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Regulations of Cell Membrane Transport Proteins in Rats with Obstructive Cholestasis: An Implication of Potential Therapeutic Target for Maintaining Digoxin Clearance

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Abstract

Drugs, such as digoxin, that undergo biliary elimination may have a decreased clearance in patients with obstructive cholestasis. The current study is to better understand the effect of extrahepatic cholestasis on regulations of membrane transporters involving digoxin and its implication for digoxin clearance. Twelve adult rats were randomly divided into bile duct ligation (BDL) and sham groups (n=6). Hepatic and renal laboratory values and digoxin pharmacokinetic (PK) studies were established before and 7 days after BDL or a sham procedure. Then, animals were sacrificed and tissue samples were taken to determine the expressions of cell membrane transport proteins by quantitative western blot and real-time PCR. Digoxin clearance was significantly decreased and liver function, but not renal function, was impaired in BDL rats. BDL resulted in increased MDR1 expression in the liver and kidney and decreased MDR1 expression the small intestine. OATP1A4 was up-regulated in the liver but down-regulated in the intestine after BDL. Expression of OATP4C1 was significantly increased in the kidney following BDL. The results suggest that cell membrane transporters of digoxin are regulated during extrahepatic cholestasis. These regulations are favorable for increasing digoxin excretion in the kidney and decreasing its absorption from the intestine to compensate for reduced digoxin clearance due to cholestasis.

Keywords: *Cholestasis; Digoxin clearance; OATP; P-glycoproteins/MDR1; Bile duct ligation*

Description

The main route of elimination of digoxin is renal excretion, which is closely correlated with glomerular filtration rate and combined with tubular secretion and reabsorption.

A smaller portion of digoxin undergoes biliary elimination with a certain degree of enterohepatic recycling [1]. Drugs with biliary elimination may have a decreased clearance in patients with cholestasis [2]. In an experimental model of cholestasis, bile duct ligation (BDL) in rabbits resulted in impaired excretion of digoxin [3,4]. These findings suggest that the administration of therapeutic dosages of digoxin during states of obstructive cholestasis may lead to its overdose in humans.

The movement of digoxin into and out of cells is mediated by different cell membrane transporters. The identification of a number of organic anions transporting polypeptides (OATP) and P-glycoproteins, also known as multidrug resistance protein 1 (MDR1), has revolutionized our understanding of the transport of biologic compounds and medications. To date, three transporters have been identified which are integral in digoxin clearance – MDR1, OATP1A4, and OATP4C1. In the rat, OATP1A4 (also known as OATP2) is found on the basolateral membrane of hepatocytes and the membrane of enterocytes serving as an influx transporter [5]. The MDR1 transporter is found in the liver, intestine, and the proximal tubule of kidney, and it acts as an efflux pump for digoxin, thus making it important in the elimination of the drug [6,7]. MDR1 is located on the canalicular membrane of hepatocytes, where it transports digoxin into the canaliculus. In the intestine, MDR1 is found on the apical membrane of enterocytes, where it serves as an effluxer to inhibit absorption of digoxin. In the kidney, MDR1 is found on the apical membrane of the proximal tubule, where it transports digoxin into the urine [8]. OATP4C1 is found in the kidney, located on the basolateral membrane of proximal tubule epithelial cells [9].

The physiological role of OATP4C1 in the kidney has been shown to be coupled with MDR1 to promote renal clearance of digoxin [9].

The distributions of the transporters vary in different tissues, and a transporter may function differently among the tissues [10]. This makes it difficult to explain the body's response to increased blood digoxin during cholestasis. Cholestasis results in increased expression of OATP1A4 and MDR1 in the liver which favors improved hepatobiliary excretion of digoxin [11,12]. The effect of cholestasis on OATP4C1 has not been studied to date. We performed this study to determine the effect of cholestasis on the expression of transporters responsible for the uptake and excretion of digoxin in the liver, kidney, and intestine. The implications of the changes in the transporters for digoxin pharmacokinetics are discussed.

Adult male Sprague Dawley (SD) rats (225-250 g) were randomly divided into a sham group (n=6) and a BDL group (n=6). BDL or sham surgery was performed and the animals were sacrificed 7 days after the surgeries. Digoxin PK study (intravenous administration of digoxin), liver and kidney function were measured before and 7 days after the surgeries. Tissue samples were collected for analyses of expressions of transporters by western blot and real-time PCR.

