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Aquaporin 3 and 8 are down-regulated in TNBS-induced rat colitis

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ABSTRACT

Aquaporins (AQPs) plays an important role in transcellular water movement, but the AQPs expression profile has not been demonstrated in 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis which closely mimics human Crohn's disease (CD) histopathologically. To solve the problem, 30 female Sprague–Dawley (SD) rats were randomly divided into a model group ($n = 18$), an ethanol control group ($n = 6$) and a normal control group ($n = 6$). On day 1, the rats in the model group received TNBS + 50% ethanol via the rectum, while the ethanol control rats received an equal volume of 50% ethanol and the normal control rats did not receive any treatment. All rats were sacrificed on day 7, and ileum, proximal colon and distal colon specimens were obtained to examine the alteration in AQP3 and AQP8 using real-time polymerase chain reaction, Western blot analysis and immunohistochemistry. As a result, exposure to TNBS + ethanol resulted in a marked decrease in both the mRNA and protein expression of AQP3 and AQP8, with the exception of AQP8 protein which was negative in the distal colon in all three groups. These reductions in AQP3 and AQP8 were accompanied by an increase in intestinal inflammation and injury. The results obtained here implied that both AQP3 and AQP8 may be involved in the pathogenesis of inflammatory bowel disease.

1. Introduction

The gastrointestinal tract is involved in bulk fluid transport. Both the fluid and ion balance are controlled, to a large extent, by the gastrointestinal epithelium. Both absorptive and secretory processes are involved in this fluid movement [1]. Water transport across the gastrointestinal epithelia involves the transcellular and paracellular pathways [1,2]. The transcellular route, which is partially mediated by a family of integral membrane proteins called aquaporins (AQPs), plays an important role in intestinal function and/or fluid homeostasis. To date, 13 AQPs have been identified in mammals. Of these, AQP1, 2, 4 and 5 are selectively permeable to water; AQP3, 7, 9 and 10 are aquaglyceroporins permeable not only to water, but also glycerol, urea and other small solutes. Others exhibit peculiar intracellular localization and functions [3]. AQP1, AQP3–6, AQP8 and AQP9 are expressed in the small intestinal epithelium [4–8] and AQP2–4 and AQP7–9 in the colonic epithelium [4–6,8–12]. In the gastrointestinal tract, incorrect expression and localization of AQPs can result in many disease conditions. An increase in both the mRNA and protein expression of AQP2 in rat distal colon was followed by water deprivation [10]. Knockdown of AQP3 is involved in intestinal barrier integrity impairment [13] and thus increases bacterial translocation in the intestine. AQP4 null mice display decreased colonic transepithelial osmotic water permeability and results in impaired fecal dehydration [9].

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the gastrointestinal tract of unknown etiology [14]. Crohn's disease (CD) and ulcerative colitis (UC) are two major forms of IBD. A complex interplay between environmental triggers, the immune system, genetically susceptible individuals and the intestinal microbiome all lead to the onset of intestinal inflammation and injury [15]. In IBD patients, the normal regulation of fluid and electrolyte flux is disrupted. Hence, alterations in AQPs in the gastrointestinal tract seem to be associated with the pathogenesis of IBD. In a previous study of dextran sodium sulfate (DSS)-induced colitis, which resembles UC histopathologically, mice lacking AQP3 displayed impaired enterocyte proliferation [16]. In addition, the expression of AQP4 and AQP8 significantly decreased after 12–24 h of DSS exposure and remained depressed throughout the treatment period [17]. However, little is known about the alteration in AQPs in 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced rat colitis, another experimental model of IBD which closely mimics human CD histopathologically. Determination of the expression profile of AQPs in TNBS-induced rat colitis will contribute to further research into the pathogenesis of CD and provide a new pathway to study potential treatment. Thus, we used real-time polymerase chain reaction (RT-PCR), Western blot analysis and immunohistochemistry to explore the alteration in AQP3

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and AQP8 at the ileum, proximal colon and distal colon in TNBS-induced rat colitis. Furthermore, the potential role of these aquaporins in the pathogenesis of IBD was clarified.

2. Materials and methods

2.1. Animals

Sprague-Dawley (SD) rats (6–8 weeks old and specific-pathogen free) were obtained from SLAC Laboratory Animal Co. (Shanghai, China) and maintained in the Experimental Animal Centre of Zhongshan Hospital (Fudan University, Shanghai, China). The rats were kept at room temperature (22 ± 2 °C) and 55 ± 5% humidity with free access to regular laboratory chow and water. Rats were allowed to acclimate to these conditions for 7 days before the experiment. All experimental procedures in this study were approved by the Institutional Ethics Committee of Fudan University and were conducted according to guidelines for experimental animals developed by Fudan University.

