Aquaporins: Their role in cholestatic liver disease

Guillermo L Lehmann, Maria C Larocca, Leandro R Soria, Raúl A Marinelli

Abstract

This review focuses on current knowledge on hepatocyte aquaporins (AQPs) and their significance in bile formation and cholestasis. Canalicular bile secretion results from a combined interaction of several solute transporters and AQP water channels that facilitate water flow in response to the osmotic gradients created. During cholestasis, hepatocytes rapidly increase their canalicular membrane water permeability by modulating the abundance of AQP8. The question was raised as to whether the opposite process, i.e. a decreased canalicular AQP8 expression would contribute to the development of cholestasis. Studies in several experimental models of cholestasis, such as extrahepatic obstructive cholestasis, estrogen-induced cholestasis, and sepsis-induced cholestasis demonstrated that the protein expression of hepatocyte AQP8 was impaired. In addition, biophysical studies in canalicular plasma membranes revealed decreased water permeability associated with AQP8 protein downregulation. The combined alteration in hepatocyte solute transporters and AQP8 would hamper the efficient coupling of osmotic gradients and canalicular water flow. Thus cholestasis may result from a mutual occurrence of impaired solute transport and decreased water permeability.

Key words: Aquaporin; Cholestasis; Estrogen; Hepatocyte; Obstructive cholestasis; Sepsis; Water transport

INTRODUCTION

Bile secretion is the main function of the exocrine liver, and the maintenance of normal bile formation and delivery into the intestinal lumen is essential for physiological processes such as digestion and absorption of dietary lipids and elimination of endo- and xenobiotics. Cholestasis is a pathologic condition defined as an impairment of normal bile formation, bile flow obstruction or both[1]. There have been major advances in the understanding of the molecular mechanisms underlying bile secretion and cholestasis, and much of this work has been focused on the study of solute membrane transporters[2,3]. However, considering that bile is composed of more than 95% water, less attention has been paid to the molecular basis and regulatory mechanisms of water transport in hepatocytes during bile formation. The cloning and functional characterization of a family of proteins that works as membrane water channels, named aquaporins (AQPs)[4], challenged the former concepts of water transport and contributed to the better understanding of bile physiology. The aim of this work is to give a concise overview of the current knowledge and recent advances in the role of AQPs during bile formation as well as the significance of AQPs in the development of bile secretory failure.

AQUAPORINS OVERVIEW-GENERAL

STRUCTURE AND FUNCTION

AQPs are small integral proteins which belong to a family of homologous tetrameric proteins widely
Aquaporins (AQP) function as a COOH-B-13 R-9 motif (asparagine-proline-alanine, NPA), which overlap in loops, two enclose the strictly conserved three-amino acid residues from the highly conserved NPA motif. The single water molecule passes through the constriction with no resistance as it forms transient hydrogen bonds with the nearby asparagines.

The first AQP was purified from human erythrocytes and was initially named CHIP28. Following expression studies in Xenopus oocytes the protein was functionally identified as a water channel and renamed AQP1[1]. The discovery of AQP1 triggered an immense number of studies which advanced the current understanding of water permeation across biological membranes. At least 13 AQP isoforms have been identified from mammalian tissues (AQP0-AQP12)[13,14]. While AQP1s function primarily as water-transporting channels, some of these proteins also exhibit permeability to certain small solutes such as glycerol, ammonia[8], hydrogen peroxide[9] and some gases such as carbon dioxide and nitric oxide[10].

AQP1 was the first member of the AQP water channel family to be identified[9]. The functional unit of AQP1 is a homotetramer, but in contrast to ion channels where the permeation site is placed in the center of the tetramer, each AQP subunit contains a distinct aqueous pore. The monomers, composed of approximately 270 amino acids, possess six transmembrane alpha-helix regions connected by five loops, with the amino- and carboxy-termini oriented towards the cytoplasm. Among the five connecting loops, two enclose the strictly conserved three-amino acid motif (asparagine-proline-alanine, NPA), which overlap in the center of the pore and are responsible for the water channel selectivity (Figure 1A and B).

Cryoelectron microscopy in combination with atomic force microscopy and X-ray analysis confirmed the so-called “hourglass model”. It consists of a wide extracellular and intracellular vestibule, joined in the center by a narrow region of approximately 20 Å in length that shapes the filter responsible for water selectivity[11]. In the vestibules water exists in bulk solution, while in the center of the channel water transits in single file. Actually it has been identified as a two-stage filter in the central region of the pore: an outer barrier termed the aromatic/arginine constriction (ar/R), and the central constriction or NPA region. Both stages of the filter add to the remarkable efficiency and selectivity of the AQP1s[12] (Figure 1C).

The filter selectivity appears to be based on size exclusion, as the central constriction of the filter is slightly wider than the water molecule (~2.8 Å) and represents a steric limit for bigger molecules through the channel. The ar/R constriction is composed of a highly conserved arginine residue in the 195 position and a nearby histidine residue in the 180 position. This filter provides supplementary criteria for solute selection, as the conserved residues have a strong positive charge that repels protonated water. In the NPA region, water-water interactions are distorted so water molecules remain isolated from their solvation shell in the bulk, a process essential for the filter selectivity[13]. As water molecules approximate to the constriction, the oxygen atom rotates towards the asparagine residues from the NPA motifs (asparagines 76 and 192) and creates new hydrogen bonds between the oxygen and the asparagine residues. The dipole reorientation breaks the hydrogen bonds among water molecules and avoids the passage of protonated water[13,14].

The hourglass model also explains the reversible inhibition caused by mercurial compounds. Mercury reacts with sulfhydryl groups from cysteine residues. Among the 4 cysteine residues present in AQP1, only that located in the 189 position reacts with mercurial compounds producing water transport inhibition[15]. This residue is located in the E loop, next to the NPA motif inside the pore[13].

