $C_{p,ss}$ is the rhodamine concentration in the plasma at steady state. Urinary clearance was determined using the equation

$Cl_u = (C_u \times V_u)/(C_{p,ss} \times time)$ where C_u is the rhodamine concentration in the urine and V_u is the urine volume.

Other assays.

Blood and urine chemistries were determined with a Hitachi 717 autoanalyser (Boehringer Mannheim Canada, Laval, PQ, Canada).

Statistical analysis.

The results are expressed as mean ± s.e. mean. Differences between groups were assessed by using an unpaired Student's *t* test or an ANOVA test. Significant ANOVA 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 II).

Table II presents the biochemicals and body weight of the two groups of animals studied. Compared to control paired-fed animals, CRF rats had higher levels of plasma creatinine and lower values of creatinine clearance (reduced by 75% ($p<0.001$)).

Protein expression of hepatic transporters in control and CRF rats (Figure 1).

As shown on Figure 1, Pgp was induced by 25% ($p<0.001$) in CRF rats compared to controls while another excretion transporter, MRP2, remained stable. On the other hand, the import transporter Oatp2 was significantly reduced by 40%. GAPDH, used as a loading control, was not affected by CRF (data not shown). We found a significant correlations between Pgp and Oatp2 protein expression and creatinine clearance ($R = 0.303$, $p<0.001$ and $R = 0.524$, $p<0.001$ respectively, data not shown).

mRNA encoding hepatic transporters in control and CRF rats (Figure 2).

Figure 2 presents the results of the analysis of mRNA coding for liver transporters.There was a significant increase in mRNA for the excretion transporters Pgp and MRP2 (50% and 40% respectively, $p<0.05$), but mRNA expression for the import transporter Oatp2 remained unchanged.

***In vivo* excretion of rhodamine 123 in control and CRF rats (Figure 3).**

To determine the repercussion of Pgp increase on the excretion of drugs, we evaluated

the *in vivo* disposition of rhodamine 123 in control and CRF rats. As shown in figure 3, the biliary clearance of rhodamine was significantly increased in CRF rats compared to controls before the addition of the Pgp inhibitor cyclosporine A. However, inhibition of Pgp with cyclosporine caused a significant decrease in the clearance of rhodamine 123 in both groups, bringing them to the same level. The urinary clearance of rhodamine, on the other hand, was significantly decreased in CRF rats (almost 100%).

Protein and mRNA expression of transporters in hepatocytes incubated with uremic serum for 48h (Figure 4)

To determine whether a circulating factor present in the serum of rats with CRF was responsible for the modulation of hepatic transporters, we incubated normal rat hepatocytes with serum from control or CRF rats for 48h. Western blot analysis of the hepatocytes showed a significant increase (34%, $p<0.01$) in Pgp protein expression as well as a significant decrease (25%, $p<0.05$) in Oatp2 protein expression in the hepatocytes incubated with serum from CRF rats. On the other hand, MRP2 remained unchanged as observed *in vivo*. No significant changes were observed in mRNA levels for any of the transporters as opposed to what as been observed *in vivo* (data not shown).

DISCUSSION

The effects of CRF on the non-renal elimination of drugs have been studied for many years. It has been well established that CRF causes a decrease in drug metabolism due to a decrease in liver and intestinal cytochrome P450 expression and activity (Pichette and Leblond, 2003; Sun et al., 2006). A decrease in non-renal drug elimination could also be attributed to a decrease in phase II elimination or in the excretion by hepatic transporters. In this study, we evaluated the effects of CRF on the hepatic expression of two excretion transporters (Pgp and MRP2) and of one uptake transporter (Oatp2) as well as its effects on Pgp activity.

Our results show that CRF causes a significant 25% increase in Pgp protein expression probably due to the 50% increase in Pgp mRNA levels. On the other hand, although we found a significant 40% increase in MRP2 mRNA expression, its protein expression was not affected by CRF. Finally, CRF causes a decrease in Oatp2 protein expression which is not associated to a decrease in mRNA levels. Similar results were obtained when we incubated normal rat hepatocytes with serum from rats with CRF for 48h. We also evaluated hepatic Pgp activity by measuring the biliary clearance of rhodamine in anesthesized rats. We found a 60% significant increase in the excretion of rhodamine that can be inhibited by cyclosporine A, a Pgp inhibitor, suggesting that the upregulation of Pgp protein expression is associated with an increase in activity.

