Renal response of solute carrier transporters and related proteins in obstructive jaundice

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ABSTRACT

Obstructive jaundice mainly takes place after cholelithiasis and neoplasms that affect the pancreas and the common bile duct. The liver and kidney eliminate toxins, pharmacotherapeutic drugs, and endogenous metabolites. It has been reported that the alteration of one route of excretion can be compensated by the other route. Modifications in the expression of several carrier proteins have been observed after the impairment of the hepatic function. The present work updates the modifications reported in the renal expression and in the urinary levels of some proteins belonging to the solute carrier family (such as Oatp1, Oat1, Oat3, Oat5, Asbt, and NKCC2) and some proteins related in some way to these ones (such as AQP2, Cav-1, and Cav-2). An increased renal expression of Oatp1, Oat1, Oat3, Oat5, and a decreased abundance of Abst was observed after 21 h of bile duct ligation, explaining the increase in the renal clearance of different compounds that could not be excreted by the liver because the biliary excretion is impaired. Moreover, the decreased expression of NKCC2 and AQP2 together with the increase in medullary renal blood flow could account for the increase in the urinary flow previously reported in this pathological state. In addition, a decreased expression of Cav-1 and an increased expression of Cav-2 in kidneys were reported in the early phase of acute cholestasis. It is well-known that renal function is altered during cholestasis and that the impairment of this organ increases with the time course of cholestasis. Increase urinary levels of NaDC1, Cav-1, and Cav-2 together with a decrease of Oat5, plus the absence of modifications of NKCC2 and AQP2 were detected after 21 h of bile duct ligation in the absence of alterations in traditional parameters of renal function. Thus, the urinary levels of these proteins were proposed as a novel panel of biomarkers of the early phase of acute obstructive jaundice.

Key words: obstructive jaundice; Oats; Oatp1; NKCC2; AQP2; caveolins

INTRODUCTION

Cholestasis is characterized by the retention of bile and bile components. Jaundice, pruritus, and an increase of bile salts, bilirubin, alkaline phosphatase, and related enzymes in serum are the main clinical indications of cholestasis. There are “intrahepatic” and “extrahepatic” forms of cholestasis, which have similar clinical manifestations. Intrahepatic cholestasis lies within the liver and has multiple origins, such as familial cholestasis, drug-induced cholestasis, and several processes that alter bile formation and excretion. Extrahepatic cholestasis is due to obstruction of large bile ducts outside the liver, which may be caused by gallstones or by tumours (mainly neoplasms in the pancreas and the common bile duct).

Ethical considerations usually preclude meaningful clinical investigation in patients with obstructive jaundice. Human cholestasis has been simulated by experimental models in animals. Double ligation and division of the common bile duct in rodents and in dogs...
is the most frequent model for obstructive jaundice.[4] In these experimental models, the decrease in bile flow is generally correlated with the increase in cholestatic serum biomarkers, modifications in liver histology, and altered liver functions associated with sinusoidal and canalicular transport.[2,3,5] Prolonged obstructive cholestasis modifies the liver function because of an altered uptake, biotransformation, and secretion of endogenous and exogenous compounds. In the presence of this pathology, adaptive mechanisms allow the urinary elimination of potentially toxic compounds that cannot be excreted by the liver. The liver and kidney have an important role in the excretion of toxic xenobiotics and endogenous metabolites.[5,7] Sometimes, the failure of one route of elimination is substituted by the other one. It is also important to take into account that alteration of liver or kidney functions can produce damage to the alternative elimination organ. It has been reported that prolonged retention of bile compounds caused by cholestasis may produce renal damage such as impairment of renal hemodynamics, alteration of renal excretion of salts and water, and sensitization of the kidney to anoxia damage, producing in some cases renal failure.[5–8]

The solute carrier (SLC) superfamily is integrated by around 60 gene subfamilies with approximately 400 family members. These genes encode several transporters (uniporters, antiporters, and symporters). The SLCs, in general transport compounds either down their concentration gradient or against their concentration gradient, coupled with the movement of a second substance down its concentration gradient.[9,10] The SLC transporter superfamily has a relevant role in the absorption, distribution, metabolism, and excretion of pharmacologically important drugs (e.g., antivirals, non-steroidal anti-inflammatory, antibiotics) and exogenous toxins (e.g., organic mercurial). They also play an important role in the elimination of numerous endogenous compounds. Several sodium transporters belong to the SLC superfamily playing an important role in renal physiology and pathophysiology.[10]

In the present revision, the expression and function of several SLC transporters in obstructive jaundice will be updated including organic anion transporting polypeptide 1 (Oatp1), organic anion transporter 1 (Oat1), organic anion transporter 3 (Oat3), organic anion transporter 5 (Oat5), sodium-dicarboxylate cotransporter 1 (NaDC1), sodium-potassium-2 chloride cotransporter (Na-K-2Cl cotransporter, NKCC2), sodium dependent bile salt transporter (Asb1). Caveolin 1 (Cav-1) and caveolin 2 (Cav-2) have a relevant role in the regulation of Oat1 and Oat3 renal expression;[11,12] thus, their behavior in extrahepatic cholestasis will also be described. As aquaporin 2 (AQP2) has an important function in water homeostasis working in collaboration with NKCC2.[13] its expression and function in this pathology will also be discussed.