HEPATOZYME AQPS: EXPRESSION AND SUBCELLULAR LOCALIZATION

There has been much interest in the localization and

---

Figure 1  Topology, organization and functioning of the aquaporin water channel molecule. A: Each AQP monomer consist of six transmembrane domains (I-VI) connected by five loops (a-e) with two NPA boxes shaping the water pore, and the amino and carboxy termini oriented toward the cytoplasm; B: Aquaporins are arranged in tetramers. The water pore does not reside in the center of the molecule, but is formed by connecting loops b and e in each subunit that functions as a unique water pore allowing bidirectional water movement; C: The hourglass model for aquaporin structure. The channel consist of an extracellular and intracellular vestibule containing water in bulk solution joined in the center by a central constriction 20 Å in length where water molecules pass in single file. The ar/R constriction delimited by arginine in the position 195 (R195) and histidine in the position 180 (H180) provides fixed positive charges which prevent proton passage. The second constriction is bounded by two asparagine residues from the highly conserved NPA motif. The single water molecule passes through the constriction with no resistance as it forms transient hydrogen bonds with the nearby asparagines.
physiological role of AQPs in the liver. We and others have recently shown that rat hepatocytes express mRNA and protein for AQP1[16], AQP8[16,28-31], AQP9[21,28] and AQP11[28]. In addition, other cells of the hepatobiliary tract such as cholangiocytes, gallbladder epithelia and peribiliary vascular endothelia also express AQPs[23-27]. The cellular and subcellular distribution and permeability characteristics of AQPs in the hepatobiliary tract are summarized in Table 1. We will focus essentially on the hepatocyte AQPs that play a role in bile formation and secretory failure, i.e. AQP8 and AQP9.

Typically, AQP8 has a varied subcellular localization, which probably correlates with broad physiological roles in the hepatocyte. Accordingly, it was estimated that under basal (unstimulated) conditions most of the hepatocyte AQP6 (75%) reside in intracellular structures, while the remainder reside in the plasma membrane[19]. Detailed biochemical, confocal immunofluorescence and immunoelectron microscopy analyses revealed that the intracellular AQP8 is mostly located in transport vesicles[17,19], smooth endoplasmic reticulum[28] and in the inner membrane of some mitochondria[29]. The protein expression in the plasma membrane is specifically located in the canalicular domain[10,13] and it is regulated by cAMP. Based on immunoblot analysis it was found that hepatocyte AQP8 can be present in two forms of different molecular weight: a N-glycosylated protein of about 34 kDa, and a non-glycosylated protein of 28 kDa[19,29]. According to this, it can be assumed that hepatocytes exhibit two intracellular subpopulations of AQP8 with different physiological functions. A 34 kDa AQP8 in transport vesicles and canalicular plasma membrane domains involved in the formation and regulation of bile (see below); and a 28 kDa AQP8, located in the inner mitochondrial membrane with still uncharacterized functions[29]. Regarding AQP8 distribution in the liver, there is evidence that it has a differential lobular localization. Accordingly, immunohistochemical studies showed staining predominantly in the hepatocytes surrounding the central vein of rat liver[10]. Different results were shown in mouse hepatic lobules, where AQP8 seems to be predominantly distributed in the perportal and midlobular hepatocytes with some immunostaining in the pericentral region[29].

AQP9 is a water channel of approximately 32 kDa that allows the passage of water and a wide variety of neutral solutes such as urea, glycerol, purines and pyrimidines[30,31]. Immunolocalization studies performed in rodent liver revealed that AQP9 is exclusively restricted to the hepatocyte sinusoidal plasma membrane domain[21], with an expression pattern strongest around the perivenous zone[8].

Hepatocytes also express AQP0, formerly named major intrinsic protein[16]. AQP0 is mainly localized to intracellular vesicular compartments, but it is not responsive to cAMP and so far, there is no evidence that its trafficking is regulated[14]. The function of AQP0 in hepatocytes has not yet been determined.

The last member to be identified was AQP11[10]. However, the study showed protein levels in total liver membranes, thus the AQP11 cellular and subcellular localization remains to be elucidated.

### REGULATION OF HEPATOCYTE AQP TRAFFICKING

Certain epithelia adjust their transport capacity in the short term (i.e. min) by rapid insertion of specific transporters in the secretory membrane. Therefore, the epithelial secretory or absorptive activity can be regulated by handling the number of transport molecules in the plasma membrane. AQP2 is the vasopressin-regulated water channel of the kidney collecting duct[32]. Accordingly, the vasopressin-induced exocytic insertion and endocytic retrieval into and out of the plasma membrane represents a rapid mechanism to regulate its water membrane permeability[33]. Likewise, AQP5 is the main AQP expressed in the acinar cells of the salivary gland. While the water channel is normally sequestered in intracellular vesicles, it redistributes to the apical plasma membrane upon stimulation by muscarinic agonists[33]. Furthermore, it was demonstrated in cholangiocytes that AQP1 is located in intracellular vesicles and undergoes secretin-induced exocytic insertion into the apical membrane[25,26]. Thus, fluid-transporting epithelia can regulate the rate of water transport across cell membranes by rapid relocation of AQP molecules.

