

Original Article

Changes in ion channel metabolism increase intestinal mucous membrane permeability in IBS

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Abstract: Objective: Our research sought to measure the expression of the ion channel in an irritable bowel syndrome (IBS) rat model in order to clarify another important mechanism of IBS, namely, the increase of intestinal mucous membrane permeability. Methods: 20 adult female Sprague-Dawley rats were divided into a control group and the IBS model group. The small intestinal propulsion rate and the stool moisture content were measured. ELISA was performed to measure the expression of D-lactic acid. Immunohistochemistry and RT-PCR were performed to quantify the expressions of SGLT1, NHE 1, and CFTR. Results: The IBS model rats were built successfully. The stool moisture content of the model group was higher than it was in the control group ($P < 0.05$). The small intestinal propulsion rate of the model group was faster than it was in the control group ($P < 0.05$). The expression of D-lactic acid in model group was higher than it was in the control group ($P < 0.05$). The expressions of SGLT1 and NHE 1 in the model group were less than they were in the control group ($P < 0.05$), and the expression of CFTR in the model group was higher than it was in the control group ($P < 0.05$). Conclusions: Our research revealed that increased of intestinal permeability is another important mechanism of IBS, and it could be related to changes in the ion channel.

Keywords: Irritable bowel syndrome, ion channel, intestinal permeability

Introduction

Irritable bowel syndrome (IBS) is a disorder with different symptoms which initially appears as abdominal pain or discomfort [1]. The global prevalence of IBS was reported to be 11.2% [2]. A survey has reported that, according to the Rome II and I criteria, the prevalence rates of IBS were 22.1% and 17.5% respectively in Taiwan [3], whereas a secondary analysis reported that the prevalence was 4.4% according to the Rome III criteria [4]. Although IBS is the most common gastroenterological diagnosis, its etiology remains unknown. It has been reported that several physical and psychological factors, such as a distorted visceral perception of sensation, a gastrointestinal motility abnormality, stress, and anxiety, are related to IBS's pathogenesis [5]. Current evidence indicates that the diagnosis of IBS is mainly according to Rome IV, but the actual underlying causes of IBS symptoms, however, remain unknown. Therefore, our research sought to

find novel pathogenic mechanisms so as to contribute to the treatment of IBS.

The Na(+)-glucose cotransporter 1 (SGLT1/SLC-5A1) is predominantly expressed in the small intestine, which transports glucose and galactose across the apical membrane driven by a Na(+) gradient created by Na(+)-K(+)-ATPase. It has been demonstrated that SGLT1 could balance the modulation to achieve therapeutic efficacy for metabolic diseases [6]. The Na⁺/H⁺ exchanger (NHE) represents an illocal membrane protein which can regulate cellular pH levels. There are a number of NHE isoforms encoded by different genes and NHE 1 is one of the most widely expressed isoforms [7]. The cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-dependent anion channel, which may cause cystic fibrosis when it mutates [8]. The aim of our study was to detect the expression of SGLT1, NHE 1, and CFTR so as to illuminate another important mechanism of IBS and the relationship between

intestinal permeability and changes in the ion channel.

Methods

Subjects

There were 20 adult female Sprague-Dawley rats, and the weight of every rat was about 200 g. The feeding environment was provided by the experimental animal center of Zhejiang Chinese Medical University. The 20 SD rats were divided randomly into two groups: ten rats in the control group, and ten rats in the model group. They were placed in a controlled environment where the temperature was 22-24°C, the humidity <60%, and the noise <50 db.