RENAL RESPONSE OF SOLUTE CARRIER TRANSPORTERS IN OBSTRUCTIVE JAUNDICE

**Organic anion transporting polypeptide 1, Slco1a1**

Oatps are a group of membrane solute transporters with a vast range of amphipathic substrates. Several members of the Oatp family are found in the brush border membrane of proximal tubule cells, where play a relevant function in the secretion/reabsorption of selected anionic compounds.[14] Oatp1 was the first discovered member of Oatps. It is a sodium-independent carrier expressed at the basolateral membrane of hepatocytes and at the brush border membrane of the S3 segment of renal proximal tubule cells. In the liver, Oatp1 mediates the uptake of amphipathic albumin-bound compounds such as bile acids, bilirubin, thyroid hormones, and conjugated steroids. In the kidney, Oatp1 participates in the secretion of certain organic substrates that were taken up into tubular cells across the basolateral membranes, and also in the reabsorption of organic compounds filtered by the glomerulus.[14–16] Oatp1 is also involved in the transport of a wide variety of pharmacotherapeutics drugs such as cardiac glycosides, β-lactam antibiotics, statins, angiotensin receptor II antagonists, and angiotensin-converting enzyme inhibitors.[14–16]

The expression and function of Oatp1 are regulated at the transcriptional level as well as at the post-transcriptional level and are tissue-specific in some cases. For example, testosterone stimulates and estrogens inhibit Oatp1 expression in renal tissue. Meanwhile, the expression of Oatp1 is not regulated by sex hormones in the liver.[17] It has also been described a functional down-regulation of Oatp1 via serine phosphorylation, which is mediated by extracellular ATP.[18] Moreover, the transport of estrone-3-sulfate (ES) is decreased by protein kinase C activation.[19]

In the experimental model of obstructive cholestasis induced by bile duct ligation (BDL) in rats, liver Oatp1 expression is down-regulated.[19] As several organic anions are taken in the liver from the systemic circulation by Oatp1, its down-regulation may be an adaptive change to limit the excess uptake of various organic anions into the hepatocytes in the presence of obstructive jaundice.

Studies from our laboratory in rats with extrahepatic cholestasis demonstrated an increase in kidney expression of Oatp1 protein at the apical membranes
Regardless of no modification in Oatp1 protein abundance in kidney homogenates.\textsuperscript{[28]} A modification in Oatp1 protein traffick produced by an increase in the recruitment of preformed transporters into the membranes or by an inhibition in the internalization of membrane carriers might be postulated. The increase in Oatp1 protein units at the brush border membrane of proximal tubule cells may be a compensatory mechanism for reducing the liver and renal damage caused by the cytotoxic compounds that circulate in obstructive cholestasis. This adaptation to hepatic dysfunction, specifically in biliary components elimination, observed in this obstructive jaundice model might explain, at least in part, the increase in the renal elimination of bromosulfophthalein (BSP, a prototypical organic anion mainly excreted in bile) observed in BDL rats.\textsuperscript{[20]}

Numerous hormonal changes have been reported in kidneys in the presence of obstructive cholestasis\textsuperscript{[21]} which might affect Oatp1 regulation. The accumulation of bilirubin, bile acids, and other potential toxins existing in this experimental model might alter the transcriptional (e.g., fetal transcription factor, pregnene X receptor) and the post-transcriptional regulation mechanisms.\textsuperscript{[22]}

**Organic anion transporter 1, Slc22a6**

Oat1 was originally named NKT for novel kidney transporter.\textsuperscript{[23]} Oat1 (NKT) is the prototypical member of the OAT subfamily of SLC22 transporters. Oat1 mediates organic anion/alpha-ketoglutarate uptake by indirect coupling of a sodium gradient produced by the activity of Na-K-ATPase.\textsuperscript{[24,25]} This carrier is nearly linked to aerobic metabolism in proximal tubule cells.\textsuperscript{[25]} In this connection, a higher urinary and a lower plasma concentration for alpha-ketoglutarate were reported in Oat1 knockout mice.\textsuperscript{[24]} Metabolic network-based predictions using transcriptomic and metabolomic data of Oat1 knockout mice suggested a relevant role for Oat1 in numerous metabolic pathways, such as tricarboxylic acid cycle, and the biosynthesis of amino acids, fatty acids, prostaglandins, cyclic nucleotides, and vitamins.\textsuperscript{[26]} Vriend \textit{et al}\textsuperscript{[26]} have experimentally supported part of these observations.