Cholestasis was induced by BDL as evidenced by elevated serum transaminase and bilirubin levels: alanine transaminase, 25 ± 8 and 192 ± 42; aspartate transaminase, 64 ± 8 and 526 ± 107; alkaline phosphatase, 142 ± 13 and 467 ± 60; total bilirubin, 0.05 ± 0.02 and 12 ± 2; unconjugated bilirubin, 0.03 ± 0.01 and 7 ± 2 (before and after BDL, respectively). BDL resulted in a significant decrease in digoxin clearance (figure 1) in correlating with prior studies in a rabbit model [3,4]. In the earliest study, BDL also resulted in elevation of serum creatinine prompting the authors to propose decreased renal excretion of orally administered digoxin as the major mechanism for decreased clearance with disruption of enterohepatic circulation as a potential complicating factor [3]. In a follow-up study, BDL led to decreased clearance of intravenously administered digoxin without alterations of serum creatinine levels. The authors postulated that impaired hepatic function and interruption of enterohepatic circulation resulted in reduced digoxin clearance [4]. In the current study, renal function remained normal following BDL (BUN, 18 ± 4 and 19 ± 6; creatinine 0.29 ± 0.03 and 0.28 ± 0.04, before and after BDL, respectively).

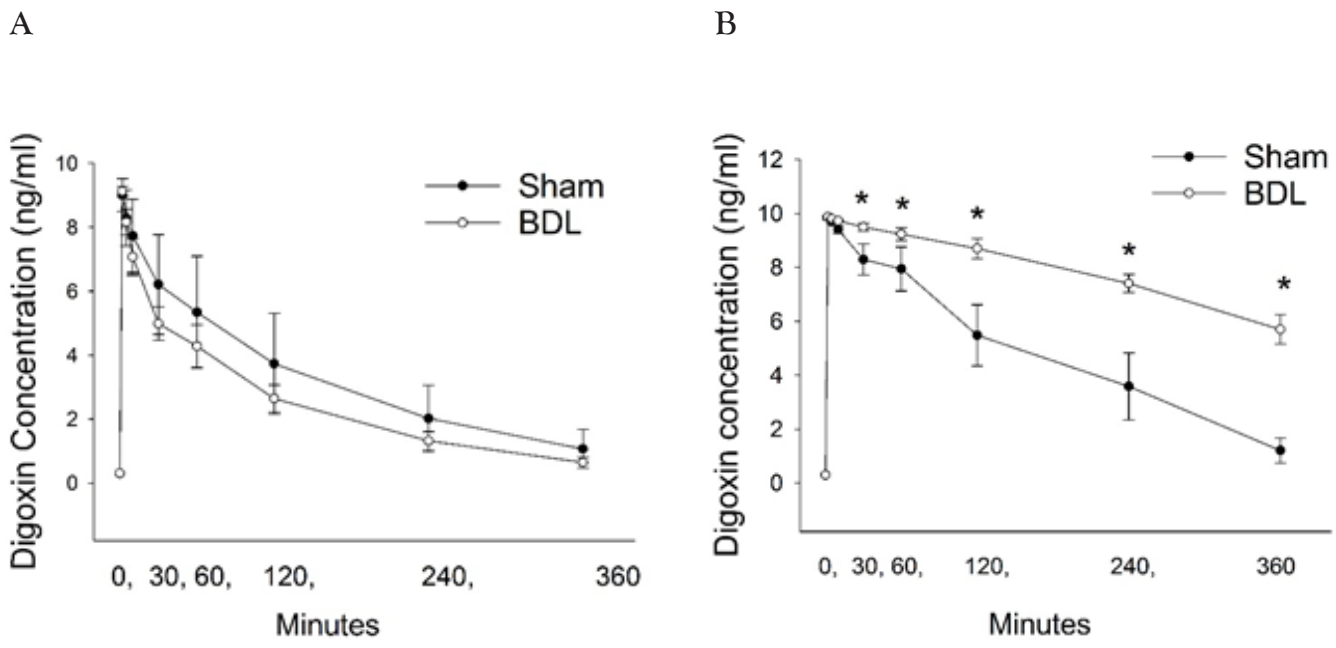

Figure 1: Effect of BDL on pharmacokinetics of digoxin in rats. Pre- (A) and post-surgery (B) digoxin pharmacokinetic studies were compared and presented as digoxin concentration-versus-time line curves. Values are expressed as means ±SD, n=6; [a]P<0.05 vs pre-surgery BDL group, [b]P<0.05 vs post-surgery sham.

