Expression of Kidney and Liver Bilitranslocase in Response to Acute Biliary Obstruction

Anabel Brandoni[a]   Gisela Di Giusto[a]   Raffaella Franca[b]   Sabina Passamonti[b]
Adriana M. Torres[a]

[a]Farmacología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, CONICET, Rosario, Argentina; [b]Department of Life Sciences, University of Trieste, Trieste, Italy

Abstract

Background/Aim: It has been recently demonstrated that acute obstructive jaundice is associated with modifications in the renal expression and function of organic anion transporters such as Oat1, Oat3, Oatp1 and Mrp2. This study examined the expression and function of bilitranslocase in liver and kidney from rats with bile duct ligation (BDL). **Methods:** Bilitranslocase expression was evaluated in renal homogenates (H), renal basolateral plasma membranes (KBLM) and liver plasma membranes (LPM) by immunoblotting. Bilitranslocase function was studied by measuring the kinetic parameters of electrogenic bromosulfophthalein (BSP) uptake in KBLM and LPM by a spectrophotometric technique. **Results:** An increased abundance of bilitranslocase in KBLM without modifications in renal H and in LPM from BDL rats was observed compared with Sham rats. BDL rats showed a higher V_{max} for BSP uptake in KBLM. No differences between groups were observed for Michaelis-Menten parameters in LPM. **Conclusion:** The higher renal expression

and function of bilitranslocase in renal basolateral membranes from rats with obstructive cholestasis might also contribute to the dramatic increase in BSP renal excretion observed in this experimental model. This would be another compensation mechanism to overcome the hepatic dysfunction in the elimination of organic anions.

Copyright © 2010 S. Karger AG, Basel

Introduction

Extrahepatic cholestasis occurs in patients suffering from cholelithiasis and neoplasms affecting the pancreas and the common bile duct [1, 2]. Altered absorption, distribution and elimination of drugs have been described in this pathology [3]. Prolonged cholestasis may not only alter liver function but also kidney function [4].

β-Lactam antibiotics, diuretics, nonsteroidal anti-inflammatory drugs, several antiviral drugs as well as endogenous compounds are classified as organic anions. The hepatic and renal organic anion transport pathway plays a key role in the pharmacokinetics of these substances. It has been demonstrated that acute obstructive jaundice is associated with increased elimination of or-

ganic anions [5–8]. The increase in the systemic and renal elimination of p-aminohippurate and furosemide (two organic anions excreted through urine) is mediated, at least in part, by the upregulation of Oat1 [5, 6]. Brandoni and Torres [7] have also described a higher expression of Oatp1 in apical membranes from kidneys of bile duct ligated animals, which might explain the observed high increase in the renal excretion of bromosulfophthalein (BSP), an organic anion principally excreted in bile.

BSP is also a substrate of bilitranslocase, which is a membrane protein originally isolated from rat livers [9, 10]. The transport activity of bilitranslocase has been largely characterized in rat liver plasma membrane vesicles. Bilitranslocase has also been found to be expressed in the absorptive epithelia of the gastrointestinal tract and in the kidney [10]. In the kidney, bilitranslocase was detected in the basolateral domain of proximal tubular cells by immunohistochemistry [11]. Electrogenic BSP uptake in renal basolateral plasma membrane vesicles was inhibited by anti-bilitranslocase antibody [11].

The aim of the present work was to determine the contribution of bilitranslocase in the increased BSP urinary excretion observed in rats with acute extrahepatic cholestasis, by evaluating its expression and function both in liver and kidneys.

Materials and Methods

Experimental Animals
Male Wistar rats aged 110–130 days were used throughout the study. For surgical procedures, the animals were anaesthetized with sulfuric ether. After an upper abdominal incision performed under sterile technique, the common bile duct was isolated and double-ligated close to the liver hilus, immediately below the bifurcation and cut between the ligatures (BDL group) as previously described [5–7, 12]. A parallel group of sham rats was processed. Animals were cared for in accordance with the Principles and Guidelines for the Care and Use of Laboratory Animals. The day of the experiments, the rats were anesthetized with sodium thiopental (70 mg/kg b.w., i.p.).

Biochemical Determinations
Blood was withdrawn from femoral artery before administering BSP to sham and BDL animals. These samples were used to measure total and direct bilirubin as parameters indicative of hepatic function. The mentioned biochemical analyses were performed with optimized spectrophotometric techniques, employing commercial kits (Wiener Laboratory, Rosario, Argentina).