Oat1 is a dynamic membrane carrier.\textsuperscript{[27]} Short-term activation of protein kinase C (PKC) promotes Oat1 ubiquitination that in turn triggers its accelerated endocytosis from the plasma membrane into intracellular endosomes.\textsuperscript{[28,29]} It has also been reported that the long-term effect of PKC on Oat1 significantly increases Oat1 degradation.\textsuperscript{[30]}

Oat1 is mainly expressed in the kidneys and weakly in the brain. Oat1 has been immunolocalized in the basolateral membrane of the proximal tubule cells. Several compounds such as dicarboxylates, nucleotides, prostaglandins, antivirals, loop and thiazide diuretics, \(\beta\)-lactam antibiotics, non-steroidal anti-inflammatory drugs, including the prototypical substrate of the classical pathway, para-aminohippurate (PAH) are transported by Oat1.\textsuperscript{[23,25,27,31,32]} Eraly \textit{et al}\textsuperscript{[24]} have generated a colony of Oat1 knockout mice, observing that the knockout mice show an important loss of organic anion transport (e.g., PAH) both \textit{ex vivo} (in isolated renal slices) as well as \textit{in vivo} (as indicated by loss of renal secretion). The loss of furosemide (FS) renal secretion in knockout animals caused altered diuretic responsiveness to this drug\textsuperscript{[24]}.

It was reported a renal up-regulation of Oat1 protein expression (both in cortical homogenates and in basolateral membranes) after 21 hours of BDL in rats which might explain the increased renal excretion of both PAH and FS\textsuperscript{[31,34]} associated with a concomitant increase of systemic and renal PAH and FS clearance. In this connection, the PAH uptake rate was increased in basolateral membranes vesicles from BDL rats (higher Vmax and no modification in Km).\textsuperscript{[33]} The up-regulation of Oat1 might suggest an increase in the synthesis or a decrease in the degradation of this carrier. The increase in Oat1 renal expression might enhance renal secretion of toxic compounds that may be harmful in the presence of the pathological state. In this connection, Tanaka \textit{et al}\textsuperscript{[35]} observed that bilirubin ditarurate, sulfate conjugated bile acids, and some components of the human bile upregulate the expression of Mrp2 in human renal tubular cells and we have reported an increased \^{}3H-PAH uptake by S2 cells expressing Oat1 in the presence of bilirubin ditarurate.\textsuperscript{[36]} Moreover, Nisetto \textit{et al}\textsuperscript{[37]} have reported that Oat1 expression in membranes from tubule renal cells is increased after the incubation with serum from BDL rats.

Brandoni \textit{et al}\textsuperscript{[38]} performed similar experiments after 3 days of bile duct ligation (the peak of elevation of serum bile acids and bilirubin) observing a reduction in the renal elimination of PAH. Oat1 protein expression decreased in basolateral membranes without modifications in kidney homogenates, suggesting internalization of membrane carriers or an inhibition in the recruitment of preformed transporters into the membranes. Thus, the evolution time of obstructive cholestasis is relevant in Oat1 regulation. It has been reported that PKC induces Oat1 downregulation mediated by carrier retrieval from the cell membrane. The peak of elevation of bile acids and bilirubin by three days of bile duct ligation can activate PKC, which may phosphorylate caveolin-2, leading to the internalization of caveolae with the Oat1 protein anchored with caveolin.\textsuperscript{[12]} Kwak \textit{et al}\textsuperscript{[11,12]} have reported that Oat1 and Oat3 colocalize with caveolins.

Caveolins are transmembrane proteins, which form specialized membrane domains in association with
cholesterol and sphingolipids. The caveolae-type lipid raft domains (LRD) function as platforms where the localization, conformational stability, and ligand affinity of the residing proteins can be regulated. Nosetto et al. analyzed the membrane distribution of Oat1 between LRD and non-LRD and showed that Oat1 is concentrated in LRD in physiological conditions. On the other hand, a shift of Oat1 from LRD to non-LRD was observed in rats with 21 h of bile duct ligation. The residence in membrane microdomains with different lipid environments might modify the transport capacity of membrane transporters. It has been reported that the shift of the hepatic sinusoidal bile salt transporter (Ntcp) from the raft to the non-raft domains causes an important increase in its transport function. Thus, the shift of Oat1 from the raft to the non-raft fraction of the membrane could be associated with the increase previously reported in its transport function in the acute phase of obstructive cholestasis. Moreover, the Oat1 shifts from the raft to the non-raft fraction could be avoiding its internalization by the caveolae-mediated pathway, allowing more Oat1 to be stably expressed in the membrane. Consequently, the kidney could excrete through Oat1, some compounds that are normally excreted by the liver, as an alternative to the biliary excretion process that is altered in the extrahepatic cholestasis.