Very few studies have evaluated the effect of CRF on liver drug transporter. A study previously published by Laouari *et al.* demonstrated a significant increase in liver MRP2 protein

and mRNA expression 6 weeks after nephrectomy was performed on rats (Laouari et al., 2001). Our results confirmed the increase in MRP2 mRNA levels but not on the protein expression, which could be explained by the specificity of antibodies used. On the other hand, Laouari *et al*. also found no changes in Pgp protein and mRNA in the liver of nephrectomized rats while we found a significant increase. The explanation for these conflicting results is not clear. It could be attributed to degree of severity of the CRF. In their study, nephrectomized rats presented a 60% decrease in creatinine clearance, while our rats have a 75% decrease in creatinine clearance. Indeed, we found a significant correlation between the severity of the renal failure as measured by the creatinine clearance and the upregulation in Pgp, suggesting that as CRF worsens Pgp increases.

Our results also demonstrate that CRF affects the hepatic transporters in different ways. We have demonstrated a decrease in the expression of the uptake transporter Oatp2 and an increase in the expression and activity of the excretion transporter Pgp. Those transporters work together to eliminate substrates into the bile. Since uptake by Oatp transporters is the first step in the elimination of many xenobiotics, making the compounds available to the detoxification enzymes and the extrusion transporters, and can be a rate-limiting step (Yamazaki et al., 1996; Sun et al., 2006), the inhibition of Oatp2 we found in rats suffering from CRF can probably explain the decrease in drug elimination by the liver, as suggested also by Sun *et al.* (Sun et al., 2004; Sun et al., 2006). The increase in Pgp expression and activity could be an adaptative response to the large quantities of organic anions circulating in CRF.

Our results also differ from those we previously published concerning intestinal transporters in CRF rats (Naud et al., 2007). We had found significant decreases in intestinal Pgp and MRP2 protein expression associated with a decrease in their transport activity across the intestinal membrane. We attributed the protein expression decrease to a post-translational mechanism since we found no changes in their mRNA expression. Also, we had found no significant change in Oatp2 and 3 protein and mRNA levels in the intestine of rats with CRF (Naud et al., 2007).

It thus appears that CRF affects the expression of drug transporters differently in the main elimination organs, *ie* liver and intestine. However, in both organs, we have demonstrated that the changes in protein and/or mRNA levels for the transporters were due to a circulating factor present in the serum of rats with CRF. Indeed, we have shown that incubating uremic serum with enterocytes or Caco-2 cells lead to a decrease in Pgp expression and function (Naud et al., 2007). Similarly, in the present study we demonstrated that uremic serum also modifies the expression of drug transporters in the hepatocytes (fig 4). The different potential factors include pro-inflammatory cytokines, parathyroid hormone (PTH) and uremic toxins, all of which are highly increased in CRF. Interestingly, the pro-inflammatory cytokine TNF-α is known to increase Pgp protein and mRNA expression and Pgp activity in cultured rat hepatocytes in a time- and dose-dependant manner (Hirsch-Ernst et al., 1998). It is also known to decrease rhodamine 123 transport in Caco-2 cells, a colon carcinoma cell line (Belliard et al., 2004) making it a prime candidate to be responsible of the observed effects of CRF on drug transporters. Sun *et al.* have also demonstrated that the uremic toxin 3-carboxy-4-methyl-5-

propyl-2-furan-propanoic acid (CMPF) can directly inhibit erythromycin uptake via a decrease in Oatp2 protein expression and activity (Sun et al., 2004).

Despite pair-feeding of the rats, we still observed a slight difference (<10%) in body weight between the CRF and control pair-fed rats. Very few studies have evaluated the effects of malnutrition on hepatic transporters. Lee *et al.* demonstrated that severe protein and caloric malnutrition caused a decrease in protein expression and activity of liver Pgp (Lee et al., 2003). Furthermore, two studies demonstrated that fasting can cause an increase in Oatp2 expression in rat (Dietrich et al., 2007) and mice (Chen et al., 2007) liver. However, since the magnitude of malnutrition of the rats involved in this study is low, and since the effects observed in extreme conditions (such as severe malnutrition or fasting) is contrary to the effects of CRF observed in the present study, we do not believe that the weight difference between our two groups could have influenced our results.