Experimental procedure and methods

Group: 1) Control group: They were normal rats. After the rats ate and drank normally for two weeks, we observed the conditions of the rats. Next, the rats' visceral sensitivity was evaluated using the abdominal withdrawal reaction technique. 2) Model group: The conditioned stimulus was a camphor ball with a special odor. The unconditioned stimulus was rectal distention pressure (>60 mmHg (1 mmHg=0.133 kPa)) combined with constrained extremities. The rats were put into a cage with a camphor ball in it, and then we fixed the extremities and trunks of the rats for 45 min. At this time, we inserted catheters into their rectums. The distance from the air balloon distal end to the anal end was about 1 cm. The catheter was fixed at the root of the tail. The balloon volume was 1.6 ml (hydrostatic pressure in the balloon's space >60 mmHg) and it lasted for 60 s, with intermittent exhaust over 3 mins and then the balloons were filled with gas 10 more times. This was a stress process. There was one process completed on the first day, and then the same process was performed on the second day at the same time. The conditioned stimulus without the unconditioned stimulus was done on the fourth day. The complete process was repeated once on the fifth day. The same process performed on the fourth day was performed again on the sixth day. The conditioned stimulus was performed on the eighth day. Then the rats' visceral sensitivity was evaluated using the abdominal withdrawal reaction technique after 2 weeks of normal eating and drinking.

Model authentication: Visceral sensitivity was evaluated using the abdominal withdrawal reaction technique (AWR). An 8F urethral catheter which was lubricated by liquid paraffin was inserted per anum and fixed at the root of the tail. The distance from the air bladder distal end to the anal end was about 1 cm. The rats were put on the platform, and after they became accustomed to the environment, we gradually affused water into the sacculus and recorded the water injection volume when the rats raised their abdomen and arched their backs into a bow shape. The rectal distention lasted for 30 s every time, and we repeated it 3 times. And then we recorded the mean values.

Experimental sample: After a laparotomy incision, a portion of the colon was removed, and the tissue samples were stored in a -80°C liquid nitrogen reserve. 2 ml blood was taken from the inferior vena cava and then placed in 4°C refrigerator.

The stool moisture content: At the 10th day of model building, we put clean filter paper in the cage and collected fresh stools over a period of 4 hours (8:00 AM to 12:00 AM). We weighed the stools and then dried them for 24 h in a desiccator. The water content per gram of stool was calculated based on the differences between the wet and dry weights.

The small intestinal propulsion rate: On the 7th day and 14th days of the experiment, we gave the experimental rats a lavage ink gum Arabic suspension (2 ml for per rat). After 20 minutes, we sacrificed the rats, and then we opened their abdominal cavities, and then we immediately removed their entire small intestines. Then we measured the ink advance percentage of small intestines respectively. The small intestine propulsion rate (%) = pylorus to ink front distance/pylorus to blind of distance × 100%.

Experimental procedure (immunohistochemical technique): To detect the expression of SGLT1, NHE 1, and CFTR in colon tissue, we did the following: 1) Three footwork: We dropwised 3% hydrogen dioxide on the tissue away from light and incubated it for 15 min. The tissue was flushed with distilled water, and then we put the chips into a PBS balanced solution and soaked them for 5 min, for 3 times. Then we dropped 50-100 ml of antibody fluid on the tissue and incubated them for 30 min at room

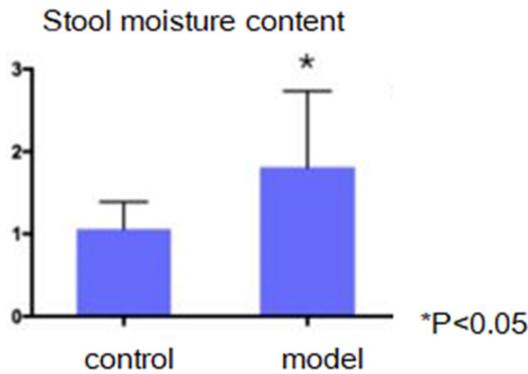


Figure 1. The stool moisture content of the two groups. *P<0.05 compared with the control group.

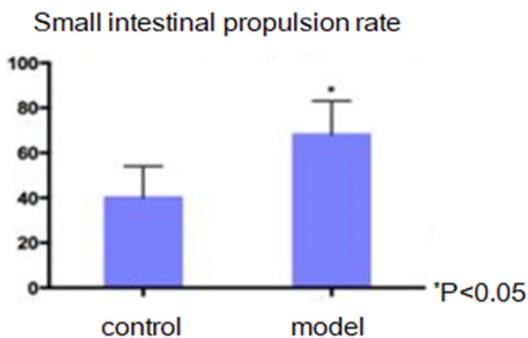


Figure 2. The small intestinal propulsion rate of the two groups. *P<0.05 compared with the control group.

