Research Communication

Adenovirus-Mediated Human Aquaporin-1 Expression in Hepatocytes Improves Lipopolysaccharide-Induced Cholestasis

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Abstract

Lipopolysaccharides (LPS) are known to cause cholestasis in sepsis. There is evidence that a defective expression of canalicular aquaporin water channels contributes to bile secretory failure in LPS-induced cholestasis. Thus, we studied whether the hepatic adenovirus-mediated transfer of human aquaporin-1 gene (*haqp1*) can improve the cholestasis induced by LPS. Adenoviral vector encoding hAQP1 (AdhAQP1) or control vector was administered to rats by retrograde intrabiliary infusion. Hepatocyte canalicular hAQP1 expression was assessed by liver immunostaining and immunoblotting in purified plasma membranes. LPS reduced bile flow and biliary bile acid excretion by 30% and 45%, respectively. AdhAQP1-treatment normalized both bile flow and biliary bile acid excretion in LPS-induced cholestasis. Moreover, markedly elevated serum bile acid levels in cholestatic rats, were also normalized with the AdhAQP1 hepatic transduction. Bile flow and serum or biliary bile acids in normal rats were not significantly altered by AdhAQP1. AdhAQP1 delivery unaffected the down-regulated protein expression of canalicular bile salt export pump (BSEP/ABCB11) in cholestasis, but improved its transport activity restoring reduced canalicular cholesterol content. Our data suggest that the adenovirus-mediated hepatocyte hAQP1 expression improves LPS-induced cholestasis in rats by stimulating the BSEP/ABCB11-mediated biliary bile acid excretion; a finding that might contribute to the understanding and treatment of sepsis-associated cholestatic diseases. © 2017 IUBMB Life, 00(0):000–000, 2017

Keywords: *aquaporin-1; LPS-induced cholestasis; biliary bile acid excretion; bile acid export pump; gene transfer*

Introduction

Lipopolysaccharides (LPS), bacterial endotoxins released from sites of infection, are key determinants of bile secretory failure in sepsis (1,2). LPS through macrophage-derived proinflammatory cytokines induce down-regulation in gene expression and functioning of hepatocyte membrane transporters directly involved in bile formation. Thus, cholestasis would ultimately be caused by the impairment of bile canalicular solute transporters, such as the bile acid export pump (BSEP/ABCB11). Aquaporin-8 channels, which facilitate canalicular osmotic water transport (3), are also down-regulated, and would contribute to LPS-induced cholestasis (2).

The adenovirus-mediated delivery of human aquaporin-1 gene (*haqp1*) water channels has been used to improve salivary flow in experimental animals (4,5) and humans (6) with irradiated salivary glands. Our recent studies show that the hepatic adenovirus-mediated delivery of *haqp1* gene to estrogen-induced cholestatic rats improves the bile secretory failure by promoting biliary excretion and choleretic efficiency of bile acids (7,8). LPS administration to rodents is a well-known experimental model used to elucidate

Abbreviations: *LPS, lipopolysaccharide; AdhAQP1, adenovirus encoding human aquaporin-1; hAQP1, human aquaporin-1; BSEP/ABCB11, bile salt export pump*

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TABLE 1 *Liver serum enzymes and body and liver weights*

	Noncholestatic + control vector	*Noncholestatic + AdhAQP1*	*LPS + control vector*	*LPS + AdhAQP1*
Serum enzymes				
ALT (U/L)	30 ± 8	22 ± 8	29 ± 3	26 ± 5
AST (U/L)	149 ± 23	162 ± 25	177 ± 14	172 ± 20
LDH (U/L)	714 ± 192	617 ± 156	861 ± 76	649 ± 85
ALP (U/L)	244 ± 34	219 ± 32	446 ± 22*	322 ± 56
GGT (U/L)	3.8 ± 0.8	3.9 ± 0.6	7.7 ± 0.7#	5.0 ± 0.5
Weights (g)				
Body (body wt)	331 ± 13	334 ± 12	344 ± 9	324 ± 19
Liver	10.7 ± 0.7	11.1 ± 0.7	11.2 ± 0.5	11.0 ± 1.1

*Data are means ± SEM (*N = *5).*
LPS, lipopolysaccharide; ALT, alanine transaminase; AST, aspartate transaminase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; GGT, γ-glutamyl transpeptidase.
**P < 0.05 as compared with noncholestatic + control vector and noncholestatic + AdhAQP1.*
#P < *0.05 as compared with noncholestatic + control vector, noncholestatic + AdhAQP1 and LPS + AdhAQP1.*

mechanistic events in sepsis-associated cholestasis. Here, we studied whether the hepatic adenovirus-mediated delivery of hAQP1 is able to improve LPS-induced cholestasis in the rat.