In conclusion, this study demonstrates that CRF is associated with important modifications in the expression and activity of the different liver drug transporters. It appears that uremic toxins or pro-inflammatory cytokines accumulated in CRF could be responsible for these modifications which seem to be affecting canalicular and basolateral transporters in a different manner.

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FOOTNOTES

This work was financed by the Canadian Institute for Health Research (CIHR) and Fonds de la Recherche en Santé du Québec (FRSQ). Vincent Pichette, Judith Naud and Josée Michaud are scholars of the Fonds de la Recherche en Santé du Québec (FRSQ).

Reprints should be sent to: Vincent Pichette MD Ph.D.
Centre de recherche de l'hôpital Maisonneuve-Rosemont
5415 boul. de l'Assomption, Montréal, Québec, Canada H1T 2M4

Legends to figures

Figure 1 Protein expression of hepatic transporters in whole liver homogenate from control (white bars) and CRF rats (black bars). Protein bands are expressed in densitometry units. The densitometry units measured for hepatic transporters were standardized by dividing them by the value obtained for GAPDH. The standardized densitometry units of control rats were arbitrarily defined as 100%. Representative blots for each transporter are shown in insert. Data are the mean ± SEM of 28 experiments in each group.

* $p<0.05$, ***$p<0.001$ as compared to control.

Figure 2 mRNA encoding hepatic drug transporters liver from control (white bars) and CRF rats (black bars) 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 GAPDH as a standard gene. The number of copies of control rats was arbitrarily defined as 100%. Data are the mean ± SEM of 28 experiments in each group.

* $p<0.05$ as compared to control rats.

Figure 3 *In vivo* excretion of rhodamine 123 in control and CRF rats. a) Biliairy excretion of rhodamine 123 in anesthesized rats infused with rhodamine 123 in the absence (white bars) and presence (black bars) of the Pgp inhibitor cyclosorine A. Biliary clearance was determined using the equation $Cl_b = (C_b \times V_b)/(C_{p,ss} \times \text{time})$ where C_b is the rhodamine concentration in the bile, V_b is the bile volume and $C_{p,ss}$ is the rhodamine concentration in the plasma at steady state. b) Urinary excretion of rhodamine 123 in anesthesized rats in the absence (white bars) and

presence (black bars) of the Pgp inhibitor cyclosporine A. Urinary clearance was determined using the equation $Cl_u = (C_u \times V_u)/(C_{p,ss} \times time)$ where C_u is the rhodamine concentration in the urine and V_u is the urine volume. Data are the mean ± SEM of 7 experiments. *p<0.05, **p<0.01, ***p<0.001, NS = no statistical difference

Figure 4 Level of Pgp, MRP2 and Oatp2 protein expression in hepatocytes incubated with 10% serum from control (white bars) and CRF rats (black bars) for 48h. Protein bands are expressed in standardized densitometry units (See figure 1). Data are the mean ± SEM of 12 experiments. Representative blots for each transporter are shown in insert. No significant changes were observed in mRNA levels in any group (data not shown).

* p<0.05, ** p<0.01 as compared to hepatocytes incubated with serum from control rats.

TABLES

Table I. Nucleotide sequences of PCR primers.

Gene	Primer sequence (5'-3')	Predicted Product size
mdr1a	Sens: ATCAACTCGCAAAAGCATCC Anti-sens : AATTCAACTTCAGGATCCGC	116 bp
mrp2	Sens: GCTGGTTGGAAACTTGGTCG Anti-sens: CAACTGCCACAATGTTGGTC	93 bp
oatp2	Sens: TGTGATGACCTGTGATAATTTTCCA Anti-sens: TTCTCCACATATAGTTGGTGCTGAA	81 bp
gapdh	Sens: TAAAGGGCATCCTGGGCTACACT Anti-sens: TTACTCCTTGGAGGCCATGTAGG	200 bp

Mdr1a, mrp2 and gapdh primers were designed based on published cDNA sequences with the help of the Jellyfish and BLASTIN 2.2.1 computer programs (Altschul et al., 1997). Oatp2 sequence was as previously published by Gao *et al.* (Gao et al., 2004). The resulting PCR products were sequenced on an ABI Prism 3100 analyzer (Applied Biosystems, Foster City, CA).