Urinary Excretion of BSP
BSP renal excretion was evaluated after administering a single bolus of BSP (10 mg/kg b.w., aqueous solution) through a femoral venous catheter. Urine was collected 40 min after administration in proper vials to measure the quantity of BSP. Concentration of BSP in urine was measured spectrophotometrically by alkalinization with 0.1 M NaOH [7].

Preparation of Basolateral Membrane Vesicles from Kidney Cortex
The preparation of the basolateral membrane vesicles from kidney cortex (KBLM) was done by a modification of the method described by Jensen and Berndt [13] as previously reported by us [6, 14]. Three different KBLM preparations were obtained for each experimental group.

Preparation of Liver Plasma Membrane Vesicles
Liver plasma membrane (LPM) vesicles were prepared according to van Amelsvoort et al. [15] in a medium containing 10 mM HEPES and 0.25 M sucrose (pH 7.40). Three different LPM preparations were obtained for each experimental group.

Preparation of Bilitranslocase Antibody
An anti-sequence bilitranslocase polyclonal antibody was used. The production and biological properties of this antibody, named antibody A, have been previously described [10, 16]. Antibody A was raised against a synthetic peptide corresponding to segment 65–75 of the predicted amino acidic sequence of bilitranslocase.

Immunoblot Technique
Immunoblotting and subsequent densitometry for bilitranslocase were performed in renal homogenates, KBLM and LPM as previously described [5–7]. The primary antibodies employed were polyclonal monospecific antibodies against bilitranslocase. Blots were processed for detection using a commercial kit (ECL enhanced chemiluminescence system, Amersham, Buckinghamshire, UK). A densitometric quantification of Western blot signal intensity of membranes was performed.

### BSP Transport Activity in Liver and Renal Plasma Membrane Vesicles
Bilitranslocase transport activity was assayed in membrane vesicles, using BSP as its standard transport substrate, as previously described in detail [10]. BSP concentration assay was recorded in real time at the wavelength pair 580–514 nm by a double-wavelength spectrophotometer (Sigma-ZWS II, Sigma Instrumente GmbH, Berlin, Germany). Briefly, 4 µl (12 µg protein) of renal or liver membrane vesicles was added to a stirred spectrophotometric polystyrene cuvette containing 2 ml of assay medium (0.1 M potassium phosphate) with different BSP concentrations (in the range of 5–80 µM), at room temperature. This addition caused an instantaneous drop in absorbance. After the attainment of a steady-state (4 s), a second drop in absorbance was brought about by valinomycin-induced K^+ diffusion potential by adding 2 ug valinomycin in 2 µl methanol. Such a K^+ diffusion potential drove the substrate into the vesicles. The slope of the linear phase of this absorbance drop, lasting about 1 s, is referred to as electrogenic BSP uptake and is related to bilitranslocase transport activity [10].

Materials
Chemicals were purchased from Sigma-Aldrich (St. Louis, Mo., USA) and were analytical grade pure.