Experimental Procedures

Animals and LPS Treatment

Adult male Wistar rats were used throughout this study. Animals had free access to water and food and were maintained under controlled temperature and humidity, alternating 12-h light and dark cycles. Animals received humane care, according to the *Guide for the Care and Use of Laboratory Animals* (NIH). Protocols complied with the local guidelines for the use of experimental animals. *Salmonella typhimurium* LPS (4 mg/kg body wt) (Sigma, St. Louis, MO) dissolved in sterile 0.9% NaCl was administered via femoral vein under ketamine (100 mg/kg body weight)/xylazine (15 mg/kg body weight). Noncholestatic controls received saline alone. Under these conditions (2), LPS induced cholestasis as indicated by 30% bile flow reduction (Fig. 2) and the increase of the serum cholestatic markers alkaline phosphatase and γ-glutamyl transpeptidase (Table 1).

Adenoviral Vectors

We used two adenovectors, AdhAQP1, which encodes hAQP1, and a similar control vector which encodes the red fluorescent protein, DsRed. AdhAQP1 was gently donated by Dr. B. Baum (NIH, Bethesda) (4).

Intrabiliary Delivery of Adenovirus

Animals were anesthetized i.p. with ketamine (100 mg/kg body weight)/xylazine (15 mg/kg body weight). 3×10^{10} pfu (plaque-forming unit) adenovirus dose per liver was retrogradely infused into the biliary tract. We previously found that this dose of adenovirus is able to improve estrogen induced cholestasis in rats (7). The surgical procedure has been detailed elsewhere (7). LPS was administered approximately 54 h later, as explained above. Experiments were started 16 h after LPS injection (i.e., 72 h after adenovirus administration). Our previous studies show that the hAQP1 protein expression in hepatic cells takes not less than 72 h (7).

Bile Secretion

Bile was collected for 30 min and bile flow assessed by gravimetry (7). Blood samples were taken by cardiac puncture, animals were killed by exsanguination and livers harvested. There were no differences in bile flow in noncholestastic or LPS-treated rats with control vector or saline alone (data not shown).

[^{3}H]Taurocholate Biliary Output

A dose of 0.1 μCi [^{3}H]taurocholate (specific activity 5 Ci/mmol, Perkin Elmer, Waltham, MA) was injected intravenously and bile collected in six 5 min periods. [^{3}H]taurocholate biliary excretion was determined from bile flow and the radioactivity values (9).

Serum and Biliary Bile Acids

Serum bile acids were determined using a commercial kit (Randox, Crumkin County Antrim, UK). Biliary bile acids were assessed using the 3-hydroxysteroid dehydrogenase procedure (10).