**Organic anion transporter 3, Slc22a8**

Oat3 is mainly expressed at the basolateral membrane of renal proximal tubules. It is also found in the liver, in the human choroid plexus, and in cerebral capillaries. Oat3 has a wide range of substrates, and it mediates the transport of PAH, ochratoxin A, ES, cimetidine, benzylpenicillin, cephaloridine, and glutarate. Oat3 selectivity overlaps that of Oat1, but affinities for several substrates appear to permit discrimination between both transporters. Oat3 knockout mice showed reduced uptake of PAH, ES, and taurocholate in renal cortical slices and nearly complete inhibition of transport of the fluorescent organic anion fluorescein in intact choroid plexus. Oat3 is regulated by PKC and by PKA. PKC increases Oat3 ubiquitination, promoting Oat3 internalization to intracellular endosomes and consequently its degradation, reducing in this way Oat3 transport activity. On the other hand, Oat3 expression and transport activity are stimulated by PKA due to the alteration in the trafficking kinetics of Oat3 through crosstalk between SUMOylation and ubiquitination. In addition, insulin-like growth factor 1 significantly increases Oat3 transport activity and SUMOylation through PKA signaling pathways. Brandoni et al. have reported an increase in Oat3 expression in renal cortex homogenates without modifications in basolateral membranes after 21 h of bile duct ligation. These results indicate an increase in the synthesis, although these performed proteins are not already anchored into membranes or a decrease in the degradation of the Oat3 protein. On the other hand, Oat3 protein expression increased both in cortex homogenates and in basolateral membranes after 3 days of bile duct ligation. This study demonstrated the key role of Oat3 expression in the impaired elimination of PAH after 3 days of obstructive cholestasis. Oat3 is expressed in various cells and all parts of the nephron, whereas Oat1 is only expressed in proximal tubule cells. Oat3 transports PAH with high affinity similar to Oat1. On the contrary, it has been reported that ES, cholate, and taurocholate are substrates for Oat3 and not for Oat1. The overexpression of Oat3 does not compensate for the down-regulation of Oat1 regarding PAH transport because after 3 days of bile duct ligation the high plasma levels of bile acids compete with PAH for Oat3 transport. Moreover, bile acids regulate the expression of several genes involved in bile salt transport. High bile acid levels may upregulate Oat3 expression without affecting Oat1 expression.

It has been reported that Oat3 is responsible for the renal secretion of bile acids during cholestasis and that cholestasis may affect the pharmacokinetic profile of Oat3 substrates. In obstructive cholestasis, the main route to excrete bile acids is urinary excretion. It has been observed that Oat3 protein expression is increased in Eisai hyperbilirubinemic rats (EHBR). EHBR are mutant rats without multidrug resistance-associated protein 2 that display higher serum and urinary concentrations of bile acids than Sprague-Dawley (SD) rats (wild type). On the contrary, Oat1 protein expression was not modified. In addition, the transport activities of rat and human Oat3, but not Oat1 were notably inhibited by bile acids such as chenodeoxycholic acid and cholic acid. Cholic acid, glycocholic acid, and taurocholic acid, which mainly increased during cholestasis are transported by Oat3. These authors also reported that cefotiam, a specific substrate for Oat3, was more increased in plasma from EHBR than in plasma from SD rats regardless of Oat3 up-regulation, which may be explained by the competitive inhibition of cefotiam transport by bile acids mediated by Oat3. These results indicate that Oat3 has an important pathophysiological role in protecting tissues from cholestatic damage by increasing the renal secretion of bile acids.

Nosetto et al. have reported that Oat3 is concentrated in LRD and that 21 h of bile duct ligation does not cause a redistribution from the LRD domain to non-LRD in contrast with that observed for Oat1. This might be related to the fact that renal Oat3 protein expression in the basolateral membrane is not altered in the acute...
phase of extrahepatic cholestasis as previously reported.