### Table 1  Cellular and subcellular localization and permeability characteristics of hepatic AQPs

| Cell type               | Aquaporin | Subcellular localization | Permeability                                           | Ref.   |
|-------------------------|-----------|--------------------------|--------------------------------------------------------|--------|
| Hepatocytes             | AQP0      | ICV                      | Water                                                  | [16]   |
|                         | AQP8      | ICV-CPM-Mitochondria-SER | Water/NH$_3$/H$_2$O$_2$                                | [9,16-20,28,29,32] |
|                         | AQP9      | BLM                      | Water/glycerol/urea/certain small uncharged molecules   | [16,21,22,30,31] |
|                         | AQP11     | ND                       | ND                                                     | [6]    |
| Cholangiocytes          | AQP1      | APM-ICV                  | Water                                                  | [23,25,36] |
|                         | AQP4      | BLM                      | Water                                                  | [26]   |
| Gallbladder epithelia   | AQP1      | APM-ICV                  | Water                                                  | [27]   |
| Peribiliary vascular endothelia | AQP1 | APM-ICV | Water | [24] |

ICV: Intracellular vesicles; CPM: Canalicular plasma membrane; BLM: Basolateral plasma membrane; APM: Apical membrane; SER: smooth endoplasmic reticulum; NH$_3$: Ammonia; H$_2$O$_2$: Hydrogen peroxide; ND: Not determined.
By using isolated rat hepatocytes, it was found that the water channel AQP8 is localized largely in intracellular vesicles and can be redistributed to the plasma membrane in a mechanism stimulated by cAMP. This was the first evidence that hepatocytes were able to regulate their membrane water permeability. Furthermore, confocal immunofluorescence microscopy and functional studies in polarized isolated rat hepatocyte couples showed that the insertion of AQP8 occurs specifically in the hepatocyte canalicular membrane domain, a mechanism that facilitates the osmotically-driven canalicular water secretion in response to a choleretic stimulus. The microtubule blocker colchicine specifically inhibits the dibutyl cAMP effect on both AQP8 translocation to plasma membrane and water transport, suggesting that the hormone-dependent AQP8 trafficking relies on microtubules.

Glucagon is a choleretic hormone, and its actions in hepatocytes are mostly mediated by cAMP-dependent protein kinase A (PKA). We found that glucagon induces the translocation of intracellular AQP8-containing vesicles to the canalicular domain in hepatocytes, and that this mechanism is dependent on the activation of PKA and the integrity of the microtubular network. Because AQP8 lacks consensus PKA phosphorylation sites, we suggested that unidentified protein mediators might be involved in the vesicle trafficking induced by glucagon. The understanding of the precise mechanisms by which glucagon stimulates AQP8 translocation requires further investigation. Because AQP1 also lacks phosphorylation sites for PKA, the mechanisms involved in AQP8 translocation are probably similar to those involved in the secretin-mediated AQP1 trafficking in cholangiocytes.

It has been shown that glucagon is able to induce phosphatidylinositol-3-kinase (PI3K) activation in rat hepatocytes. PI3K mediates several signaling transduction pathways in hepatocytes, including some involved in the regulation of vesicle trafficking and in the process of bile formation. In fact, we showed that PI3K is involved in the hepatocyte trafficking of AQP8 stimulated by glucagon. Thus, our studies indicate that there is a dual requirement of PKA and PI3K for glucagon-induced AQP8 trafficking. The cross-talk between PKA and PI3K signaling pathways has already been suggested for the regulated translocation of Bsep to the hepatocyte canalicular membrane. This may reflect the need for a cooperative action between PKA and PI3K on a single downstream effector. Consistent with this, it has been reported that in rat hepatocytes, cAMP can activate protein kinase B/Akt, a downstream PI3K effector. Interestingly, it has recently been shown that cAMP-activated PI3K mediates the endocytotic translocation of the membrane domain. The glucagon-induced AQP8 trafficking in hepatocytes seems to involve both the cAMP/PKA and PI3K signaling pathways in a cooperative manner.

Therefore, during active cholericsis, hepatocytes rapidly increase their canalicular membrane water permeability by vesicle trafficking and thus modulate the abundance of AQP8 in the membrane.

**PHYSIOLOGICAL SIGNIFICANCE OF HEPATOCYTE AQPS: BILE FORMATION**

Hepatocytes are highly polarized epithelial cells characterized by two definite plasma membrane domains: a basolateral domain in contact with the sinusoidal blood and a bile canalicular domain, defining a sealed apical compartment. The asymmetric distribution of protein transporters to the apical and basolateral membrane domains is the basis of vectorial flux of solutes and water from blood into the bile canaliculi and therefore for the generation of bile. Canalicular bile formation is an osmotic secretory process resulting from the inflow of water into the biliary space in response to osmotic gradients created by the active secretion of solutes. The excretion of bile salts via the bile salt transporter Bsep, glutathione via the organic anion transporter Mrp2, and HCO3- via the Cl-/HCO3- exchanger AE2 are known to be the major driving forces for water movement from the sinusoidal blood to the bile canaliculus. While the generation of bile flow depends on the molecular and functional canalicular expression of the aforementioned solute transporters, the molecular route for water movement has been largely disregarded albeit the majority of canalicular bile is water.

Theoretically, water can flow through the hepatocyte epithelial barrier either across tight junctions between adjacent hepatocytes (paracellular route) or across hepatocyte plasma membranes (transcellular route). The paracellular route was traditionally proposed as the major pathway for water movement. Nonetheless, the experimental data supporting this view remained limited and largely indirect. Evidence supporting the transcellular pathway came from AQP inhibitory experiments in polarized rat hepatocyte couples. Under choleretic stimuli, the AQP blockers prevented osmotically-driven water transport into the bile canaliculus. Direct osmotic water permeability assessment by stopped-flow spectrophotometry in canalicular and sinusoidal plasma membrane vesicles revealed the presence of both lipid (non-channel) and AQP-mediated pathways for sinusoidal and canalicular water movement. The study demonstrated that the canalicular plasma membrane domain has lower water permeability than the sinusoidal membrane, and thus it is rate limiting for transcellular water transport in hepatocytes. However, upon cAMP stimulus the intracellular AQP8 inserts to the canalicular domain and so this membrane becomes highly water permeable. Approximate estimations of transcellular hepatocyte water permeability suggest it to be similar to rat kidney proximal tubule, in which water flow seems to be largely transcellular. Therefore, the transcellular pathway via water channels seems to account for most of the water entering the bile canaliculus.
As stated above, hepatocytes express AQPs in intracellular compartments as well as in the basolateral and canalicular plasma membrane domain. In the canalicular membrane, AQP8 was shown to be localized in lipid microdomains (“rafts”) enriched with cholesterol and sphingolipids\(^{[50,31]}\). These rafts are thought to promote the assembly of specific proteins into definite regions of the plasma membrane. Because other canalicular transporters such as AE2 and Mrp2 are also localized in membrane microdomains\(^{[31]}\), it seems plausible that in the apical membrane, AQP8 is clustered with functionally associated solute transporters, which would generate the driving force necessary for osmotic water transport mediated by AQP8.