temperature. Next, we washed the chips with PBS, and we soaked the chips in a PBS balanced solution for 4 min, and we did this 3 times. Then we dropped appreciable proportion of diluted biotin labeling antibody (1% BSA-PBS to dilute) and incubated it for 30 min at room temperature. After that we washed the chips with PBS and soaked them in the PBS balanced solution 3 times. Then we added the corresponding biochemical secondary antibody and incubated it for 10 minutes. A streptomycin-HRP conjugate was added and incubated for 10 min at room temperature, and then we washed it with PBS for 5 min, and we did this 3 times. We dropped 50-100 ml developer DAB fluid and incubated it for 5-20 min, after complete coloration. Hematoxylin was used to counterstain for about 20 seconds and then we counterstained it with PBS. The tissue was dehydrated by 85%, 90%, 95%, 100%, 100%, 100% alcohol. Then we put the chips into xylene 3 times.

2) Negative control: We replaced the primary

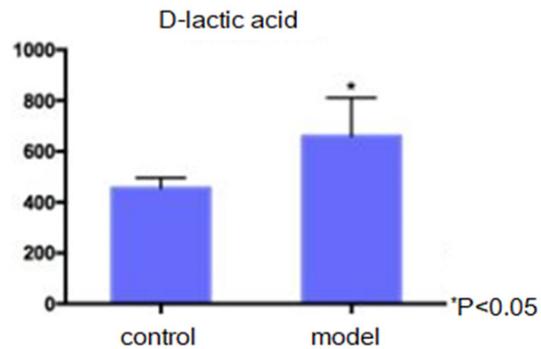


Figure 3. The D-lactic acid of the two groups. *P<0.05 compared with the control group.

antibody with PBS, and the consequence was negative. 3) Analytical method: The larger the MOD value, the stronger the positive expression. In each case, the average of the MOD values measured in 5 non-overlapping visual fields was used as the measurement.

Experimental procedure (RT-PCR technique): Total RNA was extracted from colon tissues with Trizol (Invitrogen, Gaithersburg, MD, USA) using the one-step method. After purification, the RNA concentration was analyzed using Nanodrop (Nanodrop Technologies, Wilmington, DE) and quality testing was conducted using BioAnalyzer (Agilent Technologies, Palo Alto, CA). A high-capacity cDNA synthesis kit (Applied Biosystems, Foster City, CA, U.S.A.) was used to synthesize cDNA from 1 mg of RNA. Then PCR amplification was conducted.

Experimental procedure (ELISA technique): To detect the expression of D-lactic acid in the plasma, 2 ml blood from the vena cava was removed and allowed to stand still at room temperature for 30 min. The plasma was separated by centrifugation at 10000×g for 10 min at 4°C. We purchased an ELISA kit and set up blank control wells, standard wells, and wells for the sample to be tested. The standards were added to a row of 50 ul at 0, 50, 250, 500, 1000 ng/L per well. We then added 10 ul of biotin labels to the wells of the sample to be tested and mixed them well. To the above test wells, we added 50 ul of enzyme affinity and then placed them in a 37°C incubator and incubated them for 60 min. Then we discarded the pores of the liquid, washed them repeatedly and diluted them with a cleaning solution 5 times. Each well was followed by the addition of