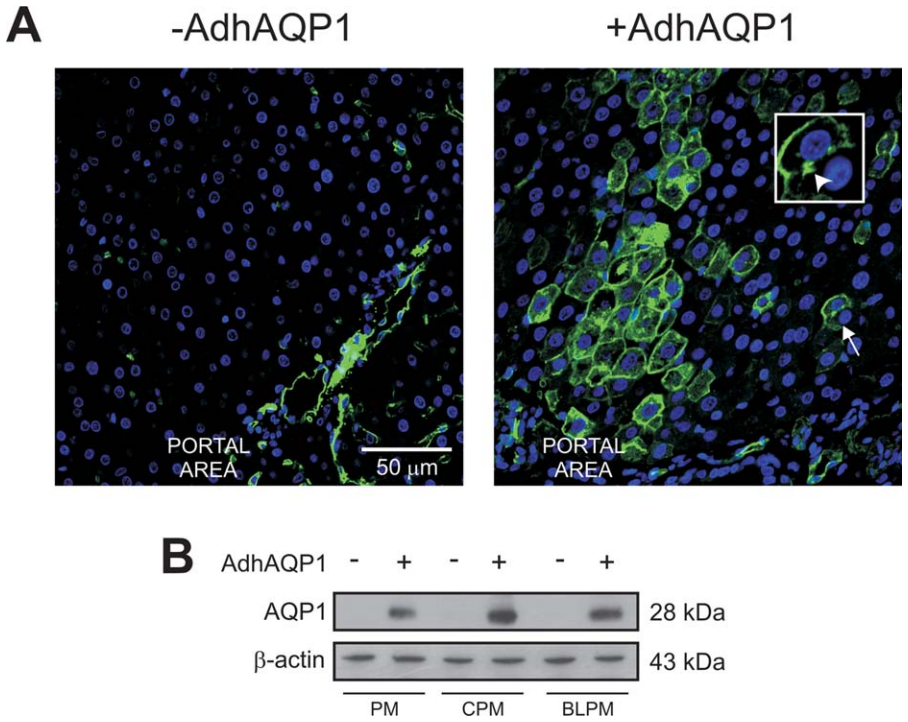

FIG 1

Hepatocyte expression of hAQP1 in LPS-cholestatic rats after retrograde intrabiliary AdhAQP1 delivery. The induction of cholestasis by LPS and the delivery of AdhAQP1 and control vectors were detailed in Materials and Methods. (A) Immunofluorescence localization of AQP1 in liver portal areas of LPS cholestatic rats transduced with control vector (–AdhAQP1) or with AdhAQP1 (+AdhAQP1) viewed by laser scanning confocal microscopy. –AdhAQP1 rats showed endogenous AQP1 staining only in peribiliary vascular endothelia and cholangiocytes, while +AdhAQP1 rats showed intense hAQP1 staining in hepatocytes. Nuclei were stained with DAPI (blue). Immunoreactivity for AQP1 in AdhAQP1-transduced rats was mostly observed on the canalicular and basolateral plasma membranes of hepatocytes (arrow, see inset). No staining was observed in experiments omitting AQP1 primary antibody (data not shown). Original magnification ×400. (B) Anti-AQP1 immunoblot of total hepatic plasma membranes (PM), and canalicular (CPM) and basolateral (BLPM) plasma membrane fractions from LPS cholestatic rats transduced with AdhAQP1 or control vector (–AdhAQP1). Lanes were loaded with 20 μg of proteins. The blots were reprobed by using anti-β-actin antibody as a control for equal protein loading.

Liver Serum Enzymes

Activities of alkaline phosphatase, aspartate transaminase, γ-glutamyl transpeptidase, alanine transaminase, and lactate dehydrogenase were assessed in serum using commercial kits (Wiener Lab, Rosario, Argentina). There were no differences in liver serum enzymes in noncholestastic or LPS-treated rats with control vector administration or saline alone data not shown

Plasma Membrane Fractions

The canalicular and basolateral plasma membrane purification procedures have been detailed elsewhere (2,7). Total proteins were measured by Lowry's method. The enrichment and purity of canalicular membranes assessed with specific markers, were comparable to those reported previously (8), and similar between LPS and control rats.

Immunoblottings

Western blottings were achieved as described (2,7). The primary affinity purified antibodies used were anti-AQP1 (1 μg/mL, Alpha Diagnostics Intl, San Antonio, TX), anti-BSEP/ABCB11 (1 μg/mL, Kamiya, Seattle), anti-caveolin-1 (1 μg/mL, Sigma, St Louis, MO); and anti-actin (1 μg/mL, Sigma, St Louis, MO). Corresponding HRP-conjugated secondary antibodies (Thermo Scientific, Rockford, IL) were used. Bands were identified by enhanced chemiluminescence detection system (ECL) and autoradiographs were acquired by exposing PVDF sheets to radiographic film. Densitometry was carried out using Image J Software (11).

Immunofluorescence and Confocal Microscopy

Immunofluorescence was performed in fixed liver sections using anti-AQP1 antibodies (10 μg/mL) and Alexa 488-conjugated secondary antibodies (Molecular Probes, Eugene, OR), and 4′-6-diamidino-2-phenylindole (DAPI) (Molecular Probes, Eugene, OR), as described (2,7). Confocal fluorescence images were taken using the same settings. Controls omitting secondary and primary antibodies showed no labeling.

Cholesterol Determination

Cholesterol content was measured using a commercial kit (Wiener Lab, Rosario, Argentina).