2.2. Model creation and sample collection

30 female SD rats were randomly divided into a model group ($n = 18$), an ethanol control group (EC group, $n = 6$) and a normal control group (NC group, $n = 6$). On day 1, 24 h-fasted rats were lightly anesthetized with sodium pentobarbital and 400 μl of 2.5 mg/ml TNBS (Sigma, MA, USA) in 50% ethanol was then administered via the rectum through a 3.5F catheter equipped with a 1 ml syringe. The catheter tip was 8–10 cm proximal to the anal verge. The rats were then held in the vertical position with head down for 30 s to ensure distribution of TNBS throughout the entire colon. The ethanol control rats were administered 400 μl 50% ethanol using the same technique. The normal control rats did not receive any treatment. All rats were sacrificed on day 7. The colon and ileum were removed and opened longitudinally. After gentle rinsing with ice-cold phosphate buffered saline (PBS), ileum, proximal colon and distal colon specimens were obtained. One half of the tissue was fixed in 4% paraformaldehyde, embedded in paraffin wax and sectioned (4 μm thick). The other half was frozen in liquid nitrogen and used for RNA extraction and Western blot analysis.

2.3. Assessment of colitis

The Disease Activity Index (DAI) scores, Colon Macroscopic Damage Index (CMDI) and the Histopathological Score (HPS) were calculated to assess the severity of induced colitis. Stool formation, rectal bleeding and body weight were monitored every day to calculate the DAI scores in accordance with the method described by Murthy et al. [18] (Table 1 [19], see Supplementary data). The CMDI was assessed using the scoring system of Wallace and Keenan [20], which takes into account the area of inflammation as well as the presence or absence of ulcers (Table 2 [21], see supplementary data). The tissue sections were stained with hematoxylin and eosin and the HPS was determined according to previously described criteria [22] (Table 3 [22], see Supplementary data).

2.4. RNA extraction and real-time polymerase chain reaction

Total RNA was isolated from the tissues using RNAiso Plus (Takara, Tokyo, Japan), and was reverse-transcribed using PrimeScript® RT Master Mix (Takara, Tokyo, Japan) on the GeneAmp® PCR system 9700 (Applied Biosystems, CA, USA). Obtained cDNAs were amplified using SYBR® Premix Ex Taq™ (Takara, Tokyo, Japan) on the Master cycler ep realplex^4 PCR system (Eppendorf, Hamburg, Germany) with the two-stage program as follows: 1 min at 95 °C, and then 40 cycles of 5 s at 95 °C and 30 s at 60 °C. Expression levels of AQPs mRNA were normalized to that of β-actin as the internal standard using the comparative method. The following primer pairs (Sangon Biotech, Shanghai, China) were used:

β-actin F: CACCCGCGAGTACAACCTTC
β-actin R: CCCATACCCACCATCACACC
AQP3 F: CCCCTTGTGATGCCTCTC
AQP3 R: CCCTAGCTGGCAGAGTTC
AQP8 F: GCCGATGTGTAGTATGGACCTA
AQP8 R: ACCCAATGAAGATGAAGAGAGC

2.5. Western blot analysis

Total membrane protein was extracted from the homogenized tissue samples, and the protein concentration was measured with a BCA protein determination kit (Beyotime, Shanghai, China). Forty micrograms of total membrane protein from each sample was loaded in 10% sodium dodecyl sulfate–polyacrylamide electrophoresis gel for separation. The separated proteins were transferred to a polyvinylidene difluoride membrane (Millipore, MA, USA). After incubation in 5% skim milk for 2 h at room temperature to block the non-specific binding, the resulting membrane was reacted for 16 h at 4 °C with rabbit anti-rat AQP3 and AQP8 antibodies (1:400, Alomone Laboratories, Jerusalem, Israel). The membrane was then washed with TBST (20 mM Tris–HCl, 137 mM NaCl, and 0.1% Tween-20, pH 7.6) for 10 min × 3 and incubated with anti-rabbit IgG-HRP (horse radish peroxidase) antibodies (1:5000, Santa Cruz, CA, USA). Finally, the membrane was washed with TBST for 10 min × 3 and reacted with the BeyoECL Plus detection reagent (Beyotime, Shanghai, China) and visualized with the Fluor-Chem®FC2 Imaging System (Alpha Innotech, CA, USA). The antibody against β-actin (Santa Cruz, CA, USA) was used as the loading control. For semiquantitative analysis, the grayscales of both AQPs and β-actin bands were measured with ImageJ software, and the ratio of AQPs to β-actin was calculated.

2.6. Immunohistochemistry

Immunohistochemistry was carried out on paraffin sections. These sections were cut, mounted on poly-L-lysine-coated glass slides, deparaffinized, and then rehydrated. To retrieve the antigen, slides were placed in EDTA solution (pH 9.0, Maixin, Fuzhou, China) and heated in a microwave oven for 20 min at 100 °C. Endogenous peroxidase activity was then blocked by 0.03% H_2O_2 for 15 min at 37 °C. The non-specific binding of antibodies was blocked by incubation with PBS containing 5% bovine serum albumin (BSA) for 30 min at room temperature. The samples were then incubated with primary antibodies (1:100) diluted in PBS containing 3% BSA overnight at 4 °C, followed by HRP-coupled secondary antibodies (Gene Tech, Shanghai, China). Diaminobenzidine (DAB) reactions were performed using a GTvision™I Immunohistochemical detection kit (Gene Tech, Shanghai, China), after which the sections were stained with hematoxylin, washed with running water, dehydrated in a graded series of ethanol and xylene, and then mounted with a coverslip. The immunoreactivity of AQP3 and AQP8 was observed under a BX51 microscope (Olympus, Tokyo, Japan) and photographed using a DP71 camera (Olympus, Tokyo, Japan).