**Organic anion transporter 5, Slc22a19**

Oat5 is an organic anion/dicarboxylate exchanger, localized in the brush border membrane of the proximal tubule S3 segment.[52–58] Oat5 interacts with numerous anionic compounds of clinical interest, such as bumetanide, FS, penicillin G, and non-steroidal anti-inflammatory drugs.[53–55] Studies performed in our laboratory have reported that the renal protein expression of Oat5 is modified in different renal pathologies (such as ischemic acute kidney injury (AKI), obstructive AKI, and nephrotoxic AKI induced by mercury, cisplatin or methotrexate),[56–63] and in vascular calcification.[64] We have also detected Oat5 in urine and urinary exosomes, and the urinary excretion of Oat5 has been postulated as a biomarker in AKI. Oat5 was proposed as a diagnostic and as early biomarker in ischemic AKI, obstructive AKI, and nephrotoxic AKI (induced by mercury, cisplatin, or methotrexate).[56–60,62,63] Meanwhile, Oat5 was also suggested as a biomarker for treatment monitoring in AKI induced by mercury and by cisplatin.[61,65,66] Moreover, Oat5 has also been proposed as an early biomarker of vascular calcification.[64]

In extrahepatic cholestasis, an increase in Oat5 protein expression was observed in brush border membranes and in homogenates from rat kidneys. No alterations in mRNA levels for Oat5 were detected.[67] Thus, a decrease in Oat5 protein degradation has been postulated. In addition, studies from our laboratory also showed a decrease of approximately 45% in urinary levels of Oat5 in rats with obstructive jaundice, which pointed to a preservation of Oat5 in renal tissue.[68] This upregulation of Oat5 expression in renal tissue would be directed toward improving the reabsorption of filtered compounds of special importance such as steroids sulfate conjugates and pharmacological agents. This is relevant for those drugs of clinical importance that are transported by Oat5 because their pharmacokinetics may be modified during obstructive jaundice.

It is important to remark that after 21 h of bile duct ligation, no modifications in traditional biomarkers of renal failure were observed.[20,33,34,67,68] In this context, the decreased urinary levels of Oat5 observed after 21 h of bile duct ligation may be postulated as an early biomarker of renal injury in obstructive cholestasis, since they are modified previously to the decreased glomerular filtration rate reported after 3 days of bile duct ligation.[38]

**Sodium-dicarboxylate cotransporter 1, Slc13a2**

NaDC1 is expressed in the apical membrane of proximal tubule cells (S1, S2, and S3 segments).[69,70] The main function of this carrier is to reabsorb filtered Krebs cycle intermediates.[70,71] These compounds, such as succinate, citrate, and α-ketoglutarate are relevant substrates in the metabolism of the kidneys because they account for 10%–15% of oxidative metabolism.[71,72] Moreover, Krebs cycle intermediates are implicated in the maintenance of the outward dicarboxylate gradient that is relevant for the activity of function of Oat exchanger proteins at both membrane domains, apical (such as Oat5) and basolateral (such as Oat1 and Oat3).[52] Studies performed in our laboratory have reported NaDC1 detection in urine and its urinary levels have been postulated as a diagnostic biomarker in ischemic AKI, obstructive AKI, and AKI induced by mercury.[56,58,73] In obstructive AKI, NaDC1 was also suggested as an early biomarker that also provides information about the duration of obstruction.[74]

In rats with BDL, it was observed an increased in renal expression of NaDC1 protein at apical membranes without modifications in homogenates, suggesting increased recruitment of preformed transporters into the apical membranes, or inhibition in the internalization of membrane transporters. No changes were reported in mRNA levels for NaDC1.[67] Studies also performed in our laboratory showed an increase of 60% in urinary NaDC1 after 21 h of bile duct ligation.

Thus, NaDC1 might contribute to preserving the outward dicarboxylate gradient required for the normal activity of Oat proteins both at apical (Oat5) and basolateral membranes (Oat1 and Oat3).[47] In this way, Oat1 and Oat3 may participate in the elimination of toxic metabolites such as bile acids and other potential toxins existing in extrahepatic cholestasis that could not be eliminated into bile. Thereafter, Oat5 may collaborate with the excretion of these substrates as well as anionic compounds that are uptaken by Oat1 and Oat3. It was also reported a relevant decrease in citrate urine levels in rats with extrahepatic cholestasis because of higher tubular citrate reabsorption. In this study, it was also reported an increase in urinary excretion of H+ in BDL rats favouring the protonation of citrate 3– to citrate 2– making it a much stronger substrate for the dicarboxylate carriers.[70] The elevated excretion of H+ in rats with extrahepatic cholestasis might justify, at least in part, the increased renal expression of NaDC1 reported in this pathology since this carrier is modulated by pH changes.[71,72,74] Moreover, the accumulation of bilirubin, bile acids, and other potential toxins existing in this experimental model of obstructive jaundice may alter post-transcriptional mechanisms.[53,76] These alterations might be part of a likely adaptation to support normal renal tubular function by increasing relevant metabolites reabsorption, such as citrate, that play different important roles in proximal tubule cell metabolism.
In a similar way to described for other pathologies of renal etiology, the increase in urinary levels of NaDC1 in the acute phase of obstructive cholestasis may be proposed as a diagnostic and as an early biomarker of the renal damage caused by a pathology of liver etiology.