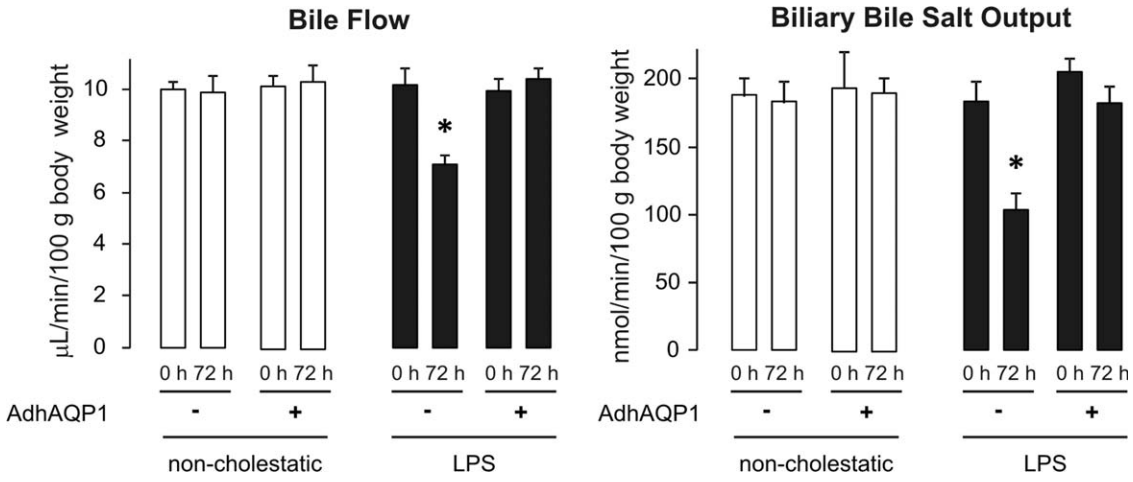

FIG 2

*Bile flow and biliary bile acid output in AdhAQP1-transduced LPS-cholestatic rats. The induction of cholestasis by LPS and the delivery of AdhAQP1 and control vectors were detailed in Materials and Methods. The figure shows bile flow and biliary output of total bile acids from noncholestatic and LPS cholestatic rats transduced with AdhAQP1 or control vector, before (0 h) and after adenoviral retrograde infusion (72 h). Data are given as mean ± SEM of four or five independent experiments per group. *P < 0.05 from noncholestatic controls and LPS + AdhAQP1.*

Statistical Analysis

Data are means ± SEM. Statistical significance ($P < 0.05$) was determined by Student's *t*-test or one-way ANOVA Tukey's test.

Results

Hepatocyte hAQP1 Expression

LPS-cholestatic animals with delivery of control adenovector did not show AQP1 expression in hepatocytes (Fig. 1A, left). Endogenous AQP1 expression in liver was observed in peribiliary vascular endothelia and cholangiocytes (12) (Fig. 1A, left). AdhAQP1 transfer to LPS-treated rats induced the hepatocyte expression of hAQP1, which was mostly localized on plasma membranes (Fig. 1A, right). As we reported (7,8), hAQP1 expression was clear in canalicular plasma membranes, the bile secretory pole in hepatocytes (Fig. 1A, right, inset). Immunoblotting studies showed a 28-kDa band corresponding to hAQP1 stronger in canalicular membranes (Fig. 1B). Moreover, in accordance to that observed in estrogen-induced cholestasis (8), hepatocyte expression and canalicular localization of hAQP1 was similar between LPS-cholestatic and noncholestatic rats (not shown), indicating that cholestasis did not affect vector infection and hAQP1 expression.

In addition, as shown in Table 1, AdhAQP1 transfer caused no cholestatic or cytolitic damage. Thus, there were no significant changes in the serum activities of transaminases and lactate dehydrogenase. Interestingly, the increased serum activities of the cholestatic markers alkaline phosphase and γ-glutamil transpeptidase in LPS rats were reduced by AdhAQP1 transfer.

Bile Flow and Biliary Bile Acid Excretion

As shown in Fig. 2, LPS treatment significantly reduced both bile flow and biliary bile acid output by 30% and 45%, respectively. As previously described for estrogen-induced cholestasis (7,8), AdhAQP1 transfer significantly improved bile flow and biliary excretion of bile acids in LPS-cholestatic rats, without significantly affecting those in noncholestatic rats (Fig. 2). In fact, the expression of hAQP1 normalized both bile flow and biliary bile acid excretion in LPS-induced cholestasis (Fig. 2).

Serum Bile Acid Levels

As reported (13), serum bile acids showed elevated levels in LPS-cholestatic rats (Fig. 3). AdhAQP1 delivery induced a

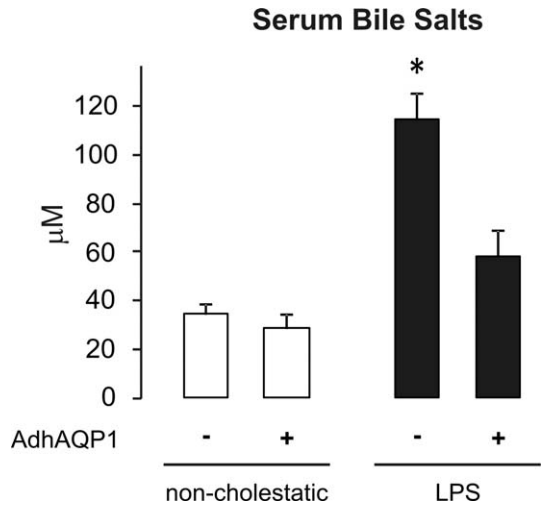

FIG 3

*Serum bile acids levels in AdhAQP1-transduced LPS-cholestatic rats. The induction of cholestasis by LPS and the delivery of AdhAQP1 and control vectors were detailed in Materials and Methods. Serum bile acids were assessed 16 h after LPS injection (i.e., 72 h after adenoviral infusion). Data are mean ± SEM of five independent experiments. *P < 0.05 from noncholestatic controls and LPS + AdhAQP1.*

