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Original Article

Expression of AQP3 gene in chronic atrophic and chronic superficial gastritis patients

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Abstract

BACKGROUND: Most studies about aquaporin 3 (AQP3) in the gastrointestinal tract were carried out on both in vivo and in vitro. The role of AQP3-mediated water transport in human gastrointestinal tract is still unclear. Our aim in this study was to explore the expression of AQP3 gene in chronic atrophic gastritis (CAG) and chronic superficial gastritis (CSG) patients and to determine its possible function in the development of gastritis.

METHODS: Twenty-two outpatients diagnosed as CSG and 12 outpatients diagnosed as CAG were selected randomly. Ten cases of healthy individuals were selected as normal control group. In all cases, AQP3 gene expression of gastric mucosa was detected by fluorescence quantitative polymerase chain reaction (FQ-PCR).

RESULTS: The AQP3 gene expression was significantly higher in gastric mucosa of CSG and healthy individuals than that in CAG ($P<0.01$). However, there was no significant difference in the AQP3 gene expression between helicobacter pylori positive patients and helicobacter pylori negative patients ($P>0.05$).

CONCLUSIONS: AQP3 expression might play certain role in the occurrence and development of gastritis.

KEY WORDS: Aquaporin 3, chronic superficial gastritis, chronic atrophic gastritis.

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Chronic superficial gastritis (CSG) is the most prevalent gastric disease, which may progress to chronic atrophic gastritis (CAG) [1-3]. Fluid movements of gastrointestinal tract mucosa are thought to be obstructive in some gastrointestinal diseases, which may potentially lead to chronic inflammation or dysfunction of the gastrointestinal tract [4-6]. In the past several years, our concepts of how water moves across epithelial barriers has dramatically changed, principally because of the discovery of aquaporins (AQPs), a family of water-channel proteins [7-9]. It has recently become clear that the epithelial cells of the digestive tract are involved in the rapid, bidirectional movement of large volumes of water; they also express AQPs [4,10,11]. As an important water channel protein that permits to drive

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osmotically water movement, aquaporin 3 (AQP3) has been identified in a large variety of tissues including gastrointestinal tract. It play an important role in water traversing epithelial barrier by both transcellular and paracellular pathways [4,11-14]. In addition, AQP3 is believed to involve in intracellular osmolality and cell volume regulation [15,16]. Although most of these studies were carried out in vivo and in vitro, the role of AQP3 in the occurrence or development of gastritis is still unclear. Therefore, we detected AQP3 gene expression in gastric mucosa by means of fluorescence quantitative polymerase chain reaction (FQ-PCR) to determine its possible function in the development of gastritis.

Methods

Patients

Patients were evaluated by diagnostic gastrointestinal endoscopy for dyspeptic symptoms at the Digestive Endoscopic Centre of GuangZhou University of Traditional Chinese Medicine and The First Affiliated Hospital of Sun Yat-Sen University from August 1, 2002 to August 1, 2004. Twenty-two CSG patients and twelve CAG patients were selected according to random numbers table (the ratio between CSG and CAG was about 2:1). Exclusion criteria included previous gastric, esophageal or intestinal resection, contraindication to performing biopsies, prior history of *H pylori* eradication therapy, and/or use of bismuth-containing compounds or antibiotics within the previous 4 weeks. Ten healthy volunteers (medical students from GuangZhou University of Traditional Chinese Medicine) with a mean age of around 20 years were selected as healthy controls, and they were confirmed as Helicobacter pylori (HP) negative and negative histology. The protocol was approved by the Ethics Committee of the hospital, and informed consents were obtained from all patients before entry.

Endoscopy and Biopsy

Upper gastrointestinal endoscopy was performed using an Olympus Optical (Tokyo, Japan) GIF-QX240 videoendoscope in a standardized manner by two experienced endoscopists. Biopsies were taken with standard biopsy forceps for histological examination from the antrum (at least 2 cm above the pyloric ring) and from the lesser and greater curvatures of the corpus above the angulus region. Two biopsy specimens from 2 cm proximal to the junction of corpus and bottom along the greater curvature were obtained for AQP3 detection.

Histological Examination

Biopsy specimens were stained with hematoxylin and eosin for conventional histologic determination and modified Giemsa for the detection of Helicobacter pylori (HP). The presence of HP in the specimens was determined in a blinded manner by two experienced pathologists according to the Sydney system [17]. Inflammation was examined for the presence and density of mononuclear cells in the lamina propria and scored on a four point scale: 0, no infiltration; 1, mild infiltration; 2, moderate infiltration; and 3, severe infiltration. In the same way, the presence of glandular atrophy and intestinal metaplasia were also quantified. The degree of atrophy was classified into four levels of severity, according to the appearance of mucosal folds and vasculature, as follows: 0, none; 1, mild (transparent fine blood vessels and yellowish discoloration limited to the lower body, with thick mucosal folds); 2, moderate (clearly transparent blood vessels and yellow-grayish discoloration up to the middle and upper body, with thinned and narrowed mucosal folds); 3, severe (clearly transparent large blood vessels and gray-greenish discoloration up to the upper body, with disappearance of mucosal folds on air insufflation).