Discovery of MDR1, OATP1A4, and OATP4C1 has allowed more in-depth investigation into the mechanisms of digoxin absorption and clearance. MDR1 is found on the apical membranes of proximal tubule cells, enterocytes, and hepatocytes, and it is responsible for efflux of digoxin [1,5]. Initial studies examined the role of MDR1 in digoxin clearance by inhibiting the protein with quinidine, which inhibited intestinal excretion of digoxin [13]. In a later study using MDR1 knock-out (KO) animals, fecal excretion of digoxin decreased and renal excretion increased compared to wild type (WT) animals; however, there was no significant change in biliary excretion [14]. The authors concluded that the lower fecal digoxin was secondary to a decrease in drug excretion by the intestinal epithelium, rather

than a decrease in biliary excretion, and that MDR1 contributes substantially to digoxin excretion via the intestinal epithelium and decreased re-uptake after biliary excretion. The increased renal excretion was surprising in the absence of MDR1 expression in the kidneys. The authors suggested that the increased renal clearance may be explained by other transporters [14].

Transport of digoxin in the liver is mediated by OATP1A4, which is responsible for uptake at the hepatocyte basolateral membrane, and MDR1, responsible for excretion into bile at the apical membrane [5, 15]. In the present study, BDL led to increased expressions of MDR1 and OATP1A4 in the liver (figure 2A, B, D and E) that could lead to increase in hepatic uptake of digoxin from the blood and increase in biliary excretion of digoxin. Although these changes would predict increased clearance of digoxin through bile, ligation of the bile duct precludes this mode of clearance.

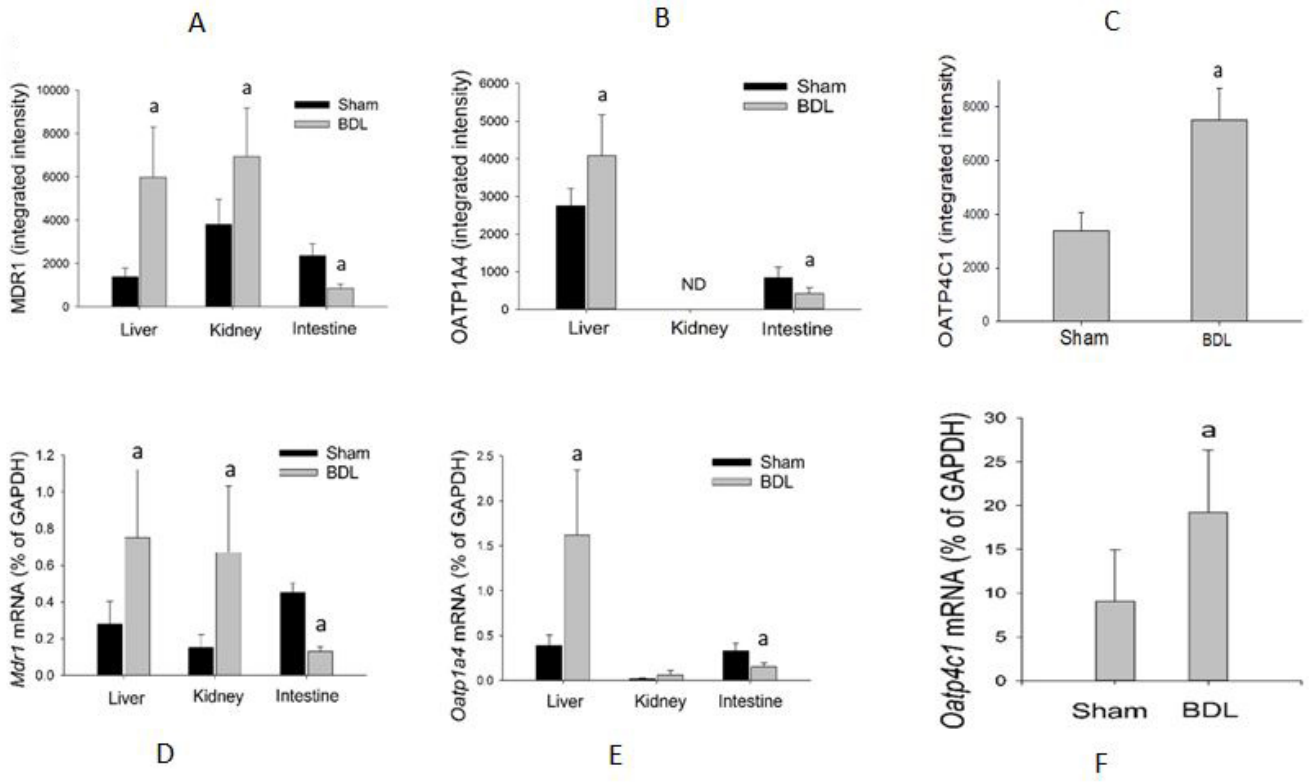

Figure 2: Effect of BDL on the expressions of *MDR1, OATP1A4* and *OATP4C1* in the liver, intestine and/or in the kidney. Transporter protein expressions of *MDR1* (A), *OATP1A4* (B) and *OATP4C1* (C, in kidney alone) were examined by quantitative western blot. Fluorescence densities of the protein bands were measured and normalized to the relative total protein amount of each sample. Values are depicted as means ± SD; n = 6; $^{a}P < 0.05$ compared with sham surgery rats. mRNA expressions were tested by real-time PCR. mRNA expressions in each sample was standardized to its GAPDH level. (A) Expressions of *Mdr1 (D), Oatp1a4 (E) and Oatp4c1 (F)* in the liver, small intestine and/or kidney. Values are depicted as means ± SD; n = 6; $aP < 0.05$ compared with sham surgery rats.