2.7. Statistical analysis

SPSS 20.0 was used for data analysis. Measurement data were expressed as mean ± standard deviation (SD). Significant differences between the NC, EC and model group were analyzed

by the Student's *t* test or Mann–Whitney U test when appropriate. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Assessment of colitis

Symptomatic parameters in all rats were observed and recorded every day during the experiment. The rats in the model group displayed significant weight loss and pasty to liquid gross bloody stools from day 1 to 7, while the NC rats gained body weight normally with normal stool consistency throughout the experiment and the EC rats suffered from mild diarrhea on day 1–2. The DAI scores were higher in the model group than in the other two groups (Fig. 1D). The CMDI was used to evaluate the gross appearance of the colon and assess the severity of intestinal injury. The colons from rats in the model group were adherent to other tissues and organs with marked hyperemia, hemorrhage, inflammation, necrosis, ulcers and bowel wall thickening, whereas the colons from the rats in the NC and EC groups showed no or only slight inflammation (Fig. 1A). To assess the severity of colonic inflammation histopathologically, HPS was calculated by two different pathologists. Ulceration, massive inflammatory cell infiltration, colon structure disorganization, goblet cell depletion, submucosa edema and extensive fibrosis were found throughout the colons in the model group, while no or very little mucosal lesions were observed in the colons from the other two groups (Fig. 1B). The CMDI and HPS in the model group were significantly higher than those in the NC and EC group, but there was no difference between the NC and EC group ($P < 0.05$, Fig. 1C).

3.2. Real-time polymerase chain reaction

We next determined the mRNA expression of AQP3 and AQP8 in the ileum, proximal colon and distal colon in the NC, EC and model groups. In all three sections, the mRNA expression of AQP3 (Fig. 2A) and AQP8 (Fig. 2B) was significantly lower ($P < 0.05$) in the model group than in the other two groups, and there was no significant difference between the NC and EC rats.

3.3. Western blot analysis

We determined the expression of AQP3 and AQP8 protein in the NC, EC and model groups by Western blot experiments. Quantification of AQPs was normalized against that of β-actin in each sample. Representative Western blots of the three groups are shown in Fig. 3. In the proximal colon, two AQP3 protein bands were detected. One appeared at 27 kDa and represented the deglycosylated form of AQP3, whereas the other appeared at 30–40 kDa and represented the glycosylated form of AQP3 (Fig. 3A). However, only the glycosylated band was observed in the ileum and distal colon (Fig. 3A). In contrast, only one AQP8 protein band at approximately