Brandoni/Di Giusto/Franca/Passamonti/ Torres
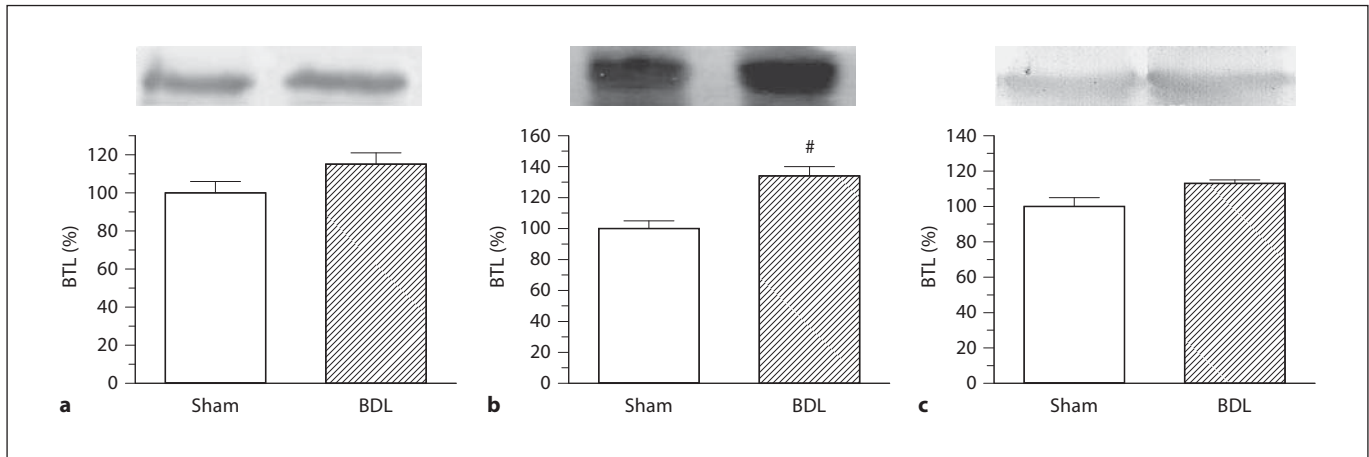
Fig. 1. Western blot analysis for bilitranslocase protein in kidney cortical homogenates (**a**), renal basolateral membranes (**b**) and liver plasma membranes (**c**) from sham and BDL rats. Bilitranslocase was identified using polyclonal antibodies as described in Materials and Methods. Sham levels were set at 100%. Each column represents mean $\pm$ SEM. [#] $p < 0.05$.
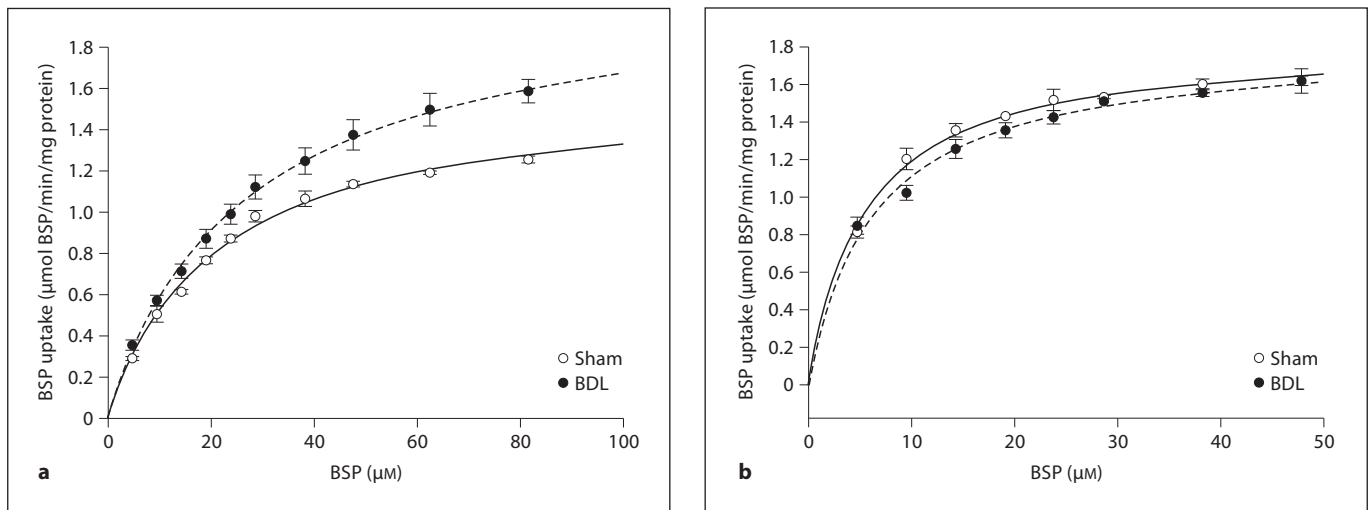
Fig. 2. Uptake of BSP into kidney basolateral plasma membrane vesicles (**a**) and into liver plasma membrane vesicles (**b**) from sham and BDL rats. Results are expressed as mean $\pm$ SEM from experiments carried out in triplicate in three different membrane preparations for each experimental group.

Statistical Analysis

Statistical analysis was performed using an unpaired t test. When variances were not homogeneous a Welch's correction was employed. $p < 0.05$ was considered significant. Values are expressed as means $\pm$ standard error (SEM). For these analyses, GraphPad software was used. Data for the characterization of the kinetics of electrogenic BSP uptake in KBLM and in LPM fitted the Michaelis-Menten equation and the apparent K_m and V_{max} values were derived with their standard errors using SigmaPlot 2001 software.

Results

Table 1 shows bilirubin serum levels and BSP urinary excretion. Total bilirubin and direct bilirubin concentrations increased in BDL rats, corroborating the adequacy of the study design. The urinary excretion of BSP greatly increased in BDL rats as previously described by Brandoni and Torres [7].

