mRNA expression of aquaporin protein 1 and 7 in rat model of slow transit constipation (STC)

Zhen Li*, Weitang Yuan and Hui Zhi

Department of Colorectal and Anal Surgery, the First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, Henan, China.

Accepted 14 September, 2012

Water channels play important role in colon, and might be involved in constipation disease. To understand the mRNA expression changes of aquaporin protein 1 and 7 (AQP1, AQP7) in a rat model of slow transit constipation (STC), a rat model of slow transit constipation was designed, and the correlated changes of aquaporin protein 1 and 7 at the mRNA level were examined. The rat model of slow transit constipation was produced with diphenoxylate administration. The mRNA expression of AQP1 and 7 from STC rats and control rats were examined with reverse transcription polymerase chain reaction (RT-PCR). AQP1 was expressed in ascending and descending colon of both control and STC rats. For the ascending colon, the AQP1 grayscale ratio in control and STC rats were 0.602 ± 0.239 and 0.344 ± 0.212 (P < 0.05), respectively; these were 0.509 ± 0.308 and 0.419 ± 0.309 (P > 0.05), respectively in descending colon. AQP7 was also expressed in ascending and descending colon of both control and STC rats, with 0.776 ± 0.642 and 0.764 ± 0.147 in ascending colon (P > 0.05), and 0.736 ± 0.42 and 0.759 ± 0.629 in descending colon (P > 0.05), respectively. We did not examine the changes of the AQPs at the protein level. The downregulation of AQP1 rather than AQP7 might be involved in the development and progression of STC.

Key words: Aquaporin protein, AQP1, AQP7, slow transit constipation, mRNA.

INTRODUCTION

Water channel proteins belong to the transmembrane channel protein family, and play important role in water absorption, liquid secretion and water transportation in and out of the cell (Verkman, 2011). The aquaporin proteins family of water channel proteins was extensively expressed in mammalian digestive system, with isoforms of AQP1, 3, 7, 8, 10, etc. The AQP 1, 3, 7, and 8 proteins were expressed in the colony (Zhi and Yuan, 2011), and have been shown to be involved in water transportation of this region.

Slow transit constipation (STC) is characterized by the slow movement of content in gastrointestinal system. The colonic absorption and secretion of water is closely related to the development and progression of STC (Jamshed et al., 2011; Leung et al., 2011). However, how AQP proteins played their roles in STC disease were not well investigated yet. The present study set out to build a rat model of STC to understand the mRNA expression changes of AQP1 and 7 proteins in this disease.

MATERIALS AND METHODS

Animals

Clean grade rats (32: male or female, weight 150 g) from Zhengzhou University Experimental Animal Research Center were randomly assigned into STC group and control group with 16 rats in each. After 1 week of adaptation to the environment, the animals were subjected to intragastric administration of distilled water at 0.32 ml/kg for 5 days for adaptation to the experimental manipulations to the gastrointestinal system. The experiments have been approved by animal research committee in Zhengzhou University.

During the experiment, the STC group was fed with rat chow containing diphenoxylate (Tianlei, He Nan) and the control group...
Table 1. The AQP1 gray scale ratio in STC and control groups ($\pm s$).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Ascending colon</th>
<th>Descending colon</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>STC</td>
<td>16</td>
<td>0.344 ± 0.212</td>
<td>0.419 ± 0.309</td>
<td>-0.688</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Control</td>
<td>16</td>
<td>0.602 ± 0.239</td>
<td>0.509 ± 0.308</td>
<td>0.825</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>t</td>
<td>-</td>
<td>2.796</td>
<td>0.719</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>-</td>
<td>&lt;0.05</td>
<td>&gt;0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

downstream: 5'-GTGA TGCGAACAGAGAC AGGC -3' (537 bp). The internal control was beta-actin. The reaction conditions: 94°C for 3 min; 94°C for 30 s, Tm (AQP 58°C, AQP7 56°C and beta-actin 61°C) for 30 s, 72°C for 1 min for 35 cycles; extension at 72°C for 5 min. The PCR products were collected and stored in the fridge.

PCR products of 5 µl with 1 µl 5X loading buffer were put on 2% agarose gel electrophoresis at 120 V for 30 min before ethidium bromide staining and imaging with the gel imaging system (BioXtal, US).

Statistics

The data were represented as mean ± standard deviation (SD) and then was analyzed by Statistical Package for Social Sciences (SPSS) 13.0 software (Chicago, US). T test was used to examine the differences between two groups and P < 0.05 was determined as statistically significant.

RESULTS

The RT-PCR results of AQP1 expression in ascending and descending colon of both groups are as shown in Table 1 and Figure 1. In ascending colon of STC group animals, the AQP1 expression was lower than control group (P < 0.05).

The RT-PCR results of AQP7 expression in ascending and descending colon of both groups are as shown in Table 2 and Figure 2. There were no significant differences between two groups, both ascending and descending colons (P > 0.05).

DISCUSSION

Constipation results from the increasing stress in current society and could contribute to the development of some severe cardiovascular disease disorders. The understanding in the pathological mechanisms of constipation is therefore important to improve the life quality of these patients. This study employed the rat model of STC induced by diphenoxylate administration, in order to observe the potential changes in water channel protein expressions of the colon. Diphenoxylate is composed of hydrochloride diphenoxylate and atropine sulfate, which could act on intestinal opioid receptors to inhibit the movement of gastrointestinal smooth muscle, and finally lead to the delayed emptying. The current rat
Table 2. The AQP7 gray scale ratio in STC and control groups (±sx).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Ascending colon</th>
<th>Descending colon</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>STC</td>
<td>16</td>
<td>0.764 ± 0.544</td>
<td>0.776 ± 0.642</td>
<td>-0.052</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Control</td>
<td>16</td>
<td>0.602 ± 0.239</td>
<td>0.736 ± 0.424</td>
<td>0.059</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Figure 2. The RT-PCR results of AQP7 expression. M represents the marker; 1 for control group ascending colon; 2 for STC group ascending colon; 3 for control group descending colon; 4 for STC group descending colon.

STC model shows neither changes in physiological functions, nor the food consumption process, with high clinical relevance. Moreover, it has been found that the changes in opioid peptide activity were involved in the pathogenesis of STC (Crowell et al., 2009; Pohl et al., 2008).

The expression changes of AQPs can be regulated upon the slowdown of the movement of gastrointestinal smooth muscle, and has been at least partially attributed to the dysfunction of ascending and descending colons. This could either be directly resulted from the pharmacological administration of diphenoxylate compound, or indirectly modulated by the movement of the smooth muscle as a compensatory mechanism. We believe that it was the latter case as there were differences between ascending and descending colon at the same time. After the slowdown of the transportation, the expression of AQP1 was down regulated in the ascending colon to reduce the water re-absorption, which facilitates the intestinal transportation. This is consistent with results obtained in a previous study showing that with 80% removal of the small intestine, the AQP1 expression in the remaining small intestine and colon increased to enhance the water reabsorption (Tsujikawa et al., 2003). The expression of AQP7 was not changed in this study, and its role in constipation is yet to be investigated in further studies.

One limiting factor in this study is that only the mRNAs of the AQPs were examined, leaving the protein level changes of these proteins uninvestigated. However the changes at transcriptional level could also directly reflect the cellular responses to environmental factors, suggesting for the future changes at the protein level. This can be clarified through western blotting of the AQP proteins in future studies.

Conclusively, this study provides some evidences that AQP1 is involved in the pathogenesis of STC. This could be a potential drug target in future experimental studies.

REFERENCES