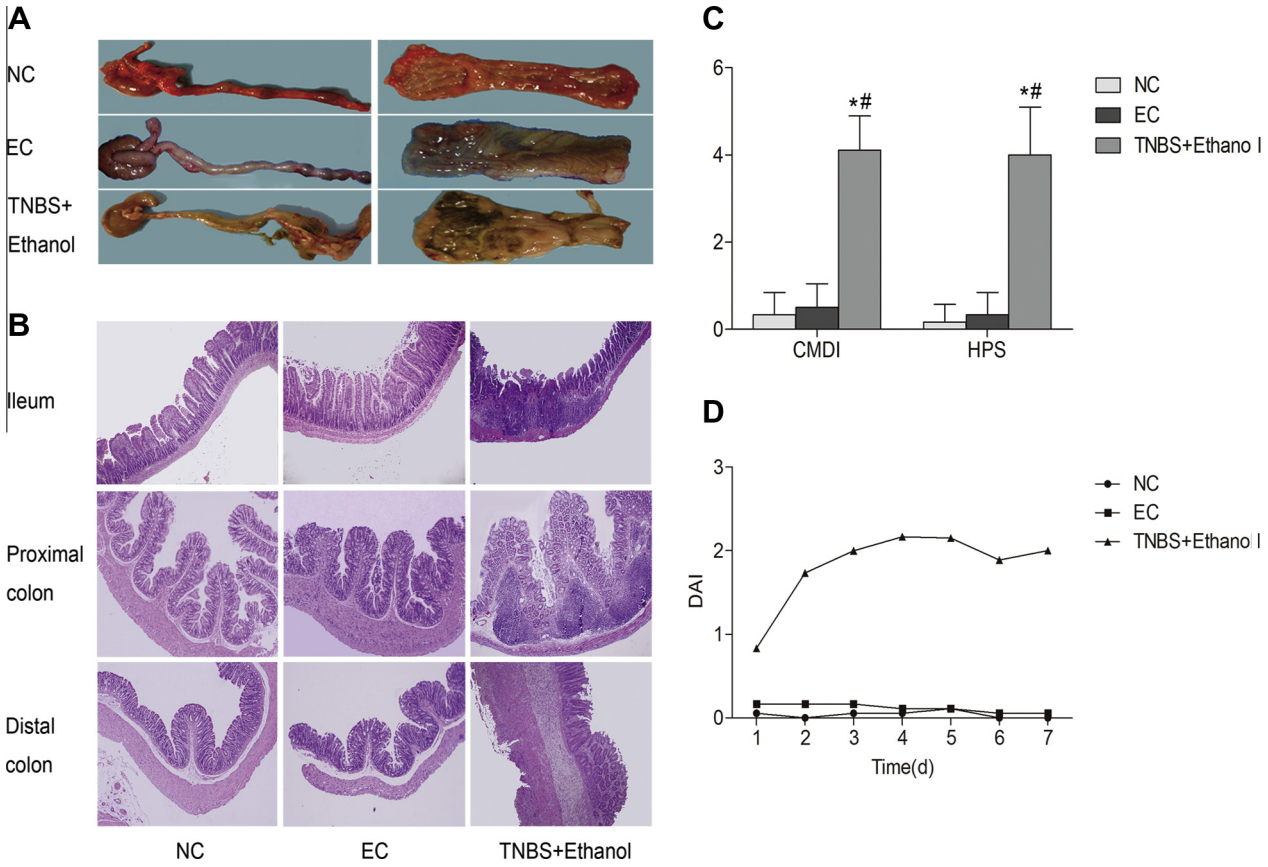

Fig. 1. Assessment of colitis using DAI, CMDI and HPS. (A) DAI scores were higher in the model group than in the other two groups during the experiment. (B) Representative gross appearance of colons from the three groups. The colons from the model group showed marked hyperemia, hemorrhage, inflammation, necrosis, ulcers and bowel wall thickening, while the colons from the rats in the NC and EC groups showed no or only slight inflammation. (C) Representative histological findings (40×) of the ileum, proximal colon and distal colon. Ulcerations, massive transmural infiltration of inflammatory cells, thickening of the colon wall, goblet cell depletion and extensive fibrosis were found throughout the colons from the model group, while no or very little mucosal lesions were observed in the colons from the other two groups. (D) CMDI and HPS were markedly higher in the model group than in the other two groups. Data are shown as mean ± standard deviation (SD) ($n = 9$ in the model group; $n = 6$ in the NC and EC group, respectively). $^{*}P < 0.05$ compared with the NC group. $^{\#}P < 0.05$ compared with the EC group.

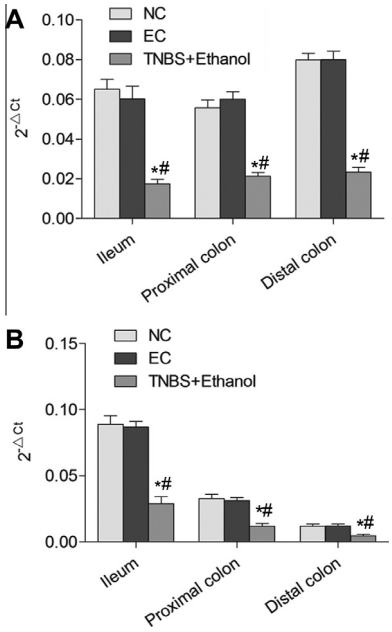

Fig. 2. Expression of AQP3 and AQP8 mRNA in the NC, EC and model group. The results were expressed as $2^{-\triangle Ct}$, $\triangle Ct = Ct_{AQP} - Ct_{\beta\text{-actin}}$. (A) AQP3 mRNA expression was lower in the model group than in the other two groups. (B) AQP8 mRNA expression was lower in the model group than in the other two groups. Data are shown as mean ± standard deviation (SD) ($n = 9$ in the model group; $n = 6$ in the NC and EC group, respectively). $^{*}P < 0.05$ compared with the NC group. $^{\#}P < 0.05$ compared with the EC group.

32 kDa was observed (Fig. 3A). Compared with the NC and EC groups, AQP3 protein expression was noticeably lower in the model group in all three examined sections ($P < 0.05$, Fig. 3B). The expression of AQP8 was lower in the ileum and proximal colon in the model group than in the other two groups ($P < 0.05$, Fig. 3C). There was no significant difference between the NC and EC groups ($P > 0.05$). However, at the distal colon in all three groups, the AQP8 protein expression was too faint to be detected.