Total RNA Isolation and Reverse Transcriptase-Polymerase Chain Reaction

A total of 50-100 mg frozen gastric mucosa tissues (taken from 2 cm proximal to the junction of corpus and bottom along the greater curvature) was pulverized in a mortar containing liquid nitrogen. The powder was dissolved in TRIzol reagent, and then chloroform was

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added to precipitate the protein. RNA was isolated by precipitation with isopropanol. RNA pellet was washed in 750 mL/L ethanol, air-dried and dissolved in water treated with diethylpyrocarbonate (DEPC). RNA was stored at -80°C until use. RNA purity and concentration were assessed spectrophotometrically. Total RNA (2 μg) was then converted to complementary DNA (cDNA) by means of the reverse transcriptase (RT) reaction with the aid of oligo (dT) 15 primer and 400 μL of each deoxyribonucleotide triphosphate (dNTP) for 60 minutes at 37°C and 3 minutes at 95°C.

AQP3 Primer and Generation of Gene-specific Real-time PCR Standards

The primer set used to amplify AQP3 was:
Forward Primer: 5′-ATAGTTTTTGGGGCTGTATTATGATGCA-3′;
Reverse Primer: 5′-GGGCCCGAAACAAAAAGC-3′;

The FAM conjugated fluorogenic probe used to quantify AQP3 gene expression was: 5′-FAM-TGGCACTTCGCCGAC-MGB-3′ with a sequence located between the PCR primers. The fluorogenic probes were synthesized by Daan Gene diagnostic center of Sun Yat-Sen University.

Five μL AQP3 cDNA fragment was amplified on PE 9600 sequence detector (Perkin Elmer) in the following reaction mixture: 5×quantitative PCR buffer 10 μL, forward and reverse primers (25 μM) 1 μL, dNTPs (10 mM) 1 μL, Taq DNA polymerase 2 μL, 5 μL cDNA, ddH2O 29 μLand the ingredient of PCR buffer (10 mM Tris-HCL (PH = 8.0), 10 mM KCL, 2 mM $MgCL_2$). The thermal cycling conditions were: 93°C for 2 minutes followed by 40 cycles at 93°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute and a final cycle at 72°C for 7 minutes in an iCycler thermal cycler (BioRad Laboratories, USA). The PCR products consisted of 68 bp AQP3 fragment. PCR fragments were run on a 2% agarose gel, excised and eluted using the QIAquick gel extraction kit. PCR fragments were quantified on a TD-360 fluorometer and the molar concentration of each PCR product was calculated on the basis of the mass concentration and the length of fragment. Equimolar quantity of standard was 10-fold serially diluted and used to generate standard curves.

Fluorescence Quantitative PCR (FQ-PCR)

FQ-PCR was carried on using a PE 7000 sequence detector (Perkin Elmer) in the following reaction mixture: 5×quantitative PCR buffer 10 μL, forward and reverse primers (25 μM) 1 μL, dNTPs (10 mM) 1 μL, fluorogenic probe (20 μM) 1 μL, Taq DNA polymerase 2 μL, 5 μL cDNA, ddH2O 29 μL and the ingredient of PCR buffer (10 mM Tris-HCL (PH8.0), 10 mM KCL, 2 mM $MgCL_2$). The thermal cycling conditions were: 93°C for 2 minutes, followed by 40 cycles at 93°C for 1 minute and 55°C for 1 minute. Real-time PCR efficiencies for each reaction were calculated using the formula: *Efficiency* (*E*) = $[10^{(1/slope)}]$ - 1, from the slope values given in the PE 7000 sequence detector System. Real time fluorescence measurements were taken and a threshold cycle (C_T) value for each sample was calculated by determining the point at which the fluorescence intensity exceeded a threshold limit (10 times the standard deviation of the baseline) by a model 7000 Sequence Detector. The C_T values for AQP3 transcripts from clinical specimens were plotted on the standard curve, and the amounts (fg) of AQP3 transcripts were calculated automatically by Sequence Detector version 1.6 (PE Applied Biosystems), a software package for data analysis. Real time quantitative PCR of all samples was performed at the same time with the same well plate. Each sample was tested in duplicate, and the average of the two values was used for calculation.

Statistical Analysis

Standard statistical measures and procedures were used. The results of quantitative RT-PCR were expressed as means ± SD and were analyzed using the Mann-Whitney U-test. Differences were considered significant at $P<0.05$.

RESULTS

Characteristics of patients

A total of 22 CSG patients, 12 CAG patients and 10 healthy volunteers were evaluated by endoscopy and gastric biopsy specimens were obtained from corpus and antrum, histopa-

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thological parameters were scored according to the updated Sydney classification system. There were no significant difference of gender and HP infection between SCG and CAG patients (table 1).

AQP3 gene expression

The AQP3 gene expression was detected in the gastric mucosa of CSG and CAG patients by quantitative RT-PCR. The AQP3 gene expression was significantly higher in CSG patients and healthy individuals than that in CAG patients (P<0.01 and P<0.05, respectively) (table 2). There was no significant difference in the AQP3 gene expression between HP positive patients and HP negative patients (P<0.05) (table 3).

