Microbial Pathogenesis 107 (2017) 430–435

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Aquaporin-3 is down-regulated in jejunum villi epithelial cells during enterotoxigenic *Escherichia coli*-induced diarrhea in mice

Di Zhang [a, *], Kaiqi Zhang [a], Weiheng Su [b], Yuan Zhao [a], Xin Ma [a], Gong Qian [a], Guijuan Qu [a], Zhihua Pei [a], Shuming Liu [a], Hongxia Ma [a, **]

[a] College of Animal Science and Technology, Jilin Agricultural University, Changchun, 130118, PR China
[b] National Engineering Laboratory for AIDS Vaccine, School of Life Science, Jilin University, Changchun, 130012, PR China

ARTICLE INFO

Article history:
Received 12 April 2017
Received in revised form
21 April 2017
Accepted 21 April 2017
Available online 23 April 2017

Keywords:
Aquaporin-3
Enterotoxigenic *Escherichia coli*
Diarrhea
Small intestine

ABSTRACT

Enterotoxigenic *Escherichia coli* (ETEC)-induced diarrhea is a complex pathological process, involving ion channel regulation and water efflux. While the mechanism underlying water efflux in ETEC-induced diarrhea is still largely unknown, aquaporins (AQPs) play important roles in transcellular water movement, but their expression profile has not been demonstrated in the murine small intestine. We identified AQP3 expression in the jejunum, but not the duodenum or ileum, using reverse transcription PCR and western blotting. Immunohistochemistry showed that AQP3 localized to the jejunum villi epithelial cells. Using an ETEC-induced murine diarrhea model, we demonstrated that both AQP3 mRNA expression and protein concentration in the jejunum were gradually but significantly decreased over 7 d compared with controls. These results suggested impaired water influx also plays an important role in ETEC-induced diarrhea.

1. Introduction

The gastrointestinal (GI) tract absorbs 9–10 L of fluid per day, while excreting 100 mL of water through feces. Fluid absorption takes place predominantly in the small intestine and to a less extent in the colon [1]. This fast, *trans*-epithelial fluid transport occurs using either transcellular pathways, paracellular pathways or both [2]. The regulation of *trans*-epithelial fluid transport in the GI tract is based on ion and water transport by aquaporin (AQP) [3]. Currently, several AQPs have been identified in the human small intestine, but details of their roles are yet to be addressed. AQP1, 3, 7, 10 and 11 transcripts are abundantly expressed in human duodenal samples [4]; however, conflicting results are often reported in studies investigating the tissue and cellular localization of AQP proteins. More recently, the AQP3 transcript was found to be abundantly expressed with the protein localizing to the epithelial cells of the upper villi [5].

Worldwide, enterotoxigenic *Escherichia coli* (ETEC) is the leading cause of bacterial diarrhea in humans and farm animals [6]. In children, ETEC is the most frequently isolated enteropathogen, accounting for approximately 200 million diarrhea episodes and 38,000 deaths annually [7]. ETEC infections are classically associated with acute watery diarrhea, while fewer patients have chronic diarrhea lasting a week or more [8]. ETEC enterotoxins are known as heat-labile (LT) and heat-stable (ST) enterotoxins, and are structurally and functionally similar to cholera toxin, which can stimulate intracellular cAMP synthesis. Elevated cAMP levels activate PKA, which subsequently phosphorylates and activates cystic fibrosis transmembrane regulator (CFTR). CFTR activation provokes Cl^- and HCO_3^- secretion [9], resulting in an osmotically-driven increased permeation of water and electrolytes. This leads to fluid accumulation in the intestinal lumen.

Considering that only a small fraction of the 6 m of the jejunum is affected by the toxins during infection, water loss due to osmosis should be well compensated for by water reabsorption in the unaffected jejunum. Could it be possible that water reabsorption is also defective during ETEC infection? Ikarashi et al. reported that diarrhea could be induced by inhibiting AQP3 in the colon [10]. Consistently, Hamabata et al. reported that when human AQP3 cDNA was injected into a *Xenopus* oocyte expression system, its expression was significantly decreased after treating the oocytes with enterotoxins [11]. Considering the small contribution of water

* Corresponding author.
** Corresponding author.
E-mail addresses: zhangdi@jlau.edu.cn (D. Zhang), hongxia0731001@163.com (H. Ma).