3.4. Immunohistochemistry

Localization of AQP3 and AQP8 in the ileum, proximal colon and distal colon in all three groups was established by immunohistochemistry. Typical photomicrographs are shown in Fig. 4. In the NC and EC groups, AQP3 staining was confined to the basolateral membrane of absorptive epithelial cells which faced the lumen directly and at the neck of crypts, and staining intensity decreased from the tip towards the base of the villus. In contrast, AQP8 immunoreactivity was present in columnar epithelial cells in the ileum and proximal colon, where the staining was mainly intracellular and seemed more intense in the subapical compartment. However, there was no apparent AQP8 reactivity observed in the crypts. Furthermore, TNBS-induced intestinal mucosal injury was accompanied by a profound change in AQP3 and AQP8 immunoreactivity. In the intestinal mucosa from the model group, total AQP3 and AQP8 immunostaining were decreased obviously and became patchy and discontinuous, however, there was no shift in their intracellular localization. Consistent with the Western blot results,

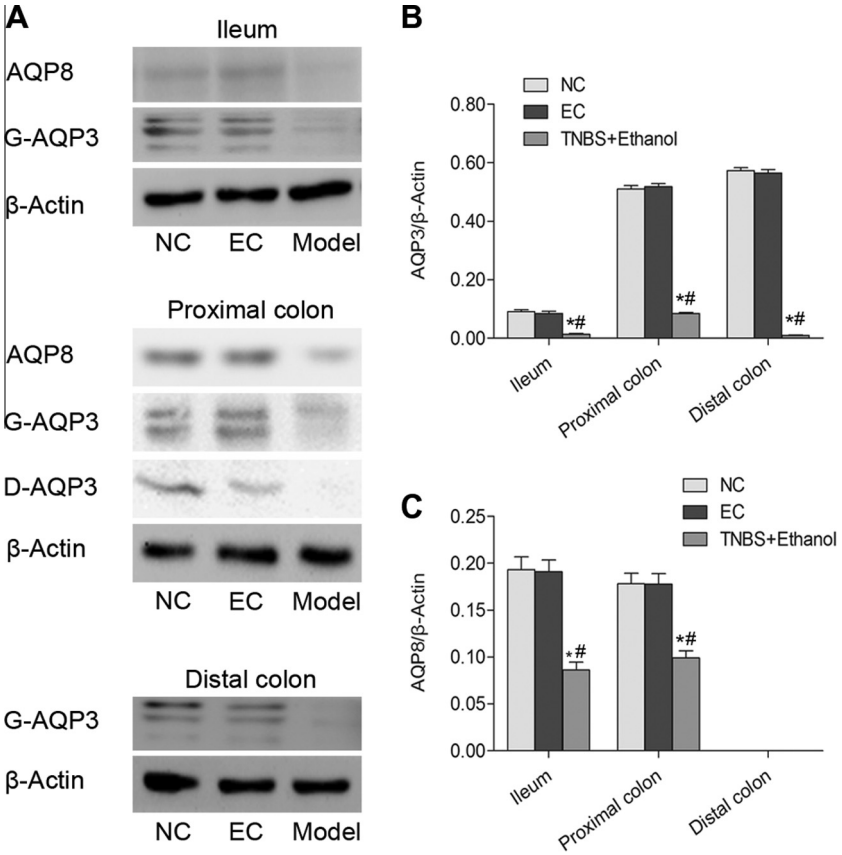

Fig. 3. Expression of AQP3 and AQP8 protein in the NC, EC and model group by Western blot. (A) The typical image of AQP3 and AQP8 Western blot in the NC, EC and model group. (B and C) The expression of AQP3 and AQP8 protein was markedly lower in the model group than in the other two groups. Data are shown as mean ± standard deviation (SD) ($n = 9$ in the model group; $n = 6$ in the NC and EC group, respectively). $^{*}P < 0.05$ compared with the NC group. $P < 0.05$ compared with the EC group. G-AQP3 represents the glycosylated form; D-AQP3 represents the deglycosylated form.