DMD Fast Forward. Published on October 16, 2007 as DOI: 10.1124/dmd.107.018192
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Table II. Characteristics of control and CRF rats

	Control (n=35)	CRF (n=35)	p-value
Body Weight (grams)	359.1 ± 6.1	322.0 ± 10.3	< 0.01
Serum creatinine (μmol/L)	52.5 ± 0.9	189.8 ± 18.8	< 0.001
Creatinine clearance (μL/100 g of body weight/min)	323.2 ± 12.2	82.1 ± 8.3	< 0.001
Serum urea (mmol/L)	5.0 ± 0.2	40.1 ± 5.8	< 0.001

Data are the mean ± SEM. Measurements were made at the time of sacrifice. Urinary collection was begun the day before. Blood urea and creatinine, and urine creatinine were determined with a Hitachi 717 autoanalyser (Boehringer Mannheim Canada, Laval, Québec, Canada).

Figure 1

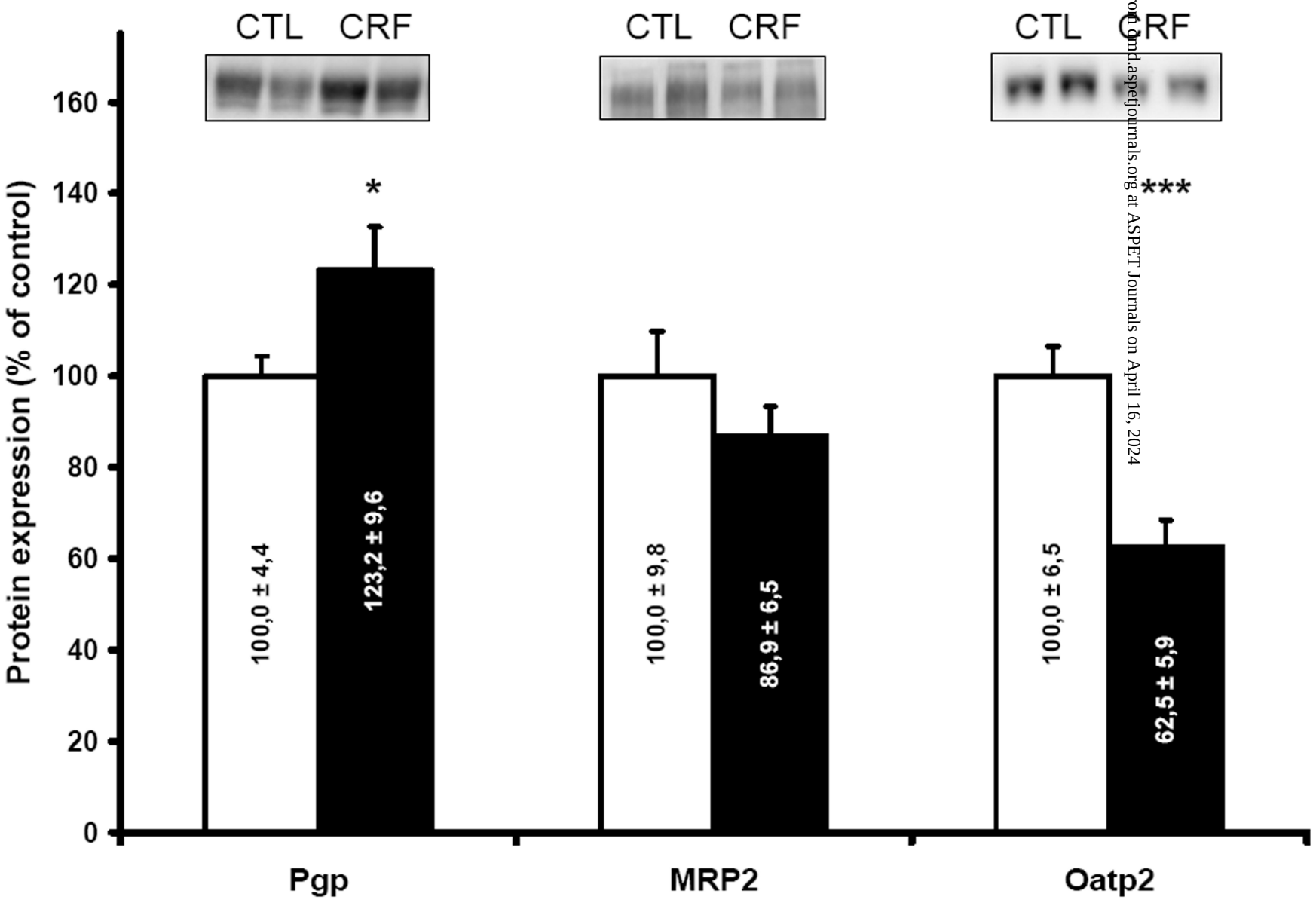

Figure 2

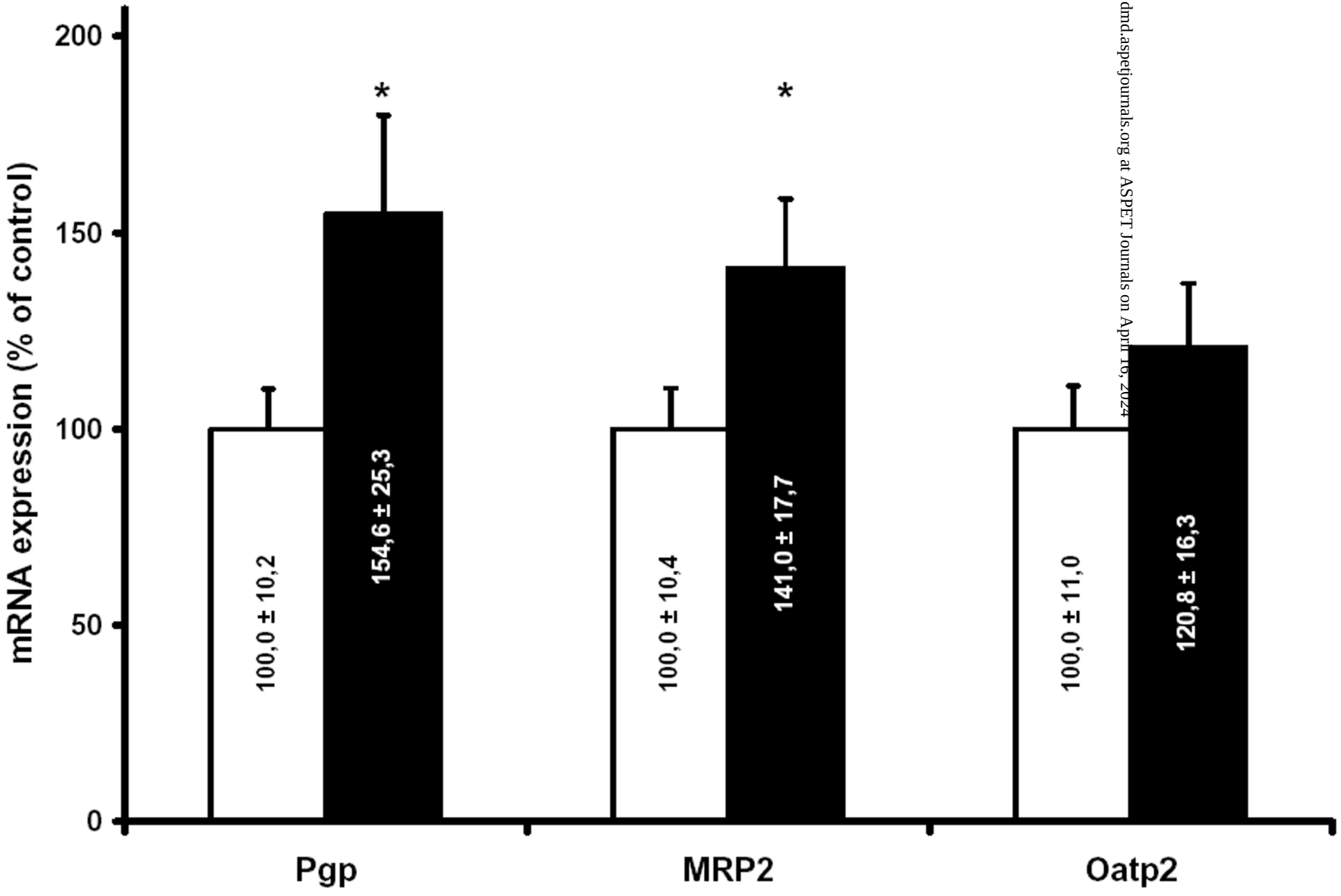