**Sodium dependent bile salt transporter, Slc10a2**

Asbt is expressed in the ileum, in the apical membrane of cholangiocytes, and the brush border membrane of proximal tubular cells. Asbt mediates the transport of conjugated and unconjugated bile acids in a sodium-dependent way.

After secretion with the bile into the small intestine, bile acids are almost completely reabsorbed in the terminal ileum. Then, with the portal venous flow they return to the liver, are taken up, and secreted again into the bile. Around 10%–50% of the reabsorbed bile acids avoid the uptake by the liver. About 10%–30% of the bile acids, present in the blood plasma, are not bound to proteins and in consequence, suffer filtration by the glomerulus and posterior nearly complete tubular reabsorption which takes place by Asbt.

A decrease in taurocholate transport in proximal tubular cells was reported after 24 h of obstructive jaundice and took place without a modification of the amount of the carrier protein in these cells. A change in the phosphorylation status of Asbt and/or redistribution of the carrier between the plasma membrane and intracellular compartments of renal cells has been postulated. Lee et al. have also reported a decrease in taurocholate transport in brush border membrane vesicles from rat kidneys after 14 days of bile duct ligation, which was associated with a decrease in renal Asbt protein expression. These results suggest a functional adaptive down regulation of Asbt activity aimed to increase renal clearance of bile acids during extrahepatic cholestasis.

**Na-K-2Cl cotransporter type 2, Slc12a1**

NKCC2 also known as “bumetanide-sensitive cotransporter” (BSC1) is a kidney specific protein expressed in the apical membrane of the thick ascending limb of the loop of Henle (TAL) where reabsorbs the 20%–25% of the total filtered NaCl load. The active transport of sodium chloride in the thick ascending limb of Henle’s loop mediated by NKCC2 creates a corticomedullary concentration gradient. This interstitial hypertonicity in the renal medulla leads to water absorption through the AQP2 water channel in the collecting duct in the presence of vasopressin. In addition, the NKCC2 transport function in the macula densa cells of the TAL is the initial step of the tubular-vascular communication within the juxtaglomerular apparatus.

After the detection of NKCC2 in urine from healthy rats, the urinary excretion of NKCC2 was evaluated in different pathological states and proposed as a biomarker when appropriate. In this connection, studies performed in our laboratory proposed urinary NKCC2 levels as an early biomarker in methotrexate-induced AKI and as a recovery biomarker in obstructive AKI.

After 21 h of bile duct ligation, studies from our laboratory reported no modifications in NKCC2 protein expression in renal cortical and medullary homogenates. On the other hand, a decreased protein expression of NKCC2 in apical membranes from renal cortical tissue without modification in apical membranes of medullary tissue was observed. Thus, at the cortical level, an increased recruitment of carrier units from the apical membrane to inside the cell or a diminished traffic of the protein to the membrane might be proposed. The downregulation of NKCC2 expression would partially contribute to the decrease of the interstitial hypertonicity by reducing electrolytes reabsorption explaining, at least in part, the increase in urinary flow and the fractional excretion of osmolytes previously reported in this experimental model.

NKCC2 expression might be altered by the action of various substances that are released in this pathology, such as bile salts, bilirubin, or cytokines. In relation to this, it was reported that the inflammatory mediator TNF-α, which levels are elevated in the plasma of rats with extrahepatic cholestasis, promotes a decrease in NKCC2 protein expression.

The urinary levels of NKCC2 were also analyzed in the early phase of acute obstructive jaundice and no modifications were observed.

**Renal response of other proteins in obstructive jaundice**

It has been reported an increase in the urinary volume in rats with extrahepatic cholestasis, which might be explained by the alteration in cortico-medullar gradient and by the urinary excretion of a great amount of osmotically active solutes that could not be eliminated by the liver, such as bile acids. This modification in the cortico-medullary gradient might be caused by the increased in medullary renal flow and partially by the decreased expression of NKCC2 that was previously reported in our laboratory in rats with 21 h of bile duct ligation. NKCC2 mediates the active transport of sodium chloride in the thick ascending limb of Henle’s loop, which contributes to creating the corticomedullary gradient. The interstitial hypertonicity achieved in the renal medulla leads to water absorption through the AQP2 water channel in the collecting duct. As both NKCC2 and AQP2 work in cooperation for the
regulation of renal water conservation,[13] the AQP2 renal expression changes observed in the presence of obstructive cholestasis deserve to be reported in this review even though AQP2 is a transporter not belonging to the SLC family.