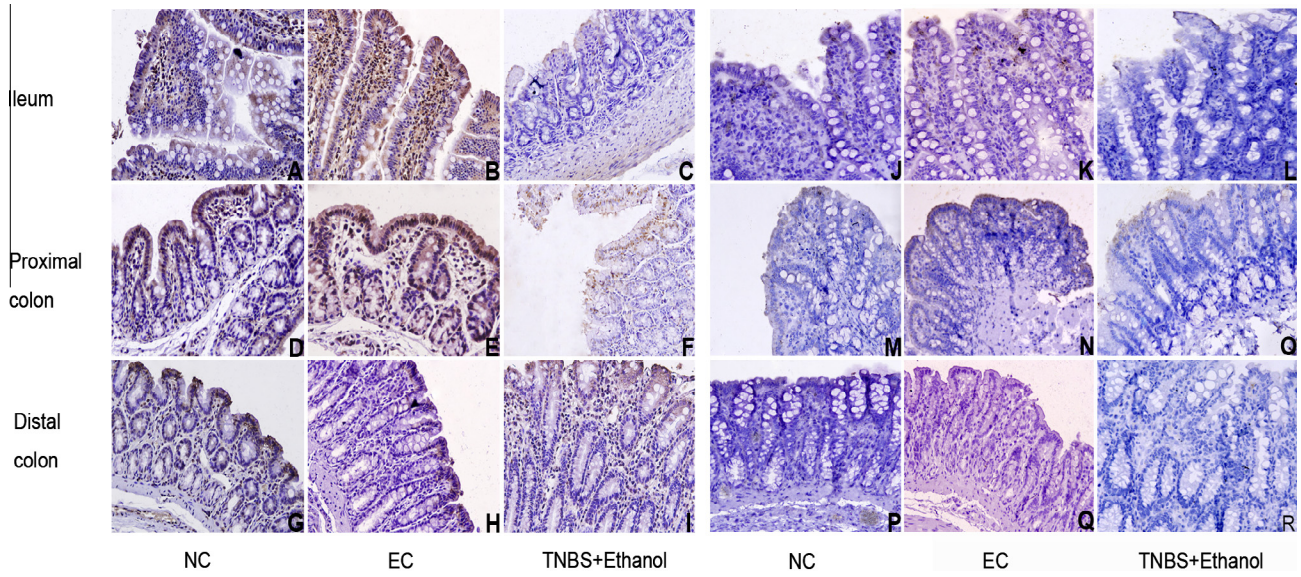

Fig. 4. A-I indicate typical photographs (200×) of AQP3 immunostaining in the ileum, proximal colon and distal colon in the three groups, while J-R represent that of AQP8. In the NC and EC groups, AQP3 staining was confined to the basolateral membrane of absorptive epithelial cells which faced the lumen directly and at the neck of crypts, and staining intensity decreased from the tip towards the base of the villus. AQP8 immunoreactivity was present in columnar epithelial cells in the ileum and proximal colon, where the staining was mainly intracellular and seemed more intense in the subapical compartment. The immunoreactivity of AQP3 and AQP8 was markedly decreased in the model group compared with the other two groups in all the examined sections (immunostaining of AQP8 was not detected in the distal colon in all three groups). However, there was no shift in its intracellular localization.

AQP8 staining was too weak to be detected in the distal colon in all three groups.

4. Discussion

Inflammatory bowel diseases (IBDs) are chronic intestinal disorders of unknown etiology [14]. It is generally accepted that the aberrant immune response against the gut microflora in genetically susceptible individuals [23] leads to the infiltration of inflammatory cells and intestinal mucosal injury. One of the main symptoms of IBD is varying degrees of diarrhea. Therefore, the disturbance of intestinal fluid flux, which is partially mediated by the aquaporins (AQPs) expressed in intestinal epithelial cells, may be associated with the pathogenesis of IBD. Evidence from a previous study [17] has highlighted the alteration in AQPs expression in DSS-induced mouse colitis compared with the control groups and suggested that DSS-induced colonic injury is associated with a downregulation in AQPs expression. However, the AQPs expression profile in TNBS-induced colitis has not been demonstrated.

Aquaporins play an important role in intestinal function and/or fluid homeostasis. In the present study, AQP3 was demonstrated in the basolateral membrane of mucosal epithelial cells in normal rat intestine, whereas AQP8 staining was intracellular and seemed more intense in the subapical compartment of the epithelial cytoplasm. The above findings suggest a role for AQP3 and AQP8 in transcellular water movement and intracellular osmoregulation. Consistent with a previous study [12], AQP8 expression was too weak to be detected in the rat distal colon by Western blot and immunohistochemistry. More studies are needed to verify the expression of AQP8 in the distal colon. We examined the alteration in AQP3 and AQP8 expression in TNBS-induced rat colitis and its associated control groups. Exposure to TNBS + ethanol resulted in a marked decrease in both the mRNA and protein expression of AQP3 and AQP8, with the exception of AQP8 protein which was negative in the distal colon in all three groups. These reductions in the expression of AQP3 and AQP8 were accompanied by aggravated mucosal injury and increased DAI scores. In the intestinal mucosa from the model group, total AQP3 and AQP8 immunostaining were decreased obviously and became patchy and discontinuous, however, there was no shift in their intracellular localization. The findings obtained here indicate that intestinal inflammation and injury may be associated with altered expression of AQPs.

The TNBS-induced model is a well established model of rat colonic inflammation and ulceration, which resembles human CD histopathologically. Thus, our current study provides a new pathway to study the pathogenesis of CD and is of great significance. An imbalance in regulatory cytokines is consistently found in this model, especially excessive production of Th1 cytokines such as TNF-α [24]. TNF-α is a pleiotropic cytokine that elicits a wide spectrum of physiologic and pathogenic events such as proliferation, differentiation, cell death, modulation of gene transcription and inflammation [25]. It has been reported that TNF-α is increased in the serum and feces of CD patients [26] and in TNBS-induced colitis [27]. In addition, administration of the anti-TNF-α antibody, infliximab, improves symptomatic parameters and resolves intestinal inflammation. A similar result was observed in a study of Sjogren's syndrome, an autoimmune disease, which is characterized by decreased secretion in lacrimal and salivary glands. Normally, AQP5 is expressed in the apical membrane of glandular acinar cells and is involved in the secretion of tears and saliva. Patients with Sjogren's syndrome display a marked reduction in apical AQP5 staining and an obvious increase in basolateral AQP5 labeling in both lacrimal and salivary gland tissue compared with control individuals [28]. In addition, in vitro experiments have confirmed that TNF-α decreases AQP5 protein and mRNA expression through a mechanism involving the p55 TNF-α receptor and NF-κB [29]. Conversely, administration of infliximab attenuates the clinical symptoms of Sjogren's syndrome and leads to a redistribution of AQP5 from the basolateral membrane back to the apical aspect of glandular acinar cells [30]. An AQP5-trafficking defect may be pivotal in the pathogenesis of Sjogren's syndrome. Similarly, TNBS-induced rat colitis also displays excessive production of Th1 cytokines, therefore, inflammatory mediators induced by the hapten (TNBS) may be involved in the regulation of AQPs in the