Figure 3

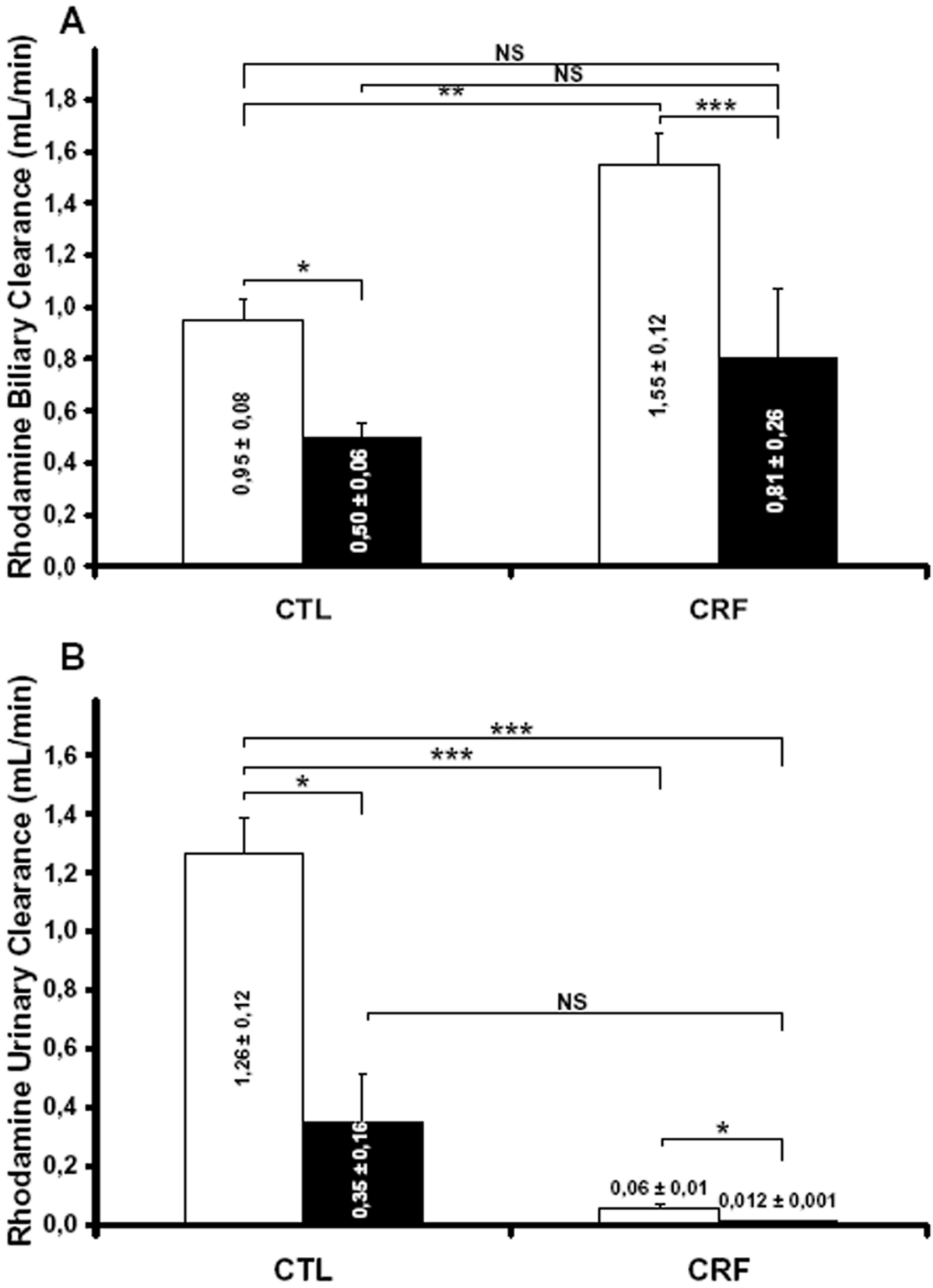
A
Rhodamine Biliary Clearance (mL/min)
1,8
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
NS
NS
**
***
*
0,95 ± 0,08
0,50 ± 0,06
1,55 ± 0,12
0,81 ± 0,26
CTL
CRF
B
Rhodamine Urinary Clearance (mL/min)
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
***
***
*
NS
*
1,26 ± 0,12
0,35 ± 0,16
0,06 ± 0,01
0,012 ± 0,001
CTL
CRF

Figure 4