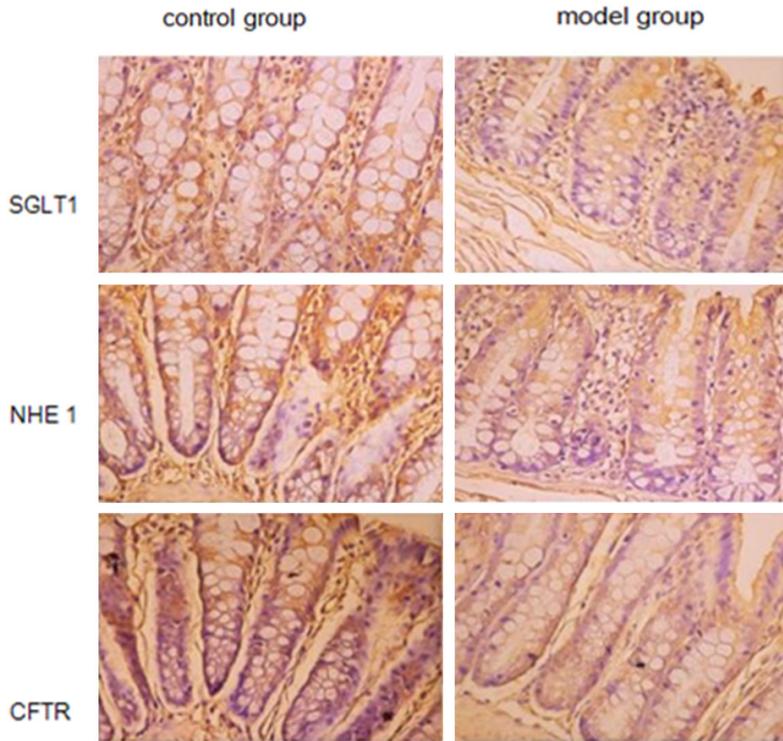


Figure 4. The expressions of SGLT1, NHE 1, and CFTR in the colon by the immunohistochemical technique (*400). SGLT1, NHE 1, CFTR positive reaction material presents as brown, the cell membrane and cytoplasm were dyed.

substrate I and II 50 ul for 15 minutes' reaction time at room temperature. Finally, 2 M H₂SO₄ 0.05 ul was added to stop the reaction. The OD value was tested by a microplate reader within 30 minutes.

Statistical analysis: The data was demonstrated as $\bar{x} \pm s$. We used the SPSS package as a statistical tool. Two samples' mean numbers were compared by *t* test. The significance level was $P < 0.05$.

Results

Model authentication

The water injection volumes of the rectums of the model rats (0.833 ± 0.143 ml) were lower than they were in the control group (1.467 ± 0.054 ml), and the difference had statistical significance ($P < 0.01$), which suggested that the model building method was successful.

The stool moisture content

The stool water content of the model group (1.807 ± 0.294) was more than it was in the con-

trol group (1.060 ± 0.105). The significance level was $P < 0.05$ (**Figure 1**).

The small intestinal propulsion rate

The small intestinal propulsion rate of the model group (67.65 ± 5.142) was higher than it was in the control group (46.60 ± 8.288). The significance level was $P < 0.05$ (**Figure 2**).

The detection of intestinal permeability

The expression of D-lactic acid in the model group (659.4 ± 53.64) was higher than it was in the control group (455.1 ± 14.57). The significance level was $P < 0.05$ (**Figure 3**).

The expressions of SGLT1, NHE 1, and CFTR in the colon by immunohistochemistry

1) SGLT1, NHE 1, and CFTR cell immunohistochemical staining: The SGLT1, NHE 1, and CFTR positive reaction material presents as brown, and the cell membranes and cytoplasm were dyed, but the negative control was not dyed (**Figure 4**).

2) The expressions of SGLT1, NHE 1, and CFTR compared among the two groups: The SGLT1, NHE 1 positive cell numbers of the model group were less than they were in the control group ($P < 0.05$), and the CFTR positive cell numbers of the model group were higher than they were in the control group ($P < 0.05$) (**Table 1**).

The expression of SGLT1, NHE 1, and CFTR in the colon using the RT-PCR technique

The expression of SGLT1, NHE 1, and CFTR compared among the two groups: The expressions of SGLT1 and NHE 1 of the model group were less than they were in the control group ($P < 0.05$), and the expression of CFTR in the model group was more than it was in the control group ($P < 0.05$) (**Table 2**).

Table 1. The expressions of SGLT1, NHE 1, and CFTR of the two groups by the immunohistochemical technique

	Control group	Model group
SGLT1	0.255±0.006	0.236±0.003 ^a
NHE 1	0.262±0.003	0.241±0.004 ^a
CFTR	0.235±0.003	0.250±0.004 ^a

^aP<0.05 compared with the control group.