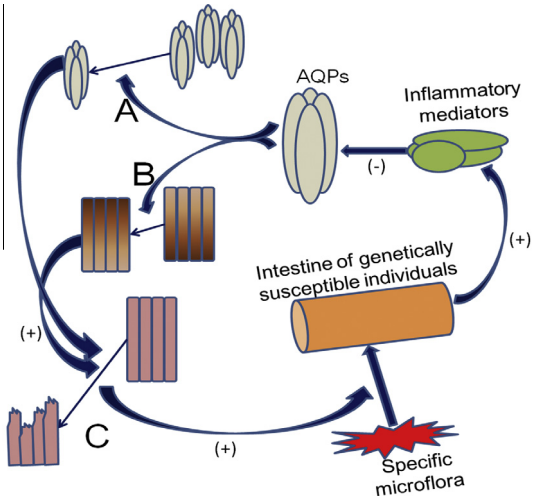

Fig. 5. The speculated vicious circle of inflammatory mediators-AQPs-inflammation. The aberrant immune response in genetically susceptible individuals against specific microflora leads to excessive production of inflammatory mediators followed by a decreased expression and an incorrect distribution of the AQPs, which results in a disturbance in fluid flux and a subsequent weakening of the mucosal defense. Additionally, decreased expression of AQP3 may impair enterocyte proliferation and cause a restoration defect in the intestinal epithelium. All of these factors increase bacterial translocation and exacerbate the intestinal inflammation with more inflammatory mediators induced subsequently. (A) The decreased expression of AQPs. (B) The incorrect distribution of AQPs. (C) The weakening of the mucosal defense and impaired enterocyte restoration.

intestinal mucosa. It could be speculated that excessive production of these mediators leads to trafficking defects and folding errors in the AQPs followed by a marked decrease and incorrect distribution of the proteins, which results in a disturbance in fluid flux and a subsequent weakening of the mucosal defense. Moreover, decreased expression of AQP3 may impair enterocyte proliferation and lead to a restoration defect in the intestinal epithelium. All of these factors worsen intestinal inflammation and injury. In a word, the vicious circle of inflammatory mediators-AQPs-inflammation (Fig. 5) may play an important role in the development of intestinal inflammation of IBDs. Further in vitro experiments on the effect of inflammatory mediators in the regulation of AQPs and clinical tests to break the vicious circle of inflammatory mediators-AQPs-inflammation are needed to determine the correlations between AQPs and IBDs.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.11.067.