The hormone glucagon is known to modulate canalicular bile formation\(^{[52]}\). Although the actual osmotic driving force involved in glucagon-induced cholestasis is currently unknown, the solute gradients are thought to be created by active HCO3- excretion mediated by the canalicular transporter AE2\(^{[2]}\). In line with this, it was shown that glucagon (via cAMP) is able to stimulate the microtubule-dependent vesicle insertion of AE2 to hepatocyte plasma membrane\(^{[52]}\). This mechanism, in association with an increased activity of the exchanger, may account for the bicarbonate-rich cholestasis induced by glucagon. Furthermore, recent immunofluorescence studies carried out in the hepatoma-derived hybrid cell line WIF-B, showed that AQP8 and AE2 are packaged in the same vesicle population, possibly conforming to a functional bile secretory unit\(^{[53]}\). Thus our findings provide evidence that AQP8 may improve the efficient coupling of canalicular water transport to the HCO3- secreted by AE2 during glucagon-stimulated hepatocyte bile formation.

Further evidence for the role of AQP8 in bile secretion comes from ontogenic expression studies during mice liver development. It was shown that at the time of weaning, there is a rapid increase in AQP8 mRNA and protein expression when the hepatobiliary transport systems complete their maturation, suggesting that AQP8 is necessary for canalicular bile formation\(^{[50]}\).

Although the mentioned studies support a role for AQP8 in canalicular water secretion, conclusive evidence should come from studies performed in hepatocytes lacking AQP8 expression. With regard to this, experimental evidence against a role for AQP8 in canalicular bile formation came from data obtained from AQP8 knockout mice\(^{[54]}\). This study revealed that AQP8-null mice challenged with a high-fat diet did not show a significantly different phenotype when compared to their wild-type counterpart. The lack of dietary fat misprocessing could suggest that the excretion of bile salts required for proper lipid digestion was at least preserved. However, direct studies on bile formation in AQP8-null mice are mandatory, in order to determine if these animals develop cholestasis. On the other hand, as hepatocytes express several members of the AQP water channel family, the normal AQP8-null mouse phenotype could result from a compensatory overexpression or functional modification of other genes.

Functional studies from our laboratory provided further evidence supporting the notion that canalicular water transport during bile secretion is AQP8-mediated. Indeed, we found that AQP8 gene suppression by RNA interference is able to inhibit osmotically-driven and cAMP-induced canalicular water secretion in the human hepatocyte cell line HepG2\(^{[50]}\).

Therefore, while according to our model, AQP8 modulates the canalicular, rate limiting water flow, AQP9 would contribute to the sinusoidal uptake. In agreement with this, functional studies in rat hepatocyte basolateral membrane indicate that sinusoidal water transport is AQP-mediated\(^{[53]}\). As bile secretion requires the transcellular movement of water to the bile canaluli and AQP9 is the only sinusoidal water channel described so far, it is logical to believe that water moves from the sinusoidal blood, at least in part, through AQP9.

### PATHOPHYSIOLOGICAL SIGNIFICANCE OF HEPATOCYTE AQPS: CHOLESTASIS

Bile secretion failure is a consequence of several pathologic conditions with the risk of producing severe liver injury and systemic disease. There have been major advances in the understanding of the molecular pathogenesis of bile secretory failure\(^{[54]}\). It is well known that hepatocyte canalicular bile secretion results from the coordinated interaction of several solute membrane transport systems together with, as detailed above, AQP water channels. Hence, it is conceivable that defective AQP membrane expression may lead to alterations in normal bile physiology. The significance of liver AQPs in bile secretory failure are summarized in Table 2.

Extrahepatic cholestasis is a pathologic condition caused by a mechanical obstruction of the biliary tree secondary to a wide variety of acute and chronic conditions including gallstones, pancreatic carcinoma and cholangiosarcoma\(^{[56]}\). If uncorrected, the obstruction may lead to hepatocyte damage, secondary biliary cirrhosis and portal hypertension. The experimental model of bile duct ligation (BDL) in the rat has been extensively used to assess modifications in the molecular expression of hepatocyte membrane transporters in obstructive cholestasis. In a recent study, we examined the effect of BDL on the protein expression and subcellular localization of the hepatocyte water channel AQP8\(^{[57]}\). Biochemical and immunohistochemical studies determined that BDL-induced extrahepatic cholestasis caused downregulation of hepatocyte AQP8 at the protein level (Figure 2). In opposition, the AQP8 mRNA steady-state levels in BDL were increased, possibly as a compensatory mechanism in response to AQP8 protein reduction. The fact that AQP8 protein downregulation was not associated with reduced levels of the mRNA may indicate the involvement of posttranscriptional regulatory mechanisms. Additionally, the AQP8 translocation to the hepatocyte plasma membrane in BDL was found to be impaired. Hence it was concluded that the defective hepatocyte AQP8 functional expression as well as impaired translocation...
may contribute to the secretory dysfunction caused by obstructive cholestasis.