Recent studies demonstrated that intestinal *OATP1A4* is a carrier protein that transports drugs from the gut into the portal circulation [16], and digoxin has been shown as a substrate of *OATP1A4* [17]. Our results demonstrated that BDL resulted in decreased *OATP1A4* expression in the intestine (figure 2B and E). Decreased expression of intestinal *OATP1A4* favors decreased absorption predicting improved drug clearance in the feces. Although the change in *OATP1A4* favors digoxin clearance in the gut, clearance of intravenously administered digoxin is limited to renal excretion in the BDL model.

In the kidney *MDR1* is responsible for excretion of digoxin across the apical membrane of renal cells into urine [8]. Our pilot study revealed that *OATP1A4* is not expressed in the kidney of SD rats suggesting another transporter is responsible for transport of digoxin across the basolateral membrane into renal cells [9]. Mikkaichi T, et al. (2004) isolated an organic acid transporting peptide denoted *OATP4C1* both in humans and rats [9]. *OATP4C1* is localized on the basolateral membrane of the renal proximal tubules where it has been shown to be the primary transporter of digoxin into renal cells. *MDR1* is co-localized with *OATP4C1* in the proximal tubule. Renal failure resulted in decreased expression of renal *OATP4C1* but has no effect on expression of *MDR1* suggesting that decreased digoxin clearance in renal failure is due to loss of *OATP4C1* activity [9,18]. Our findings demonstrated that BDL resulted in increased expression of both *MDR1* and *OATP4C1* in the kidney (figure 2A, D, C and F) favoring enhanced vectorial transport of digoxin from blood to urine by proximal tubule cells. To the best of our knowledge, the current report is the first study to investigate the regulation of *OATP4C1* in the kidney in a pathological model in vivo. The results from the current study are summarized in table 1.

Table 1: Summary of the regulations of cell membrane transporters and potential effects on digoxin clearance.

	Efflux	Influx	Effects
Kidney	*MDR1* up-regulated	*OATP4C1* up-regulated	Increase tubule exclusion
Intestine	*MDR1* down-regulated	*OATP1A4* down-regulated	Decrease intestinal absorption
Liver	*MDR1* up-regulated	*OATP1A4* up-regulated	Increase exclusion into bile duct

It is interesting that MDR1 and OATP1A4 participate in transport of both bile acids and digoxin [19]. Similarly, OATP4C1 may also participate in the excretion of bile acids by the kidney through increased uptake at the basolateral membrane. Bile acids activate the nuclear hormone receptors farnesoid-X-receptor (FXR) and pregnane-X-receptor (PXR). Both MDR1 and OATP1A4 are PXR-responsive, and their expression increased in cholestasis [20,21]. OATP4C1 expression is indued through transitional factor Aryl hydrocarbon receptor (AhR) by binding of the xenobiotic responsive element (XRE) [22]. Previous studies have shown that AhR is activated in cholestasis [23] through the action of PXR [24]. We suggest that the up-regulation of OATP4C1 in cholestasis is best explained by this mechanism.

This is exploratory research to examine how the body responds to increased digoxin during cholestasis. Further studies are needed to confirm our finding by measuring digoxin tissue distributions and digoxin concentrations in urine and along the intestinal tract from the duodenum to the ileum. We believe that the findings from the current study will serve as a base for future studies of digoxin clearance mediated by renal-expressed OATP4C1 during cholestasis.

Conclusion

Under physiological conditions, the main route of elimination of digoxin is renal excretion which closely correlates with glomerular filtration rate. Biliary excretion is the major non-renal route. Enterohepatic cycle has minor importance [1]. Our finding demonstrated that under pathological conditions, such as cholestasis in the current study, cell membrane digoxin transporters are regulated which is in favor of an increase in digoxin excretion in renal proximal tubules and a decrease in its absorption from the intestine. These changes compensate for reduced digoxin clearance due to cholestasis. This finding could have clinical application by modifying the transporters' activities through pharmaceutical approaches for improving digoxin clearance during cholestasis.

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