Table 2. The expressions of SGLT1, NHE 1, and CFTR of the two groups by the RT-PCR technique

	Control group	Model group
SGLT1	1.692±0.362	0.684±0.096 ^a
NHE 1	1.333 0.209	0.778±0.098 ^a
CFTR	0.712±0.076	1.417±0.201 ^a

^aP<0.05 compared with the control group.

Discussion

As we know, several hypotheses have been proposed to explain the pathogenesis of IBS, including visceral hypersensitivity, altered gut microbiota, dysmotility, dysregulation of brain-gut axis, gastrointestinal infection, psychological factors, and so on [9]. Despite the growing number of studies on this topic, IBS pathophysiology remains poorly understood. The intestinal epithelial barrier including the mucus layer, the intestinal epithelial cell layer, and the tight junctions of intestinal epithelial cells is the first-line of mucosal immune defense [10]. D-lactic acid was thought to be one of the best markers to evaluate intestinal mucous membrane permeability [11]. Our study has revealed that the small intestinal propulsion rate, the stool moisture content, and the expression of D-lactic acid of the IBS model rats were higher than they were in the control group, which suggests that the intestinal mucous membrane permeability was elevated in the colons of the IBS rats. The barrier function of the intestine is essential for maintaining the normal homeostasis of the gut and mucosal immune system. It has been reported that various gastrointestinal disorders such as IBS were observed to be connected with abnormalities in the intestinal barrier function as expressed by increased intestinal permeability [12]. Accordingly, our study reached the same conclusion. What's more, it has also been reported that small intestine permeability increases in IBS-D patients are related to mucosal inflammation and immunity [13]. This study revealed that small intestinal permeability, but not colonic permeability, is increased in IBS-D patients. On the contrary, our study suggests the colonic permeability is increased in IBS rats. As we performed animal experiments and as other researchers performed human tests, that might be the reason why we got different results, so further studies should be done to confirm the findings.

Our study showed that the expressions of SGLT1 and NHE 1 were down-regulated, and CFTR was up-regulated in IBS rats. It has been reported that SGLT1 is highly active in pruruminant animals but is down-regulated in ruminating animals due to a lack of substrate in the upper small intestines [14]. One research reported that amino an acid-based oral rehydration solution (AA-ORS) increased villus height which can improve electrolyte and nutrient absorption and increase the expression of NHE3 and SGLT1 in the brush border membrane which is connected with intestinal epithelial proliferation [15]. It is also reported that implications of the sodium glucose cotransporter SGLT1 in either pumping water or passively channeling water contrast with its water transporting capacity [16]. Our study suggests that NHE 1 inhibition reduces nuclear factor-kB (NF-kB) activation, which means that NHE 1 plays an important role in intestinal permeability through the regulation of the inflammatory response [17]. CFTR is a unique member of the ATP-binding cassette (ABC) transporter family that forms a novel Cl-channel, and it is located predominantly in the apical membrane of the epithelia where it mediates transepithelial salt and liquid movement [18]. Another study showed that the CFTR protein is an ion channel regulated by cAMP-dependent phosphorylation and is expressed in many types of epithelial cells, and CFTR-mediated chloride and bicarbonate secretion play a key role in the respiratory and gastrointestinal systems [19]. Thus, according to our study, we speculated that the regulation of SGLT1, NHE 1, and CFTR are involved in the mechanism of IBS, and they are connected with an increase in intestinal mucous membrane permeability.

This is the first study about IBS and its connection with the ion channel. Our study reveals that the regulation of the ion channel might play an important role in IBS. Although the mechanism

of IBS is still unclear, an increase in intestinal mucous membrane permeability could be another mechanism of IBS and the regulation of the ion channel. We hope our findings provide new ideas for research on the future treatment of IBS. However, there are several limitations in our study. We still don't know the specific mechanism involved in increasing intestinal mucous membrane permeability by the regulation of the ion channel. As a result, further research should focus on this particular question.

Conclusion

An increase in intestinal mucous membrane permeability might be another important mechanism of IBS, and it could be regulated by changes in the ion channel. However, further research should be done to clarify the mechanisms involving IBS and the ion channel.

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Disclosure of conflict of interest

None.

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