In addition, very recent work suggested a potential involvement of sinusoidal AQP9 in the pathogenesis of obstructive cholestasis. Detailed biochemical studies demonstrated that in BDL there is a decrease in AQP9 protein in basolateral membranes with a simultaneous intracellular increase of the protein. In addition, functional studies performed in sinusoidal hepatocyte membrane found a close correspondence between the AQP9 decreased membrane protein levels and impaired osmotic water permeability. It was concluded that downregulation of AQP9 in the hepatocyte basolateral plasma membrane affects sinusoidal water uptake during bile formation, thus contributing along with AQP8 downregulation to the bile flow dysfunction in obstructive cholestasis.

Estrogens are known to cause intrahepatic cholestasis in susceptible women. The most common clinical features of this disorder are oral contraceptive-induced cholestasis and cholestasis associated with pregnancy or postmenopausal replacement therapy. Experimental cholestasis induced by 17α-ethinylestradiol (EE) has been widely used to investigate in vivo alterations in the expression of hepatocyte membrane transporters in this pathological condition. In a recent work, we found that the protein expression of hepatocyte AQP8 is downregulated in estrogen-induced cholestasis possibly by posttranscriptional mechanisms, without significant changes in the sinusoidal AQP9. In fact, complementary studies in primary cultured rat hepatocytes with protease inhibitors indicated that estrogen-induced AQP8 downregulation was mediated by increased lysosomal degradation. Of note, the canalicular AQP8 downregulation was correlated with a 22% reduction in the canalicular membrane water permeability. Previous reports have estimated that under basal conditions the AQP-mediated water

| Experimental model          | AQP8 | AQP9 | Ref.   |
|-----------------------------|------|------|--------|
|                             | Protein | mRNA | CPM | Protein | mRNA | BLM P |       |
| Obstructive cholestasis     | ↓↓   | ↑    | ND  | ↓↓↓   | ↓↑   | ↓     | [57,58]|
| BDL                          |       |      |     |       |      |       |        |
| Intracellular cholestasis   | ↓↓↓  | ↑    | ↓   | ↔     | ND   | ND    | [59]   |
| EE-induced cholestasis      | ↓↓↓  | ↑    | ↓   | ↔     | ND   | ND    | [64]   |
| LPS-induced cholestasis     | ↓↓↓  | ↑    | ↓   | ↔     | ND   | ND    | [64]   |
| CLP-induced cholestasis     | ↓↓↓  | ND   | ND  | ↔     | ND   | ND    | [66]   |

Arrows depict significant protein and mRNA changes in treated rats compared with controls: ↑, increased; ↓, decreased; ↔, without change. ND: Not determined; P: Osmotic membrane water permeability; CPM: Canalicular plasma membrane; BLM: Basolateral plasma membrane; BDL: Bile duct ligature; EE: 17α-ethinylestradiol; LPS: Lipopolysaccharide; CLP: Cecal ligation and puncture.
pathway contributes to approximately 30% of the total canalicular water transport\cite{65}. For that reason, the 22% water permeability decrease caused by estrogens may be enough to impair the efficient canalicular coupling between osmotic solutes and water transport during bile formation. On the other hand, it is worth mentioning that the contribution of AQP8 in acute cholestasis appears to be less significant. A recent study showed that experimental acute cholestasis in rats caused by the estrogen metabolite estradiol-17-\( \alpha \)-glucuronide (E\(_1\)7G) failed to cause endocytic internalization of canalicular AQP8\cite{66}, in contrast to that observed for Bsep and Mrp2\cite{61,62}. Therefore the rapid retrieval of solute transporters, but not that of AQP8, seems to be the main cause of acute cholestasis induced by E\(_1\)7G.

It is well known that sepsis, a systemic inflammatory response secondary to bacterial infection, is frequently associated with intrahepatic cholestasis\cite{65,66}. Lipopolysaccharides (LPS) are endotoxins released into the circulation from bacterial sites of infection and are responsible for the macrophage secretion of proinflammatory cytokines, primarily tumor necrosis factor \( \alpha \) (TNF\( \alpha \)), interleukin 1-\( \beta \) and interleukin-6. These cytokines are the principal mediators of bile secretory failure\cite{66}. In a study performed using a rodent model of endotoxemia, we demonstrated that LPS reduced the functional expression of hepatocyte canalicular AQP8\cite{66}. As shown in Figure 2, a decrease in canalicular AQP8 protein expression of approximately 70% was associated with a 25% decrease in water canalicular permeability measured by stopped-flow spectrophotometry. This result is in good agreement with the above-mentioned studies of estrogen-induced cholestasis. Thus, LPS-induced cholestasis may ultimately be caused by impaired transient osmotic gradients generated by defective canalicular expression of the solute transporters Bsep and Mrp2\cite{66}, together with reduced canalicular water permeability secondary to defective AQP8 expression. The impairment in AQP8 expression was found to be posttranscriptional and mediated by the cytokine TNF\( \alpha \). Indeed, the passive immunization \textit{in vivo} with anti-TNF\( \alpha \) antibody prevented LPS-induced cholestasis and AQP8 protein downregulation. These results were confirmed in cultured rat hepatocytes treated with recombinant TNF\( \alpha \). Complementary \textit{in vitro} studies using lysosome and proteasome inhibitors showed that AQP8 degradation was mediated \textit{via} both lysosomal and proteasomal pathways. It was concluded that LPS induces posttranscriptional AQP8 downregulation and an associated decrease in canalicular membrane water permeability, a mechanism that is likely to contribute to the molecular pathogenesis of LPS-induced cholestasis.

As clinical sepsis is commonly polymicrobial, the above-mentioned results were further confirmed in an animal model of peritoneal sepsis characterized by a focus of infection with mixed intestinal flora instead of an endotoxin challenge. Thus liver AQP8 expression was studied in rats with sepsis induced by cecal ligation and puncture (CLP)\cite{66}. In agreement with the endotoxic model, immunoblotting and immunohistochemical studies revealed a significant decrease in AQP8 protein level in canalicular membranes, without any significant reduction in AQP9 expression. These results are in agreement with the findings in LPS-treated rats, and further support the notion that the defective expression of hepatocyte AQP8 contributes to the development of bile secretory dysfunction in sepsis.