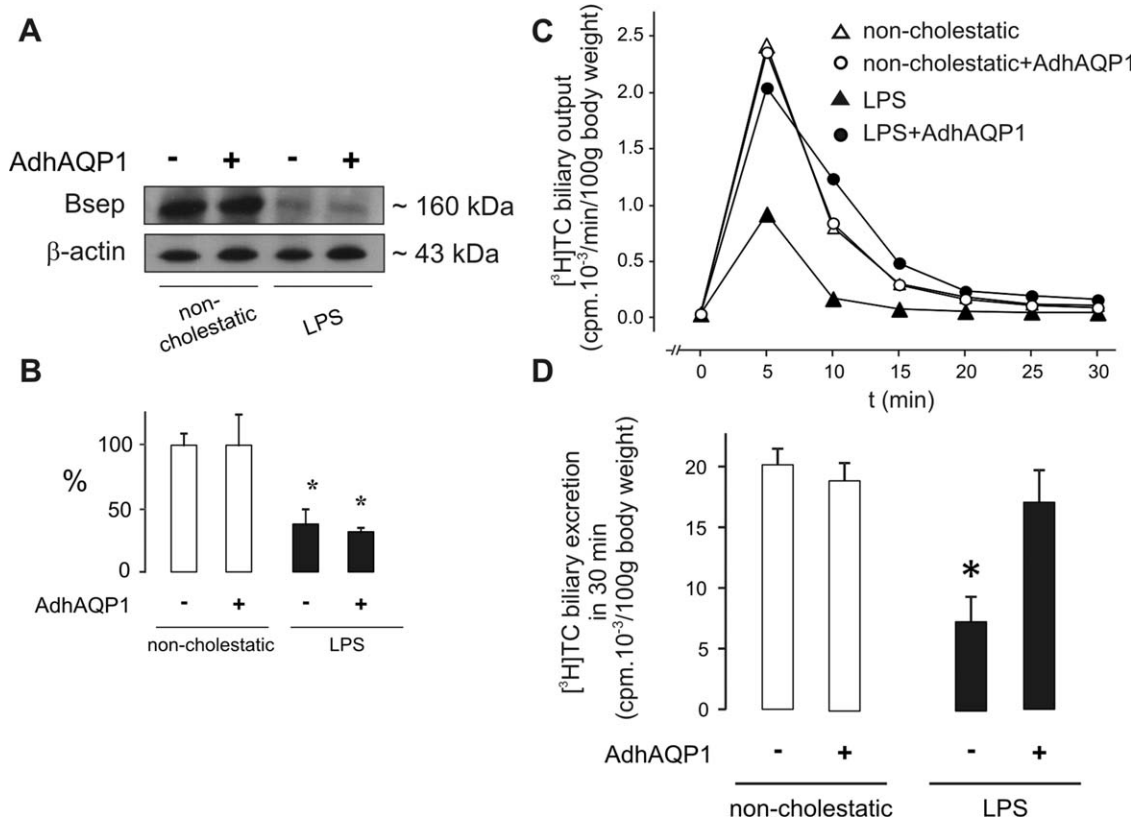

FIG 4

*Hepatocyte functional expression of BSEP/ABCB11 in AdhAQP1-transduced LPS-cholestatic rats. The induction of cholestasis by LPS and the delivery of AdhAQP1 and control vectors were detailed in Materials and Methods. Liver canalicular plasma membrane fractions were isolated and subjected to immunoblotting as described in Materials and Methods. (A) Representative immunoblot for BSEP/ABCB11 in canalicular membranes (25 μg proteins/lane). The blots were reprobed by using anti-β-actin antibody as a control for equal protein loading. (B) Densitometric analysis of four separate experiments in each group. Data (means ± SEM) are expressed as percentage of noncholestatic controls. *P < 0.05 from noncholestatic controls. (C) Representative time course of biliary [3H]TC output and (D) 30-min cumulative biliary [3H]TC output after a single i.v. administration of 0.1 μCi [3H]TC. Data are given as means ± SEM of three independent experiments per group. *P < 0.05 from noncholestatic controls and LPS + AdhAQP1.*

significant decrease in bile acid serum levels in cholestasis with no apparent effects in normal noncholestatic rats (Fig. 3).

BSEP/ABCB11 Functional Expression

In LPS-induced cholestasis (14), canalicular BSEP/ABCB11 expression was down-regulated by about 40% (Fig. 4A,B). Canalicular BSEP/ABCB11 protein expression was not altered by AdhAQP1 (Fig. 4A,B). Likewise BSEP/ABCB11, the sodium/taurocholate cotransporting polypeptide (NTCP; Slc10a1), which is the primary mechanism for hepatocyte bile acids uptake, was also down-regulated in LPS-induced cholestasis (15) and unaffected by AdhAQP1 (data not shown).