Table 1. Characteristics of chronic gastritis patients and histopathological grading according to the Sydney system.

Classification of chronic gastritis		Gender (M/F)	Average age	HP positive
CSG	**Mild**	2 (1/1)	41.5	1
	Moderate	17 (8/9)	31.8	9
	Severe	3 (2/1)	34.1	2
CAG	**Mild**	2 (1/1)	41.7	1
	Moderate	9 (5/4)	45.5	6
	Severe	1 (0/1)	49.3	1
	Healthy controls	10 (4/6)	21.8	0

SCG: chronic superficial gastritis; CAG: chronic atrophy gastritis.

Table 2. AQP3 gene expression in CSG patients and CAG patients (mean ± SD).

Gastritis	n	AQP3 (log copies/μg)
CSG	22	4.32 ± 1.76*
CAG	12	2.25 ± 1.55
Healthy controls	10	3.30 ± 1.32†

*P<0.01 vs. AG; †P<0.05 vs. CSG and CAG.
n: number; CSG: chronic superficial gastritis; CAG: chronic atrophic gastritis.

Table 3. AQP3 gene expression in HP associated patients (mean ± SD).

	n	AQP3 (log copies/μg)
HP positive	20	3.78 ± 1.63*
HP negative	14	3.17 ± 1.68

n: number. *P<0.05 vs. HP negative.

Discussion

Chronic gastritis is characterized by neutrophilic and mononuclear cell infiltration of the lamina propria [18,19]. When there was neutrophil transepithelial migration in gastric mucosa, enhanced paracellular permeability was observed, sequentially led to the movement of water in the direction of osmotic gradient [20-22]. Very often this process will lead to the loss of gastric glands (atrophy) over the years [3,23]. Although some studies have been carried out on the regulation mechanism of the water and salt metabolism in gastritis patients, the mechanism of water and salt metabolism in the development of gastritis remains obscure [24,25]. As one important member of AQP family, AQP3 is a small, membrane-spanning protein that acts as a highly selective water channel

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[8,9,26]. The earlier studies of AQP HP in kidney showed that AQP3 is localized at the basolateral membrane of the renal collecting duct principal cells and is involved in water reabsorption. The expression of AQP3 could be upregulated by hypertonicity and hyperosmolality [27,28]. AQP3 plays an important role in intracellular osmolality and cell volume regulation [15,16]. However, the roles of AQP3 in digestive tract have received more and more attention [4,12,13,21,22]. It is suggested that the digestive organs of the adult human secret about 7 L of fluid into the lumen of gastrointestinal tract and approximately 2 L of water is secreted by the stomach [21]. In addition, approximately 2 L of water enter the gastrointestinal tract from the diet each day. Therefore, a large amount of fluid transfer happens in digestive tract each day. AQP3 has been reported to express widely in the epithelia of the upper digestive tract from the oral cavity to the stomach and intestine [11,13,21], which suggests that AQP3 plays critical role in fluid transfer. Although most of these studies of AQP3 in the gastrointestinal tract were carried out on both in vivo and in vitro, the physiologic and pathophysiologic relevance of AQP3-mediated water transport in human gastrointestinal tract is still unclear [4,9-12].

Our previous study showed that the AQP3 gene expression was closely associated with grumme secretion and AQP3 increments in superficial mucosa may contribute to defensive mechanisms in mucosal lesions [6]. Also, in present study, the AQP3 gene expression detected by quantitative RT-PCR was significantly higher in gastric mucosa of chronic superficial gastritis patients and healthy individuals than that in atrophy gastritis. As we all know, chronic gastric mucosal lesion may cause abnormal changes of gastric permeability, and subsequently the abnormal expression of AQP3 [1,29,30]. Therefore, we suggest that AQP3 might play certain role in the natural history of gastritis, which may present as inflammation in the mucosa of stomach, and finally lead to atrophy [5,7,8]. H pylori has been widely accepted as a major cause of gastritis [1-3]. In some subjects, the HP infection has played an important role in the occurrence and development of gastritis. In our study, there was no significant difference in the AQP3 gene expression between HP positive patients and HP negative patients. We still do not know the exact reason, because a large amount of observations have shown that HP infection has close relation to gastric mucosa damage[1,2]. However, taking into account that different genotypes of HP may have different cytotoxins in gastric mucosa, HP infection may not be associated with the severity of gastritis, which is in agreement with our present observation [2,31-33]. However, our study had a limitation because we did not check antiparietal cell antibodies and anti-intrinsic factor antibodies. As we all know there are two main kinds of atrophic gastritis, diffuse corporal atrophic gastritis and multifocal atrophic gastritis. Since these two kinds of atrophic gastritis have different etiologies (autoimmune and H. pylori infection, respectively [34-37]) and also different distributions in the stomach, the expression of AQP3 may also be affected by these factors which need to be studied further.

In conclusion, our results suggested that AQP3 might play certain role in the occurrence and development of gastritis. A better understanding of these events will hopefully provide new insights into the mechanisms of AQP3 in regulating fluid transfer in chronic gastritis.

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