References

[1] U. Laforenza, Water channel proteins in the gastrointestinal tract, Mol. Aspects Med. 33 (2012) 642–650.
[2] J. Fischbarg, Fluid transport across leaky epithelia: central role of the tight junction and supporting role of aquaporins, Physiol. Rev. 90 (2010) 1271–1290.
[3] A. Rojek, J. Praetorius, J. Frokiaer, S. Nielsen, R.A. Fenton, A current view of the mammalian aquaglyceroporins, Annu. Rev. Physiol. 70 (2008) 301–327.
[4] A.I. Masyuk, R.A. Marinelli, N.F. LaRusso, Water transport by epithelia of the digestive tract, Gastroenterology 122 (2002) 545–562.
[5] C. Silberstein, A. Kierbel, G. Amodeo, E. Zotta, F. Bigi, et al., Functional characterization and localization of AQP3 in the human colon, Braz. J. Med. Biol. Res. 32 (1999) 1303–1313.
[6] M.L. Elkjaer, L.N. Nejsum, V. Gresz, T.H. Kwon, U.B. Jensen, et al., Immunolocalization of aquaporin-8 in rat kidney, gastrointestinal tract, testis, and airways, Am. J. Physiol. Renal Physiol. 281 (2001) F1047–F1057.
[7] M.N. Parvin, K. Tsumura, T. Akamatsu, N. Kanamori, K. Hosoi, Expression and localization of AQP5 in the stomach and duodenum of the rat, Biochim. Biophys. Acta 1542 (2002) 116–124.
[8] S. Okada, T. Misaka, I. Matsumoto, H. Watanabe, K. Abe, Aquaporin-9 is expressed in a mucus-secreting goblet cell subset in the small intestine, FEBS Lett. 540 (2003) 157–162.
[9] K.S. Wang, T. Ma, F. Filiz, A.S. Verkman, J.A. Bastidas, Colon water transport in transgenic mice lacking aquaporin-4 water channels, Am. J. Physiol. Gastrointest. Liver Physiol. 279 (2000) G463–G470.
[10] P. Gallardo, L.P. Cid, C.P. Vio, F.V. Sepulveda, Aquaporin-2, a regulated water channel, is expressed in apical membranes of rat distal colon epithelium, Am. J. Physiol. Gastrointest. Liver Physiol. 281 (2001) G856–G863.
[11] T. Ma, A.S. Verkman, Aquaporin water channels in gastrointestinal physiology, J. Physiol. 517 (1999) 317–326.
[12] G. Calamita, A. Mazzone, A. Bizzoca, A. Cavalier, G. Cassano, et al., Expression and immunolocalization of the aquaporin-8 water channel in rat gastrointestinal tract, Eur. J. Cell Biol. 80 (2001) 711–719.
[13] W. Zhang, Y. Xu, Z. Chen, Z. Xu, H. Xu, Knockdown of aquaporin 3 is involved in intestinal barrier integrity impairment, FEBS Lett. 585 (2011) 3113–3119.
[14] D.K. Podolsky, Inflammatory bowel disease, N. Engl. J. Med. 325 (1991) 928–937.
[15] A. Spor, O. Koren, R. Ley, Unravelling the effects of the environment and host genotype on the gut microbiome, Nat. Rev. Microbiol. 9 (2011) 279–290.
[16] J.R. Thiagarajah, D. Zhao, A.S. Verkman, Impaired enterocyte proliferation in aquaporin-3 deficiency in mouse models of colitis, Gut 56 (2007) 1529–1535.
[17] J.A. Hardin, L.E. Wallace, J.F. Wong, E.V. O'Loughlin, S.J. Urbanski, et al., Aquaporin expression is downregulated in a murine model of colitis and in patients with ulcerative colitis, Crohn's disease and infectious colitis, Cell Tissue Res. 318 (2004) 313–323.
[18] S.N. Murthy, H.S. Cooper, H. Shim, R.S. Shah, S.A. Ibrahim, et al., Treatment of dextran sulfate sodium-induced murine colitis by intracolonic cyclosporin, Dig. Dis. Sci. 38 (1993) 1722–1734.
[19] Y. Zhang, R. Zhou, F. Zhou, H. Cheng, B. Xia, Total glucosides of peony attenuates 2,4,6-trinitrobenzene sulfonic acid/ethanol-induced colitis in rats through adjustment of Th1/Th2 cytokines polarization Cell Biochem. Biophys. Unpublished results, http://dx.doi.org/10.1007/s12013-013-9696-3.
[20] J.L. Wallace, C.M. Keenan, An orally active inhibitor of leukotriene synthesis accelerates healing in a rat model of colitis, Am. J. Physiol. 258 (1990) G527–G534.
[21] J.L. Wallace, W.K. MacNaughton, G.P. Morris, P.L. Beck, Inhibition of leukotriene synthesis markedly accelerates healing in a rat model of inflammatory bowel disease, Gastroenterology 96 (1989) 29–36.
[22] G. Lahat, D. Halperin, E. Barazovsky, I. Shalit, M. Rabau, et al., Immunomodulatory effects of ciprofloxacin in TNBS-induced colitis in mice, Inflamm. Bowel Dis. 13 (2007) 557–565.
[23] L. Mayer, Evolving paradigms in the pathogenesis of IBD, J. Gastroenterol. 45 (2010) 9–16.
[24] I.J. Fuss, T. Marth, M.F. Neurath, G.R. Pearlstein, A. Jain, et al., Anti-interleukin 12 treatment regulates apoptosis of Th1 T cells in experimental colitis in mice, Gastroenterology 117 (1999) 1078–1088.
[25] T. Qidwai, F. Khan, Tumour necrosis factor gene polymorphism and disease prevalence, Scand. J. Immunol. 74 (2011) 522–547.
[26] B. Yin, X. Hu, J. Wang, H. Liang, X. Li, et al., Blocking TNF-alpha by combination of TNF-alpha- and TNFR-binding cyclic peptide ameliorates the severity of TNBS-induced colitis in rats, Eur. J. Pharmacol. 656 (2011) 119–124.
[27] S.M. Da, S. Sanchez-Fidalgo, E. Talero, A. Cardeno, S.M. Da, et al., Anti-inflammatory intestinal activity of Abarema cochliacarpos (Gomes) Barneby & Grimes in TNBS colitis model, J. Ethnopharmacol. 128 (2010) 467–475.
[28] S. Steinfeld, E. Cogan, L.S. King, P. Agre, R. Kiss, et al., Abnormal distribution of aquaporin-5 water channel protein in salivary glands from Sjogren's syndrome patients, Lab. Invest. 81 (2001) 143–148.
[29] J.E. Towne, C.M. Krane, C.J. Bachurski, A.G. Menon, Tumor necrosis factor-alpha inhibits aquaporin 5 expression in mouse lung epithelial cells, J. Biol. Chem. 276 (2001) 18657–18664.
[30] S.D. Steinfeld, T. Appelboom, C. Delporte, Treatment with infliximab restores normal aquaporin 5 distribution in minor salivary glands of patients with Sjogren's syndrome, Arthritis Rheum. 46 (2002) 2249–2251.