BSEP/ABCB11-mediated canalicular bile acid efflux constitutes the rate limiting step of the hepatocellular bile acids transport (16). Thus, in line with the downregulation of BSEP/ABCB11 expression, control vector-transduced cholestatic rats showed a significant reduction in the time course (Fig. 4C) and cumulative (Fig. 4D) biliary excretion of an intravenous tracer dose of [^{3}H]taurocholate (17,18). Nevertheless, biliary [^{3}H]taurocholate output in cholestatic rats was restored to normal values after hepatic transduction with AdhAQP1 (Fig. 4C,D). This suggests an hAQP1-induced increase in BSEP/ABCB11 transport activity specific for cholestasis since AdhAQP1 induced no significant change in biliary [^{3}H]taurocholate output in noncholestatic rats (Fig. 4C,D).

As BSEP/ABCB11 activity has been demonstrated to be dependent on cholesterol (19,20), we assessed canalicular cholesterol content and expression of cholesterol-binding protein caveolin-1. We found that in LPS-cholestasis, canalicular cholesterol content is reduced by 28% which is almost normalized after hAQP1 expression (Fig. 5A). Canalicular caveolin-1 expression, which was markedly down-regulated in LPS-cholestasis, was restored by hAQP1 expression (Fig. 5B).

Discussion

The main finding in this study relates to the functional significance of hepatocyte hAQP1 gene transfer to bile secretion in LPS-induced cholestatic rats.

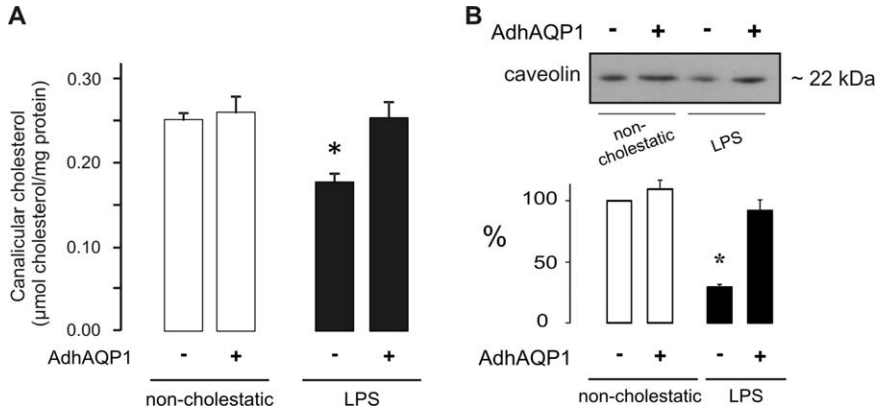

FIG 5

*Canalicular cholesterol content and caveolin-1 expression in AdhAQP1-transduced LPS-cholestatic rats. (A) Canalicular cholesterol content (μmol/mg protein). *P < 0.05 from noncholestatic controls and LPS + AdhAQP1. (B) Representative immunoblot for caveolin-1 in canalicular membranes (25 μg proteins/lane) and densitometric analysis of four separate experiments in each group. Data (means ± SEM) are expressed as percentage of noncholestatic controls. *P < 0.05 from noncholestatic controls.*

The retrograde intrabiliary AdhAQP1 infusion induced hAQP1 expression predominantly in periportal hepatocytes (i.e., the primary cells for bile formation) (Fig. 1A, right). Hepatocyte bile formation results from the canalicular secretion of water (mainly via AQP8) in response to osmotic gradients created by the active transport of solutes, primarily bile acids via BSEP/ABCB11 (21,22). Cholestasis in sepsis is mainly caused by a functional decline in bile formation at hepatocyte level. LPS-induced release of proinflammatory cytokines is thought to be determinant in the pathogenesis of sepsis-associated cholestasis, although noncytokine-mediated mechanisms seem to be also involved (23). The impairment of the functional and molecular expression of BSEP/ABCB11 is a key event leading to the development of LPS-induced cholestasis as well as non-inflammatory hepatocellular cholestasis such as that induced by estrogens (22). BSEP/ABCB11 deficiency impairs biliary bile acid excretion, which in turn leads to accumulation of bile acids in liver and blood with known deleterious consequences (22). Present study on LPS-induced cholestasis and previous one on estrogen-induced cholestasis (8) suggest that canalicularly expressed hAQP1 promotes biliary bile acid excretion by improving BSEP/ABCB11 functioning without changing its protein expression. Accordingly with the improvement in biliary bile acid excretion, serum bile acid levels are normalized in AdhAQP1-transduced cholestatic rats (8) (Fig. 3). The hAQP1-induced canalicular BSEP/ABCB11 activity in LPS-induced cholestasis, it is likely related to changes in canalicular cholesterol content as BSEP/ABCB11 activity is critically dependent on cholesterol (19,20). It has been shown that a small increase in the amount of cholesterol in liver plasma membranes (around 20%) is able to markedly induce BSEP/ABCB11 activity by over 100% (19). We found that in LPS-cholestasis, canalicular cholesterol content is diminished and nearly normalized by hAQP1 expression (Fig. 5A). Thus the observed increase of canalicular cholesterol content in AdhAQP1-transduced cholestatic rats would explain the hAQP1-induced BSEP/ABCB11 activity, nevertheless, an increased concentration of BSEP/ABCB11 in cholesterol-rich microdomains may also contribute (8). hAQP1 might induce a change in membrane cholesterol by means of its binding to caveolin-1 (24) present in liver canalicular membranes (25). Caveolin-1 is thought to induce changes in the canalicular lipid (cholesterol) environment of BSEP/ABCB11 that favor its transport activity without changing its protein expression (26). In support of this is the finding that canalicularly expressed hAQP1 restored the downregulated canalicular caveolin-1 expression in LPS-cholestasis (Fig. 5B).