Based on the cumulative evidence described above, a schematic model for the hepatocyte AQP8 contribution to the development of bile secretory failure is depicted in Figure 3.

**CONCLUSION**

In conclusion, this review summarized recent progress in research and current available data on the expression and pathophysiological significance of AQP water channels in the hepatocyte. It has long been established that canalicular bile secretion is the result of a combined interaction of several solute transporters. However, in the last few years further insight has been provided on the molecular basis of water movement during bile secretion. The functional expression of AQP8 is impaired in several experimental models of cholestasis such as extrahepatic obstructive cholestasis, estrogen-induced cholestasis and sepsis-induced cholestasis. A combined alteration in solute transporters and AQP8 would hamper the efficient coupling of osmotic gradients and canalicular water flow. Therefore, the common association of impaired solute transport together with decreased water permeability would ultimately lead to bile secretory failure. Nevertheless, more research is needed to expand the current knowledge underlying AQP expression regulation and water transport in cholestasis.

**REFERENCES**

1. Zollner G, Trauner M. Mechanisms of cholestasis. \textit{Clin Liver
Molecular aspects of bile formation

Assaf Yano M, Trauner M. Molecular aspects of bile formation and cholestasis. Trends Mol Med 2003; 9: 558-564

Roma MG, Crocanzi FA, Sanchez Pozzi EA. Hepatocellular transport of bile: new insights into functional, regulatory and therapeutic aspects. Clin Sci (Lond) 2008; 114: 567-588

King LS, Kozono D, Agre P. From structure to disease: the evolving tale of aquaporin biology. Nat Rev Mol Cell Biol 2004; 5: 687-698

Preston GM, Carroll TP, Guggino WB, Agre P. Appearance of water channels in Xenopus oocytes expressing red cell CHIP28 protein. Science 1992; 256: 385-387

Gorelick DA, Praetorius J, Tsenunari T, Nielsen S, Agre P. Aquaporin-11: a channel protein lacking apparent transport function expressed in brain. BMC Biochem 2006; 7: 14

Itoh T, Rai T, Kuwahara M, Ko SB, Uchida S, Sasaki S, Ishibashi K. Identification of a novel aquaporin, AQPI2, expressed in pancreatic acinar cells. Biochem Biophys Res Commun 2005; 330: 832-838

Saparov SM, Liu K, Agre P, Pohl P. Fast and selective ammonia transport by aquaporin-8. J Biol Chem 2007; 282: 5296-5301

Bienert GP, Moller AL, Kristiansen KA, Schulz A, Moller IM, Schjoerring JK, Jahn TP. Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. J Biol Chem 2007; 282: 1138-1192

Wu B, Beitz E. Aquaporins with selectivity for unconventional permeants. Cell Mol Life Sci 2007; 64: 2413-2421

Jung JS, Preston GM, Smith BL, Guggino WB, Agre P. Molecular structure of the water channel through aquaporin CHIP. The hourglass model. J Biol Chem 1994; 269: 14648-14654

Beitz E, Wu B, Orm K, Schultz JE, Zeuzen T. Point mutations in the aromatic/arгинine region in aquaporin 1 allow passage of urea, glycerol, ammonia, and protons. Proc Natl Acad Sci USA 2006; 103: 269-274

de Groot BL, Engel A, Grubmuller H. A refined structure of human aquaporin-1. FEBS Lett 2001; 504: 206-211

Murata K, Mitsuoka K, Hirai T, Walz T, Agre P, Heymann JB, Engel A, Fujiyoshi Y. Structural determinants of water permeation through aquaporin-1. Nature 2000; 407: 599-605

Savage DF, Stroud RM. Structural basis of aquaporin inhibition by mercury. J Mol Biol 2007; 367: 607-617

Huebert RC, Splinter PL, Garcia F, Marinelli RA, LaRussa NF. Expression and localization of aquaporin water channels in rat hepatocytes. Evidence for a role in canicular bile secretion. J Biol Chem 2002; 277: 22710-22717

Calamita G, Mazzone A, Bizzoca A, Cavalier A, Cassano G, Thomas D, Svelto M. Expression and immunolocalization of the aquaporin-8 water channel in rat gastrointestinal tract. Eur J Cell Biol 2001; 80: 711-719

Elkjaer ML, Nejsum LN, Gresz V, Kwon TH, Jensen UB, Frokjaer J, Nielsen S. Immunolocalization of aquaporin-8 in rat kidney, gastrointestinal tract, testis, and airways. Am J Physiol Renal Physiol 2001; 281: F1047-F1057

Garcia F, Kierbel A, Larocca MC, Gradilone SA, Splinter P, LaRussa NF, Marinelli RA. The water channel aquaporin-8 is mainly intracellular in rat hepatocytes, and its plasma membrane insertion is stimulated by cyclic AMP. J Biol Chem 2001; 276: 12147-12152

Tan1 T, Koyama Y, Niheki K, Hatakeyama S, Ohshiro K, Yoshida Y, Yaoita E, Sakai Y, Hatakeyama K, Yamamoto T. Immunolocalization of aquaporin-8 in rat digestive organs and testis. Arch Histol Cytol 2001; 64: 159-168

Elkjaer M, Vajda Z, Nejsum LN, Kwon T, Jensen UB, Amiry-Moghaddam M, Frokjaer J, Nielsen S. Immunolocalization of AQPI9 in liver, epididymis, testis, spleen, and brain. Biochem Biophys Res Commun 2000; 276: 118-128