http://dx.doi.org/10.1016/j.micpath.2017.04.031
0882-4010/

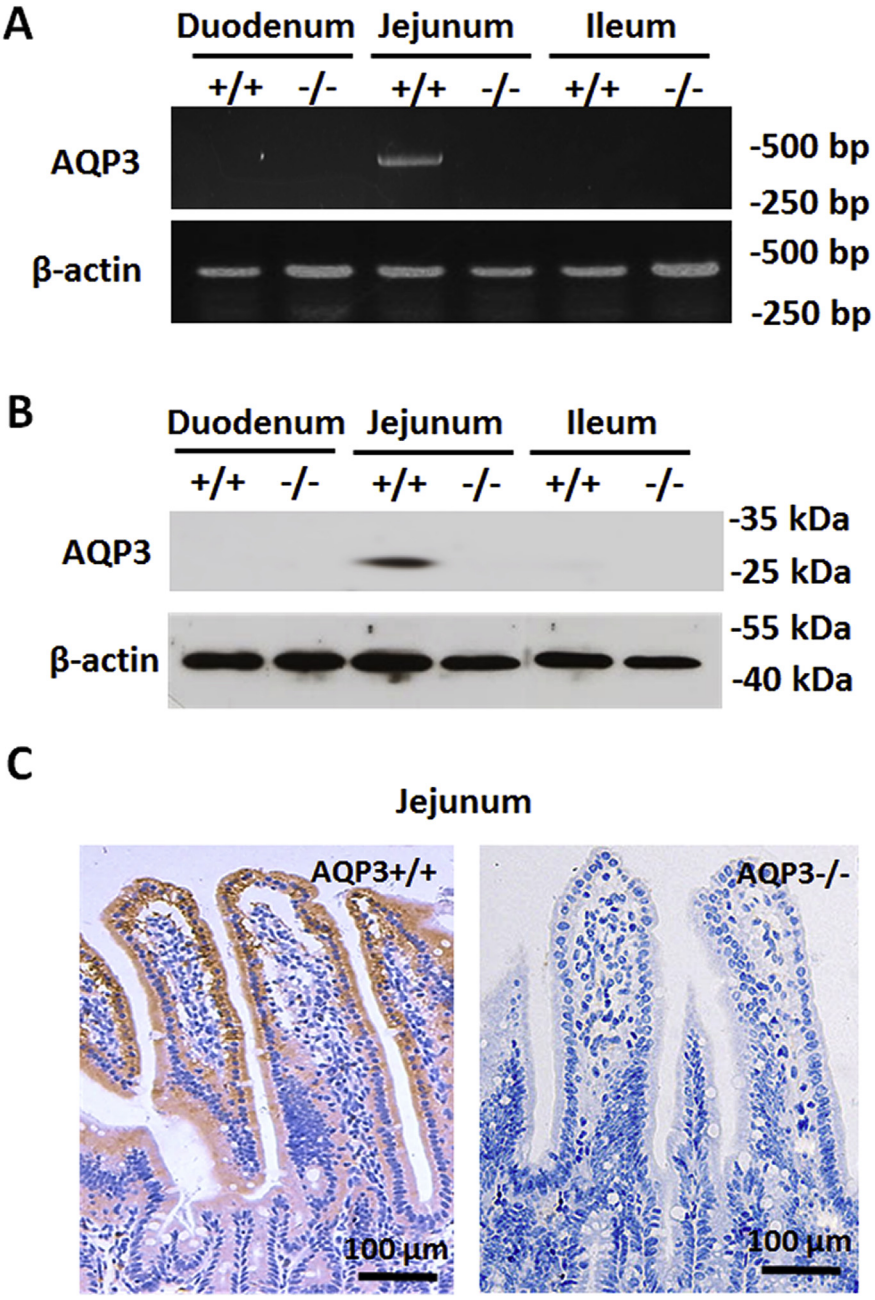

Fig. 1. AQP3 expression and localization in the murine small intestine. (A) RT-PCR detection of AQP3 mRNA from the duodenum, jejunum and ileum. (B) Western blot detection of AQP3 from the duodenum, jejunum and ileum. (C) Immunohistochemical localization of AQP3 in the jejunum epithelial cell villi. Scale bars = 100 μm.

transport in the colon, the role of AQP3 could be more significant in the small intestine during diarrhea. Collectively, these findings suggested a potential connection between ETEC-induced diarrhea and AQP3-induced water transport in the small intestine.

In this study, the expression and localization of AQP3 were investigated in different regions of the small intestine. Additionally, AQP3 expression was quantified in a time-dependent manner in an ETEC-induced murine diarrhea model.

2. Materials and methods

2.1. Animals

The AQP3-deficient ($AQP3^{-/-}$) mice in the C57BL/6 background used in this study were described *else*where [12]. Age-matched $AQP3^{-/-}$ and wild-type mice were used in all experiments. Mice were maintained in a SPF (Specific-pathogen-free) room kept at a constant temperature (22 ± 2 °C) and humidity (55 ± 5%) on a 12h light/dark cycle. All experiments were approved by the Animal Care Committee at Jilin Agricultural University.

2.2. Reverse transcription-PCR and quantitative PCR (qPCR)

Total RNA was extracted from small intestines using the RNeasy micro kit (QIAGEN, Hilden, Germany). cDNA was generated from 2 μg of total RNA using the SuperScript First-strand Synthesis System (Invitrogen/Thermo Fisher Scientific, Waltham, MA, USA). For PCR, the yielded cDNA was used as a template using primers