Table 1. Serum total and direct bilirubin levels and BSP urinary excretion in sham and BDL rats

	Sham (n = 4)	BDL (n = 4)
Total bilirubin, mg/l	4.9 ± 0.2	43.1 ± 3.0*
Direct bilirubin, mg/l	1.8 ± 0.2	33.4 ± 2.9*
BSP urinary excretion, μg	2.02 ± 0.32	31.30 ± 12.50*

Results are expressed as means ± SEM. * $p < 0.05$.

Table 2. Michaelis-Menten kinetic parameters of BSP in KBLM and in LPM vesicles

	Sham (n = 3)	BDL (n = 3)
KBLM		
K_m, μM	21 ± 2	26 ± 1
V_{max}, μmol BSP/min/mg protein	1.61 ± 0.04	2.12 ± 0.04*
LPM		
K_m, μM	5.4 ± 0.4	6.3 ± 0.6
V_{max}, μmol BSP/min/mg protein	1.84 ± 0.03	1.82 ± 0.04

Results are expressed as means ± SEM from experiments carried out in triplicate in three different membrane preparations for each experimental group. * $p < 0.05$.

Kidney cortex homogenates (H), KBLM and LPM from sham and BDL animals were subjected to immunoblot analysis for bilitranslocase protein. Figure 1 shows an increased abundance of bilitranslocase in renal KBLM without modifications in renal H and in LPM from BDL rats as compared with sham rats.

BSP uptake in KBLM and in LPM is shown in figure 2. BDL rats showed a higher V_{max} for BSP uptake in KBLM as compared with sham rats. No differences between groups were observed for Michaelis-Menten parameters in LPM (table 2).

Discussion

Extraction of organic anions from circulation is an essential function of the liver and the kidneys [17]. It has been found that cholestasis alters the transport of bile salts and of other organic anions [1, 2]. Previous studies performed in our laboratory showed modifications in the expression of organic anion transporters as Oat1, Oat3 and Oatp1 [5–7].

Renal secretion of organic anions has a critical role in regulating their plasma concentrations and in clearing the body of potentially toxic xenobiotic agents, drugs of pharmacological importance and endogenous compounds [18, 19]. The transepithelial transport involves an entry step at the basolateral plasma membrane and an exit step at the luminal plasma membrane of renal tubular cells. Several proteins have been cloned and functionally characterized as the organic anion transporters involved in these steps. BSP is an organic anion mainly excreted by the liver which is a substrate for several carrier proteins such as Oat3, Oatp1, Mrp2 and bilitranslocase.

We have demonstrated an increase in BSP urinary excretion in rats with extrahepatic cholestasis associated with a higher expression of Oatp1 in apical membranes and an unchanged expression of Oat3 in basolateral membranes from kidneys [5–7]. Moreover, Mrp2 upregulation in kidneys from BDL rats has been described [20, 21].

Bilitranslocase is not a bile acid transporter, but rather a bile pigment transporter [9, 10, 16]. Bilitranslocase has been indicated as the protein responsible for the electrogenic hepatic uptake of cholephilic organic anions, such as BSP and thymol blue, the tetrapyrrole bilirubin, and flavonoids (the anthocyanin malvidin 3-glucoside and the flavonol quercetin) [10]. Bilitranslocase has also been detected in basolateral plasma membranes from kidney cells and has been demonstrated to be involved in the renal transport of BSP, bilirubin and anthocyanins [11, 22]. In this way, bilitranslocase contributes to the hepatic and renal elimination of exogenous organic anions (such as BSP), endogenous metabolites (such as bilirubin) and anthocyanins (flavonoid-based pigments that are present in many fruits and vegetables in the human diet, which have been reported to be positively implicated in human health [22]).

To evaluate the functional activity of bilitranslocase, we measured BSP electrogenic uptake in LPM and in KBLM vesicles prepared from sham and BDL rats. No modifications were observed in bilitranslocase activity and abundance in LPM vesicles from BDL rats. On the contrary, the extrahepatic cholestasis resulted in a marked increase of renal BSP uptake and this was due to an important increase in V_{max} (capacity). The capacity of a transfer is principally determined by the total number of active carriers presented in KBLM. The difference in V_{max} indicates that a higher number of functional carrier units exists in KBLM vesicles from BDL rats, which is in agreement with the higher expression of bilitranslocase in KBLM. The observation that BDL rats have a higher

Brandoni/Di Giusto/Franca/Passamonti/Torres

renal expression of bilitranslocase at the basolateral membranes despite no change in bilitranslocase abundance in kidney H suggests an alteration in bilitranslocase trafficking that might be caused by an increased recruitment of preformed transporters into the membranes or an inhibition in the internalization of membrane transporters. The same behavior was observed for renal Oatp1 expression in the same experimental model of acute extrahepatic jaundice [7].