Niechta A, Nico R, Ricco G, Svelto M. Tissue distribution and membrane localization of aquaporin-9 water channel: evidence for sex-linked differences in liver. J Histochem Cytochem 2001; 49: 1547-1556

Robert S, Yano M, Ueno Y, Pham L, Alpini G, Agre P, LaRussa NF. Cholangiocytes express the aquaporin CHIP and transport water via a channel-mediated mechanism. Proc Natl Acad Sci USA 1994; 91: 1306-1309

Nielsen S, Smith BL, Chiang J, de Groot BL, Agre P. Distribution of the aquaporin CHIP in secretory and absorptive epithelia and capillary endothelia. Proc Natl Acad Sci USA 1993; 90: 7275-7279

Marinelli RA, Pietz TS, Pham LD, Rueckert L, Agre P, LaRussa NF. Secretin induces the apical insertion of aquaporin-1 water channels in rat cholangiocytes. Am J Physiol 1999; 276: C280-C286

Marinelli RA, Pham LD, Tietz PS, LaRussa NF. Expression of aquaporin-4 water channels in rat cholangiocytes. Hepatology 2000; 31: 1313-1317

Calamita G, Ferri D, Bazzini C, Mazzone A, Bottagio G, Liquori GE, Paulmichl M, Fortincasa P, Meyer G, Svelto M. Expression and subcellular localization of the AQPI8 and AQPl water channels in the mouse gall-bladder epithelium. Biol Cell 2005; 97: 415-423

Calamita G, Ferri D, Gena P, Liquori GE, Cavalier A, Thomas D, Svelto M. The inner mitochondrial membrane has aquaporin-8 water channels and is highly permeable to water. J Biol Chem 2005; 280: 17149-17153

Tsukaguchi H, Shayanukul C, Berger UV, Mackenzie B, Devidas S, Guggino WB, van Hoek AN, Hediger MA. Molecular characterization of a broad selectivity neutral solute channel. J Biol Chem 1998; 273: 24737-24743

Tsukaguchi H, Weremowicz S, Morton CC, Hediger MA. Functional and molecular characterization of the human neutral solute channel aquaporin-9. Am J Physiol 1999; 277: F685-F696

Carbrey JM, Gorelick-Feldman DA, Kozono D, Praetorius J, Nielsen S, Agre P. Aquaglyceroporin AQP9: solute permeation and metabolic control of expression in liver. Proc Natl Acad Sci USA 2003; 100: 2945-2950

Nielsen S, DiGiovanni SR, Christensen EL, Knepper MA, Harris HW. Cellular and subcellular immunolocalization of vasopressin-regulated water channel in rat kidney. Proc Natl Acad Sci USA 1993; 90: 11663-11667

Nielsen S, Chou CL, Marples D, Christensen EL, Kishore BK, Knepper MA. Vasopressin increases water permeability of kidney collecting duct by inducing translocation of aquaporin-CD water channels to plasma membrane. Proc Natl Acad Sci USA 1995; 92: 1013-1017

Ishikawa Y, Yuan Z, Inoue N, Skowronski MT, Nakae Y, Shono M, Cho G, Yasui M, Agre P, Nielsen S. Identification of AQPI5 in lipid rafts and its translocation to apical membranes by activation of M3 mAChRs in interlobular ducts of rat parotid gland. Am J Physiol Cell Physiol 2005; 289: C1303-C1311

Marinelli RA, Pham L, Agre P, LaRussa NF. Secretin promotes osmotic water transport in rat cholangiocytes by increasing aquaporin-1 water channels in plasma membrane. Evidence for a secretin-induced vesicular translocation of aquaporin-1. J Biol Chem 1997; 272: 12984-12988

Gradilone SA, Garcia F, Huebert RC, Tietz PS, Larocca MC, Kierbel A, Carreras FL, Larossa NF, Marinelli RA. Glucagon induces the plasma membrane insertion of functional aquaporin-8 water channels in isolated rat hepatocytes. Hepatology 2003; 37: 1435-1441

Ishibashi K, Kuwahara M, Kagayaema Y, Tohsaka A, Marumo F, Sasaki S. Cloning and functional expression of a second new aquaporin GP, abundantly expressed in testis. Biochem Biophys Res Commun 1997; 237: 714-718

Tietz PS, McNiven MA, Splinter PL, Huang BQ, Larusso NF. Cytoskeletal and motor proteins facilitate trafficking of...
AQPI-containing vesicles in cholangiocytes. *Biol Cell* 2006; 98: 43-52

40 Zhao AZ, Shinhara MM, Huang D, Shimizu M, Eldar-Finkelman H, Krebs EG, Beavo JA, Bornfeldt KE. Leptin induces insulin-like signaling that antagonizes cAMP elevation by glucagon in hepatocytes. *J Biol Chem* 2000; 275: 11348-11354

41 Blommaart EF, Krause U, Schellens JP, Vreeling-SindelaroVA H, Meijer AJ. The phosphatidylinositol 3-kinase inhibitors wortmannin and LY294002 inhibit autophagy in isolated rat hepatocytes. *Eur J Biochem* 1997; 243: 240-246

42 Follì F, Alvaro D, Gigliozzi A, Bassotti C, Kahn CR, Pontiroli AE, Capocaccia L, Jezequel AM, Benedetti A. Regulation of endocytic-transcytotic pathways and bile secretion by phosphatidylinositol 3-kinase in rats. *Gastroenterology* 1997; 113: 954-965

43 Misra S, Ujhazy P, Varticovski L, Arias IM. Phosphoinositide 3-kinase lipid products regulate AP-dependent transport by sister of P-glycoprotein and multidrug resistance associated protein 2 in bile canalicular membrane vesicles. *Proc Natl Acad Sci USA* 1999; 96: 5814-5819