On the other hand, it has been described that Oat1 and caveolin-2 (Cav-2) are co-expressed in the plasma membrane of rat kidneys and that Oat1 transport function is upregulated by Cav-2 in normal physiological conditions. Moreover, Oat3 was reported to share a cellular expression with caveolin-1 (Cav-1), which increases the Oat3 mediated transport of organic anionic compounds under normal physiological conditions.[11,12] In addition, a colocalization of Cav-1 with AQP2 has been reported.[93] Thus, the modifications in the renal expression of Cav-2 and Cav-1 in rats with extrahepatic cholestasis also deserve to be described in this review even though both proteins are not members of the SLC family.

**Aquaporin 2**

AQP2 is a water channel protein expressed at the connecting tubule and in the collecting duct from the renal cortex and medulla. AQP2 plays a relevant role in urine concentration and body-water homeostasis. Short-term and long-term (adaptive) mechanisms are involved in the regulation of AQP2.[94]

The renal water excretion is regulated by the insertion of AQP2 channels in the apical membranes of collecting ducts principal cells.[95] AQP2 has an important role in the development of water retention linked to different pathologies, such as congestive heart failure and liver cirrhosis.[96-97] Defective regulations of AQP2 in response to vasopressin are very important in diseases with alterations in the balance of water.[98,94,95,99]

AQP2 is also a biomarker that can be readily measured in urine, and that has been exploited in studies of various water-balance disorders.[98-100] Studies from our laboratory proposed urinary AQP2 as a diagnostic and as an early biomarker in methotrexate-induced AKI and as a recovery biomarker in obstructive AKI.[88,101]

Nosetto et al.[89] have recently reported that after 21 h of bile duct ligation, AQP2 protein expression was not modified in homogenates and apical membranes from rat kidney cortex. On the contrary, a downregulation of AQP2 protein expression was observed in homogenates and apical membranes from the renal medulla, suggesting a decrease in its synthesis or an increase in its degradation. Thus, the decrease in AQP2 expression could contribute to the increment in the urine output reported in the acute phase of cholestasis, by decreasing water reabsorption of water in the collecting duct. It has been reported that bile salts could have a regulatory role in the AQP2 expression via modulation of the transcription factor, the nuclear receptor farnesoid X receptor (FXR).[102] The mediators of the inflammatory response that are released in obstructive jaundice and can reach the collecting duct via the bloodstream could also influence the regulation of AQP2. It has been reported that the cytokine TNF-α decreases the protein and mRNA expression of AQP2.[103,104] AQP2 urinary excretion was also evaluated in our laboratory in rats with obstructive jaundice and no modifications were observed.[93]

**Cav-1 and Cav-2**

Caveolins are a family of approximately 22 kDa integral membrane proteins that are the main constituents of caveolae. Caveolae are small invaginations, containing cholesterol, sphingolipids, and the proteins caveolins and cavins.[39] Caveolae and caveolins are expressed in most cell types and have been reported to play a relevant role in vesicular transport, signal transduction mechanisms, the homeostasis of lipids, and the protection of cells from mechanical stress.[103,106] Caveolin family is integrated by three members, Cav-1, Cav-2, and Cav-3. Cav-1 and Cav-2 are co-expressed in a variety of cell tissue, principally in type-1 alveolar cells, fibroblasts, endothelial cells, and adipocytes, while Cav-3 is expressed specifically in smooth and skeletal muscle cells.[106] Cav-1 and Cav-2 have also been found in renal tissue.[11,12,107] Even though Cav-2 colocalizes with Cav-1 in different cell types, their expressions can be independently regulated in response to a wide variety of cellular responses.[108,109] In the kidney, several authors demonstrated that Cav-1 and Cav-2 are expressed in renal collecting duct principal cells, distal tubule cells, connecting tubule cells, and in glomerular and vascular structures.[107,110] Moreover, some authors described the expression of Cav-1 and Cav-2 in proximal tubule cells.[11,12,111] Works performed in our laboratory demonstrated that Cav-2 is expressed in the proximal tubule cells under physiological conditions. Cav-2 was found in both plasma membrane domains, with a greater expression in apical membranes than in basolateral membranes of proximal tubule cells.[112] Cav-2 presence in urine and urinary exosomes in physiological conditions was also first reported in our laboratory.[112] Concerning to Cav-1, it was detected in urine from mice with renal failure,[113] and it was reported for the first time in urine from healthy rats in our laboratory.[92] Studies also performed in our laboratory proposed urinary Cav-2 as a diagnostic biomarker in obstructive AKI and mercury-induced AKI.[113] On the other hand, we have postulated urinary Cav-2 as a biomarker of renal recovery in cisplatin-induced AKI.[114]

Nosetto et al.[115] reported a decreased expression of Cav-1 in renal homogenates from rats with obstructive cholestasis and in homogenates from renal cells.
incubated with serum from rats with bile duct ligation of 21 h. A significant increase in urinary Cav-1 was also reported in rats with obstructive jaundice.\(^{[92,115]}\) The decrease of Cav-1 expression in renal tissue may be caused by a decrease in synthesis or an increase in degradation, and/or an increased dumping of Cav-1 in the urine. In renal tissue of rats with extrahepatic cholestasis, the decreased expression of Cav-1 might be related to the down-regulation of AQP2 previously reported in rats with extrahepatic cholestasis. In this connection, Aoki et al.\(^{[93]}\) have reported a colocalization of AQP2 and Cav-1 suggesting a relevant role of Cav-1 in the internalization process of AQP2. Thus, it is possible to assume that the numerous mediators liberated in extrahepatic cholestasis, can modulate the expression of AQP2 through a Cav-1-dependent pathway.