We previously reported that hepatocyte expression of endogenous AQP8 and canalicular osmotic water permeability are down-regulated in LPS-induced cholestasis (2). AdhAQP1 delivery unaltered AQP8 expression (data not shown), nevertheless, as we described for estrogen-induced cholestasis, canalicularly-expressed hAQP1 is expected to increase membrane water permeability and to contribute in part to the bile flow improvement in LPS-induced cholestasis (7).

The retrograde biliary infusion allows the gene transfer into the liver with negligible leakage because of the hepatobiliary tract anatomy (27), and eventually, the repetitive gene transduction without immunosuppression (28). Besides, as the intrabiliary delivery is an approach that would be clinically practicable via endoscopic retrograde cholangiography, AdhAQP1 administration might be helpful to treat some cholestatic disorders.

In conclusion, we found that the hepatic adenovirus-mediated transfer of hAQP1 improves LPS-induced cholestasis by stimulating the biliary excretion of bile acids. This finding might contribute to new therapeutic approaches for endotoxin-induced cholestatic diseases.

Acknowledgements

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References

[1] Bolder, U., Ton-Nu, H. T., Schteingart, C. D., Frick, E., and Hofmann, A. F. (1997) Hepatocyte transport of bile acids and organic anions in endotoxemic rats: impaired uptake and secretion. Gastroenterology 112, 214–225.

[2] Lehmann, G. L., Carreras, F. I., Soria, L. R., Gradilone, S. A., and Marinelli, R. A. (2008) LPS induces the TNFα-mediated down-regulation of rat liver aquaporin-8: role in sepsis-associated cholestasis. Am. J. Physiol. Gastrointest. Liver Physiol. 294, 567–575.

[3] Larocca, M. C., Soria, L. R., Espelt, M. V., Lehmann, G. L., and Marinelli, R. A. (2009) Knockdown of hepatocyte aquaporin-8 by RNA interference induces defective bile canalicular water transport. Am. J. Physiol. Gastrointest. Liver Physiol. 296, 93–100.

[4] Delporte, C., O'Connell, B. C., He, X., Lancaster, H. E., O'Connell, A. C., et al. (1997) Increased fluid secretion after adenoviral-mediated transfer of the aquaporin-1 cDNA to irradiated rat salivary glands. Proc. Natl. Acad. Sci. USA 94, 3268–3273.

[5] Baum, B. J., Zheng, C., Cotrim, A. P., McCullagh, L., Goldsmith, C. M., et al. (2009) Aquaporin-1 gene transfer to correct radiation-induced salivary hypofunction. Handb. Exp. Pharmacol. 190, 403–418.

[6] Baum, B. J., Alevizos, I., Zheng, C., Cotrim, A. P., Liu, S., et al. (2012) Early responses to adenoviral-mediated transfer of the aquaporin-1 cDNA for radiation-induced salivary hypofunction. Proc. Natl. Acad. Sci. USA 109, 19403–19407.

[7] Marrone, J., Lehmann, G. L., Soria, L. R., Pellegrino, J. M., Molinas, S., et al. (2014) Adenoviral transfer of human aquaporin-1 gene to rat liver improves bile flow in estrogen-induced cholestasis. Gene Ther. 21, 1058–1064.

[8] Marrone, J., Soria, L. R., Danielli, M., Lehmann, G. L., Larocca, M. C., et al. (2016) Hepatic gene transfer of human aquaporin-1 improves bile acid secretory failure in rats with estrogen-induced cholestasis. Hepatology 64, 535–548.