It has also been found that urinary excretion of bile acids is markedly increased in obstructive liver diseases [23]. The increase in bile acids urinary excretion may be a consequence of the fact that the amount of filtered bile acids in cholestasis exceeds the maximum capacity for tubular reabsorption. But it may also involve adaptive mechanisms of the kidneys, such as a decline of tubular apical reabsorption of bile acids and an enhanced tubular basolateral uptake. Mrp2 and the sodium-dependent bile salt transporter ABST are two renal apical bile acids carriers; Mrp2 mediates the exit of bile acids from the cells and ABST is involved in their reabsorption. As was previously mentioned, Mrp2 is upregulated [20] and there is a fall in the function of ABST [24] in the presence of acute extrahepatic cholestasis. Both mechanisms may also contribute to the increased excretion of bile acids in the presence of this pathology. On behalf of the basolateral domain, Oat3 transports bile acids from the blood into the cells. As has been mentioned earlier, its expression does not change [6].

These results suggest that the complex series of hormonal changes induced in kidneys by extrahepatic cholestasis [25] might influence the regulation of bilitranslocase and Oatp1 in a similar way. The characteristic accumulation of bile acids, bilirubin, and other potential toxins in cholestasis may affect transcriptional and post-transcriptional regulatory mechanisms [26, 27]. In this connection, bilirubin, sulfate-conjugated bile acid and human bile upregulated the expression of Mrp2 in renal tubular cells but not in liver cells [20].

The increase in bilitranslocase protein units at the basolateral membrane together with the increased expression of Oatp1 and Mrp2, and the fall in ABST function, at the apical domain of renal cells may be a compensatory mechanism for protecting hepatocytes or kidney cells from cytotoxic substances that accumulate in the presence of obstructive cholestasis. The upregulation observed in the three protein carriers might explain the dramatic increase in BSP renal elimination displayed in this pathology.

Acknowledgments

References

1 Koopen NR, Müller M, Vonk RJ, Zimniak P, Kuipers F: Molecular mechanisms of cholestasis: causes and consequences of impaired bile formation. Biochim Biophys Acta 1998;1408:1–17.

2 Reichen J, Simon FR: Cholestasis; in Arias M, Jakoby WB, Popper H, Schachter D, Shafritz DA (eds): The Liver: Biology and Pathology, ed 2. New York, Raven Press, 1988, pp 1105–1124.

3 Sugi K, Inoue M, Morino Y, Sato T: Effect of obstructive jaundice on the fate of a nephrophilic organic anion in the rat. Biochim Biophys Acta 1989;987:217–221.

4 Kramer HJ: Impaired renal function in obstructive jaundice: roles of the thromboxane and endothelin systems. Nephron 1997;77:1–12.

5 Brandoni A, Quaglia NB, Torres AM: Compensation increase in organic anion excretion in rats with acute biliary obstruction: role of the renal organic anion transporter 1. Pharmacology 2003;68:57–63.

6 Brandoni A, Villar SR, Picena JC, Anzai N, Endou H, Torres AM: Expression of rat renal cortical OAT1 and OAT3 in response to acute biliary obstruction. Hepatology 2006;43:1092–1100.

7 Brandoni A, Torres AM: Characterization of the mechanisms involved in the increased renal elimination of bromosulfophthalein during cholestasis: involvement of Oatp1. J Histochem Cytochem 2009;57:449–456.

8 Torres AM: Renal elimination of organic anions in cholestasis. World J Gastroenterol 2008;14:6616–6621.

9 Tiribelli C, Lunazzi G, Luciani M, Panfili E, Gazzin B, Liut G, Sandri G, Sottocasa GL: Isolation of a sulfobromophthalein-binding protein from hepatocyte plasma membrane. Biochem Biophys Acta 1978;531:105–112.