On the other hand, Cav-2 expression showed no changes in renal homogenates and an increase in basolateral membranes from rats with obstructive jaundice.\(^{[116]}\) In this connection, Cav-2 expression did not change in homogenates and increased in membranes from cells incubated with serum from rats with bile duct ligation of 21 h.\(^{[116]}\) Thus, increased recruitment of Cav-2 into the membranes or an inhibition in the internalization of the protein, regulated by bile salts or by other mediators compounds liberated in the bloodstream of rats with extrahepatic cholestasis may be postulated. In this regard, it was demonstrated that Oat1 and Cav-2 are co-expressed in plasma membranes from rat kidneys.\(^{[12]}\)

Thus, the increased expression in renal membranes of both Oat1 and Cav-2 in the acute phase of extrahepatic cholestasis demonstrates a cooperative functional role of both proteins in a pathological state.

Nosetto et al.\(^{[92,115]}\) observed a significant increase of urinary Cav-1 and Cav-2 after 21 h of biliary obstruction in rats when traditional parameters of renal function and urinary NGAL were not altered at this time point. Thus, Cav-1 and Cav-2 have been postulated as early biomarkers of extrahepatic cholestasis.\(^{[92,115]}\)

**CONCLUSION**

The liver and kidneys work in cooperation in the metabolism and the excretion of many endogenous compounds and pharmaceutical drugs. Modulations in the expression of proteins presented in both organs could be an organism approach to mitigate the effects of the disease and to maintain the homeostasis of small molecules, like organic anions or bile salts, to conserve the optimal degree of inter-organic communication. In proximal tubule cells, the increased expression of Oat1, Oat3, Oat5, and the decreased abundance of Abst observed in the early phase of obstructive jaundice are leading to an increase in the renal clearance of bile acids and harmful compounds that could not be excreted by the liver because the biliary excretion is damaged. On the other hand, the decreased expression of NKCC2 and AQP2 together with the increase in medullary renal blood flow could explain the increase in urinary flow, which is in direct correlation with the increased urinary excretion of different osmolytes reported in this pathological state. In the above context, it is important to take into account the modulation in the expression of Cav-1 (co-expressed with Oat3 and with AQP2) and of Cav-2 (co-expressed with Oat1) which might have an important role in the expression and function of these transporters and many other proteins after 21 h of bile duct ligation.

Measurements of renal excretion of different proteins are exploited for the study of certain disease states. A big number of biomarkers have been evaluated in the last times as tools for the early detection of renal diseases. Moreover, markers of tubular damage could be potentially useful in the evaluation of prognosis and for monitoring the effectiveness of a treatment. Renal function is impaired during cholestasis and the damage increase with the time of cholestasis duration. The increased urinary levels of NaDC1, Cav-1, and Cav-2 together with the decrease of Oat5, and the absence of modifications of NKCC2 and AQP2 might be proposed as a novel urinary proteomic signature of the early phase of obstructive jaundice. This protein panel might be useful to reliably identify the renal damage induced by obstructive cholestasis at an early time, using a noninvasive approach. The urinary levels of NaDC1, Cav-1, Cav-2, Oat5, NKCC2, and AQP2 could be considered as an excellent panel of early biomarkers for the detection of renal damage produced in extrahepatic cholestasis; since traditional parameters of renal function, such as uremia, creatininaemia, glomerular filtration rate (evaluated as the clearance of creatinine and as the clearance of inulin), glucosuria, proteinuria, NGAL in urine, and urinary activity of alkaline phosphatase are not modified at this time point.\(^{[20,33,34,37,67,92]}\) This panel of early and non-invasive biomarkers would have the potential to predict subsequent renal alterations as those reported in our laboratory after 3 days of bile duct ligation (such as a decrease in glomerular filtration rate and remark histopathological alterations).\(^{[10]}\)

**DECLARATIONS**

**Source of funding**

Supported by Grants from: Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) (PICT 2017-N°0936), Consejo Nacional de Investigaciones

**Author contributions**
Torres AM contributed all to this work.

**Conflicts of interest**
The author has no conflicts of interest to disclose.

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