[9] Hung, D. Y., Siebert, G. A., Chang, P., and Roberts, M. S. (2005) Hepatic pharmacokinetics of taurocholate in the normal and cholestatic rat liver. Br. J. Pharmacol. 145, 57–65.

[10] Talalay, P. (1960) Enzymic analysis of steroid hormones. Methods Biochem. Anal. 8, 119–143.

[11] Schneider, C.A., Rasband, W.S., Eliceiri, K.W. (2012) NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675.

[12] Marinelli, R. A., Tietz, P. S., Pham, L. D., Rueckert, L., Agre, P., et al. (1999) Secretin induces the apical insertion of aquaporin-1 water channels in rat cholangiocytes. Am. J. Physiol. 276, 280–286.

[13] Han, X., Fink, M. P., Uchiyama, T., Yang, R., and Delude, R. L. (2004) Increased iNOS activity is essential for hepatic epithelial tight junction dysfunction in endotoxemic mice. Am. J. Physiol. Gastrointest. Liver Physiol. 286, 126–136.

[14] Lee, J. M., Trauner, M., Soroka, C. J., Stieger, B., Meier, P. J., et al. (2000) Expression of the bile salt export pump is maintained after chronic cholestasis in the rat. Gastroenterology 118, 163–172.

[15] Green, R. M., Beier, D., and Gollan, J. L. (1996) Regulation of hepatocyte bile salt transporters by endotoxin and inflammatory cytokines in rodents. Gastroenterology 111, 193–198.

[16] Stieger, B. (2011) The role of the sodium-taurocholate cotransporting polypeptide (NTCP) and of the bile salt export pump (BSEP) in physiology and pathophysiology of bile formation. Handb. Exp. Pharmacol. 201, 205–259.

[17] Mühlfeld, A., Kubitz, R., Dransfeld, O., Häussinger, D., and Wettstein, M. (2003) Taurine supplementation induces multidrug resistance protein 2 and bile salt export pump expression in rats and prevents endotoxin-induced cholestasis. Arch. Biochem. Biophys. 413, 32–40.

[18] Hojo, M., Sano, N., and Takikawa, H. (2003) Effects of lipopolysaccharide on the biliary excretion of bile acids and organic anions in rats. J. Gastroenterol. Hepatol. 18, 815–821.

[19] Paulusma, C. C., de Waart, D. R., Kunne, C., Mok, K. S., and Elferink, R. P. (2009) Activity of the bile salt export pump (ABCB11) is critically dependent on canalicular membrane cholesterol content. J. Biol. Chem. 284, 9947–9954.

[20] Kis, E., Ioja, E., Nagy, T., Szente, L., Heredi-Szabo, K., et al. (2009) Effect of membrane cholesterol on BSEP/ABCB11 activity: species specificity studies for substrates and inhibitors. Drug Metab. Dispos. 37, 1878–1886.

[21] Marinelli, R. A., Lehmann, G. L., Soria, L. R., and Marchissio, M. J. (2011) Hepatocyte aquaporins in bile formation and cholestasis. Front Biosci. 6, 2642–2652.

[22] Boyer, J. L. (2013) Bile formation and secretion. Compr. Physiol. 3, 1035–1078.

[23] Recknagel, P., Gonnert, F. A., Halilbasic, E., Gajda, M., Jbeily, N., et al. (2013) Mechanisms and functional consequences of liver failure substantially differ between endotoxaemia and faecal peritonitis in rats. Liver Int. 33, 283–293.

[24] Jablonski, E. M., and Hughes, F. M. Jr. (2006) The potential role of caveolin-1 in inhibition of aquaporins during the AVD. Biol. Cell 98, 33–42.

[25] Fernandez-Rojo, M. A., and Ramm, G. A. (2016) Caveolin-1 Function in Liver Physiology and Disease. Trends Mol. Med. 22, 889–904.

[26] Moreno, M., Molina, H., Amigo, L., Zanlungo, S., Arrese, M., et al. (2003) Hepatic overexpression of caveolins increases bile salt secretion in mice. Hepatology 38, 1477–1488.

[27] Jiang, X., Ren, Y., Williford, J.M., Li, Z., and Mao, H.Q. (2013) Liver-targeted gene delivery through retrograde intrabiliary infusion. In Nanotechnology for Nucleic Acid Delivery: Methods and Protocols (Ogris, M., Oupicky, D., eds.). pp. 275–284, Humana Press, Totowa, NJ.

[28] Tominaga, K., Kuriyama, S., Yoshiji, H., Deguchi, A., Kita, Y., et al. (2004) Repeated adenoviral administration into the biliary tract can induce repeated expression of the original gene construct in rat livers without immunosuppressive strategies. Gut 53, 1167–1173.