10 Passamonti S, Terdoslavich M, Franca R, Vanzo A, Tramer F, Braidot E, Petrussa E, Vianello A: Bioavailability of flavonoids: a review of their membrane transport and the function of bilitranslocase in animal and plant organisms. Curr Drug Metab 2009;10:369–394.

11 Elías MM, Lunazzi GC, Passamonti S, Gazzin B, Miccio M, Stanta G, Sottocasa GL, Tiribelli C: Bilitranslocase localization and function in basolateral plasma membrane of renal proximal tubule in rat. Am J Physiol 1990;259:F559–F564.

12 Brandoni A, Torres AM: Extrahepatic cholestasis model; in Rigalli A, Di Loreto V (eds): Experimental Surgical Models in the Laboratory Rat. Boca Ratón, CRC Press Taylor and Francis Group, 2009, pp 139–141.

13 Jensen RE, Berndt WO: Epinephrine and norephinephrine enhance p-aminohippurate transport into basolateral membrane vesicles. J Pharmacol Exp Ther 1988;244:543–549.

14 Di Giusto G, Torres AM: Transport studies in plasma membrane vesicles isolated from renal cortex; in Rigalli A, Di Loreto V (eds): Experimental Surgical Models in the Laboratory Rat. Boca Ratón, CRC Press Taylor and Francis Group, 2009, pp 189–194.

15 van Amelsvoort JMM, Sips HJ, van Dam K: Sodium-dependent alanine transport in plasma-membrane vesicles from rat liver. Biochem J 1978;174:1083–1086.

16 Battiston L, Passamonti S, Macagno A, Sottocasa GL: The bilirubin-binding motif of bilitranslocase and its relation to conserved motifs in ancient biliproteins. Biochem Biophys Res Commun 1998;247:687–692.

17 Fleck C, Bräunlich H: Renal handling of drugs and amino acids after impairment of kidney or liver function: influences of maturity and protective treatment. Pharmacol Ther 1995;67:53–57.

18 Miyazaki H, Sekine T, Endou H: The multi-specific organic anion transporter family: properties and pharmacological significance. Trends Pharmacol Sci 2004;25:654–662.

19 Sekine T, Miyasaki H, Endou H: Molecular physiology of renal organic anion transporters. Am J Physiol 2006;290:F251–F261.

20 Tanaka Y, Kobayashi Y, Gabazza EC, Higuchi K, Kamisako T, Kuroda M: Increased renal expression of bilirubin glucuronide transporters in a rat model of obstructive jaundice. Am J Physiol 2002;282:G656–G662.

21 Villanueva SSM, Ruiz ML, Soroka CJ, Cai S-H, Luquita MG, Torres AM, Sánchez Pozzi EJ, Pellegrino JM, Boyer JL, Catania VA, Mottino AD: Hepatic and extrahepatic synthesis and disposition of dinitrophenyl-S-glutathione in bile duct-ligated rats. Drug Metab Dispos 2006;34:1301–1309.

22 Vanzo A, Terdoslavich M, Brandoni A, Torres AM, Vrhovsek U, Passamonti S: Uptake of grape anthocyanins into the rat kidney and the involvement of bilitranslocase. Mol Nutr Food Res 2008;52:1106–1116.

23 Ostrow JD: Metabolism of bile salts in cholestasis in humans; in Tavolin N, Berk PD (eds): Hepatic Transport and Bile Secretion: Physiology and Pathophysiology. New York, Raven Press, 1993, pp 673–712.

24 Schlattjan JH, Winter C, Greven J: Regulation of renal tubular bile acid transport in the early phase of an obstructive cholestasis in the rat. Nephron Physiol 2003;95:49–56.

25 Leung N, Croatt AJ, Haggard JJ, Grande JP, Nath KA: Acute cholestatic liver disease protects against glycerol-induced acute renal failure in the rat. Kidney Int 2001;60:1047–1057.

26 Donner MG, Schumacher S, Warskulat U, Heinemann J, Häussinger D: Obstructive cholestasis induces TNF-a- and IL-1b-mediated periportal downregulation of Bsep and zonal regulation of Ntcp, Oatp1a4, and Oatp1b2. Am J Physiol 2007;293:G1134–G1146.

27 Geier A, Dietrich CG, Trauner M, Gartung C: Extrahepatic cholestasis downregulates Oatp1 by TNF-alpha signalling without affecting Oatp2 and Oatp4 expression and sodium-independent bile salt uptake in rat liver. Liver Int 2007;27:1056–1065.