44 Webster CR, Anwer MS. Role of the PI3K/PKB signaling pathway in cAMP-mediated translocation of rat liver Ntcp. *Am J Physiol* 1999; 277: G1165-G1172

45 Gradilone SA, Carreras FI, Lehmann GL, Marinelli RA. Phosphoinositide 3-kinase is involved in the glucagon-induced translocation of aquaporin-8 to hepatocyte plasma membrane. *Biol Cell* 2005; 97: 831-836

46 Kagawa T, Varticovski L, Sai Y, Arias IM. Mechanism by which cAMP activates PI3-kinase and increases bile acid secretion in WIF-B9 cells. *Am J Physiol Cell Physiol* 2002; 283: C1655-C1666

47 Cosentino C, Di Domenico M, Porcellini A, Cucuzza C, De Gregorio G, Santillo MR, Agnese S, Di Stasio R, Feliciello A, Migliaccio A, Avvedimento EV. p85 regulatory subunit of PI3K mediates cAMP-PKA and estrogens biological effects on growth and survival. *Oncogene* 2007; 26: 2095-2103

48 Masyuk AI, Marinelli RA, La Russo NF. Water transport by epithelia of the digestive tract. *Gastroenterology* 2002; 122: 545-562

49 Marinelli RA, Tietz PS, Caride AJ, Huang BQ, La Russo NF. Water transporting properties of hepatocyte basolateral and canalicular plasma membrane domains. *J Biol Chem* 2003; 278: 43157-43162

50 Mazzone A, Tietz P, Jefferson J, Pagano R, La Russo NF. Isolation and characterization of lipid microdomains from apical and basolateral plasma membranes of rat hepatocytes. *Hepatology* 2006; 43: 287-296

51 Tietz P, Jefferson J, Pagano R, Larusso NF. Membrane microdomains in hepatocytes: potential target areas for proteins involved in canalicular bile secretion. *J Lipid Res* 2005; 46: 1426-1432

52 Banales JM, Prieto J, Medina JF. Cholangiocyte anion exchange and biliary bicarbonate excretion. *World J Gastroenterol* 2006; 12: 3496-3511

53 Gradilone SA, Tietz PS, Spliet PL, Marinelli RA, La Russo NF. Expression and subcellular localization of aquaporin water channels in the polarized hepatocyte cell line, WIF-B. *BMC Physiol* 2005; 5: 13

54 Yang B, Song Y, Zhao D, Verkman AS. Phenotype analysis of aquaporin-8 null mice. *Am J Physiol Gastrointest Liver Physiol* 2005; 288: C1161-C1170

55 Hofmann AF. Cholestatic liver disease: pathophysiology and therapeutic options. *Liver* 2002; 22 Suppl 2: 14-19

56 Larocca MC, Soria LR, Espelt MV, Lehmann GL, Marinelli RA. The knockdown of hepatocyte aquaporin-8 by RNA interference induces defective bile canalicular water transport. *Am J Physiol Gastrointest Liver Physiol* 2009; 296: G93-G100

57 Carreras FI, Gradilone SA, Mazzone A, Garcia F, Huang BQ, Ochoa JE, Tietz PS, Larusso NF, Calamita G, Marinelli RA. Rat hepatocyte aquaporin-8 water channels are down-regulated in extrahepatic cholestasis. *Hepatology* 2003; 37: 1026-1033

58 Calamita G, Ferri D, Gena P, Carreras FI, Liguori GE, Portincasa P, Marinelli RA, Svelto M. Altered expression and distribution of aquaporin-9 in the liver of rat with obstructive extrahepatic cholestasis. *Am J Physiol Gastrointest Liver Physiol* 2008; 295: G682-G690

59 Carreras FI, Lehmann GL, Ferri D, Tioni MF, Calamita G, Marinelli RA. Defective hepatocyte aquaporin-8 expression and reduced canalicular membrane water permeability in estrogen-induced cholestasis. *Am J Physiol Gastrointest Liver Physiol* 2007; 292: G905-G912

60 Mottino AD, Carreras FI, Gradilone SA, Marinelli RA, Vore M. Canalicular membrane localization of hepatocyte aquaporin-8 is preserved in estradiol-17beta-D-glucuronide-induced cholestasis. *J Hepatol* 2006; 44: 232-233

61 Crocenzi FA, Mottino AD, Cao J, Veggi LM, Pozzi EJ, Vore M, Coleman R, Roma MG. Estradiol-17beta-D-glucuronide induces endocytic internalization of Bsep in rats. *Am J Physiol Gastrointest Liver Physiol* 2003; 285: G449-G459

62 Mottino AD, Cao J, Veggi LM, Crocenzi F, Roma MG, Vore M. Altered localization and activity of canalicular Mrp2 in estradiol-17beta-D-glucuronide-induced cholestasis. *Hepatology* 2002; 35: 1409-1419

63 Geier A, Fickert P, Trauner M. Mechanisms of disease: mechanisms and clinical implications of cholestasis in sepsis. *Nat Clin Pract Gastroenterol Hepatol* 2006; 3: 574-585

64 Lehmann GL, Carreras FI, Soria LR, Gradilone SA, Marinelli RA. LPS induces the TNF-alpha-mediated downregulation of rat liver aquaporin-8: role in sepsis-associated cholestasis. *Am J Physiol Gastrointest Liver Physiol* 2008; 294: G567-G575

65 Lee JM, Trauner M, Soroka CJ, Stieger B, Meijer PJ, Boyer JL. Expression of the bile salt export pump is maintained after chronic cholestasis in the rat. *Gastroenterology* 2000; 118: 163-172

66 Lehmann GL, Marinelli RA. Peritoneal sepsis downregulates liver expression of Aquaporin-8: a water channel involved in bile secretion. *Liver Int* 2009; 29: 317-318

S-Editor Cheng JX L-Editor Webster JR E-Editor Ma WH