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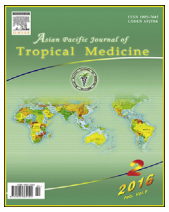

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Effect of tight junction protein of intestinal epithelium and permeability of colonic mucosa in pathogenesis of injured colonic barrier during chronic recovery stage of rats with inflammatory bowel disease

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ABSTRACT

Objective: To discuss the changes in the tight junction protein of intestinal epithelium and permeability of colonic mucosa and its possible mechanism by building the rat mode of inflammatory bowel disease at the chronic recovery stage.

Methods: A total of 36 SD rats were divided into the model group and control one according to the random number table, with 18 rats in each group. Rats in the model group were given the 3% dextran sulfate sodium solution by the way of drinking for 7 d to build the rat model of inflammatory bowel disease, while rats in the control group were given free drinking of water. Six rats were executed at day 7, 14 and 21 respectively. The colonic tissues were collected from rats to observe the pathological changes of colonic mucosa. The activity of myeloperoxidase was detected and the white blood count was performed for rats in each group. The Ussing chamber technique was employed to detect the transepithelial electrical resistance (TER) and short-circuit current (SC) of colonic mucosa of rats in different time intervals; the quantum dots labeling technique was employed to detect the expression level of claudin-1 and claudin-2 in the colonic tissues.

Results: After the successful modeling, the weight of rats in the model group was significantly reduced, while the disease activity index score was increased. The weight was at the lowest level at day 14 and then it began to increase afterwards. The disease activity index score was at the highest level at day 12 and then it began to decrease gradually. The activity of myeloperoxidase and WBC for rats in the model group all reached the peak value at day 14 and then decreased gradually. There was no significant difference in the changes of TER and SC in different time intervals for rats in the control group ($P > 0.05$). TER of model group was at the lowest level at day 14 and then increased gradually; SC was at the highest level at day 14 and then decreased gradually. TER of model group at day 7, 14 and 21 was significantly lower than that of control group, while SC of model group was significantly higher than that of control group ($P < 0.05$). There was no significant difference in the change of mean fluorescence intensity of claudin-1 and claudin-2 in different time intervals for rats in the control group ($P > 0.05$). The claudin-1 and claudin-2 for rats in the model group reached the highest level at day 14 and then decreased gradually. The claudin-1 and claudin-2 of model group at day 7, 14 and 21 was significantly higher than that of control group ($P < 0.05$).

Conclusions: After the acute stage, the inflammatory bowel disease is then in the chronic recovery stage; the increased permeability of colonic mucosa and increased expression of tight junction protein of intestinal epithelium are closely related to the pathogenesis and development of disease. The tight junction protein plays a key role in the pathogenesis of injured colonic barrier of inflammatory bowel disease.

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1. Introduction

The intestinal epithelium is the main defensive barrier of intestine, which can inhibit the free passing of pathogens, virus and microorganisms to protect the intestine [1,2]. The tight junction protein of intestinal epithelium is an important part of colonic mucosa barrier. After being injured, it will lead to the increased permeability of colonic mucosa and then make the microorganisms and toxic substances that cannot pass through enter in the intestine to induce the inflammatory response and eventually cause the inflammatory bowel disease. At present, there have been a great number of clinical researches on the mechanism of injured colonic mucosa, including the cytokine pathway and small guanosine triphosphatase pathway [3,4], but the findings are still controversial. In this study, by building the rat mode of inflammatory bowel disease at the chronic recovery stage, it is to discuss the changes in the tight junction protein of intestinal epithelium and permeability of colonic mucosa and the pathogenesis of inflammatory bowel disease, with the findings shown below.

2. Materials and methods

2.1. Materials

A total of 36 SPF healthy SD rats were purchased from the laboratory animal center, with equal amount of females and males, weight of 120–150 g and age of 2–3 months. They were fed in the cage with the temperature of (22 ± 2) °C and relative humidity of 55%, 12 h day and night alternate. The drinking water for all rats was treated with high-temperature sterilization. All rats were treated with fasting, but free water-drinking.

2.2. Instruments and reagents

The dextran sulfate sodium for modeling of colitis was purchased from MPBIO (Item No.: 120317); the myeloperoxidase was purchased from Shanghai Xin Yu Biotech Co., Ltd; the rabbit anti-rat claudin-1 and claudin-2 polyclonal antibody was purchased from Santa Cruz Biotechnology, Inc.; the goat anti-mouse IgG secondary antibody was purchased from Shanghai Biovol Technologies Co., Ltd.; Olympus IX70 fluorescence inverted microscope was adopted.

2.3. Methods

2.3.1. Rat mode of inflammatory bowel disease

After 1 week of adaptive feeding, 36 rats were divided into the model group and control one according to the random number table, with 18 rats in each group. The animal model was referred to related literature [5]: The dextran sulfate sodium was prepared into 3% solution. Rats were fed by the drinking water for 7 d, then the sterile drinking water at day 8, and then free drinking water for 14 d. Rats in the control group were given the sterile drinking water during the whole process. The general conditions of hair, diet and weight of rats in both groups were recorded. The rat droppings were collected every 2 d. The fecal occult blood test kit was used to detect the fecal occult blood fraction and the disease activity index (DAI) [6] to evaluate the animal growth. Weight: constant weight as 0 point, weight loss by 1%–5% as 1 point, weight loss by 5%–10% as 2 points, weight loss by 10%–15% as 3 points and weight loss over 15% as 4 points; the normal stool consistency as 0 point, loose stool as 2 points and diarrhea as 4 points; the normal stool bleeding as 0 point, positive occult blood as 2 points and revealed hemorrhage as 4 points. The comprehensive evaluation was performed on these three conditions and then the total scores of these three results were divided by 3 to obtain the DAI value, namely DAI = (weight index + stool form + bleeding situation)/3. Six rats were selected at day 7, 14 and 21 respectively. After being anesthetized with 10% chloral hydrate, the colon was sampled at the place about 2 cm from the anus and then embedded with the paraffin after the fixation using the formaldehyde. They were sliced into pieces by 5 μm, stained with hematoxylin-eosin and then observed under the common microscope.

2.3.2. Detection of myeloperoxidase

The colonic tissues of rats were collected at day 7, 14 and 21 respectively. After the homogenization, they were centrifuged at 15 000 r/min for 10 min. The precipitate was removed and supernatant was collected. According to the instruction manual of myeloperoxidase kit, it was then incubated at 37 °C for 20 min. Its absorbance was measured at 460 nm and then the enzymatic activity was calculated. The whole blood was sampled from rats of two groups in different time intervals by 2 mL. The automatic biochemical analyzer was employed to detect the white blood cell (WBC) of rats in each group, with three repeats of measurement. The average of results was than calculated.

2.3.3. Detection of permeability of colonic mucosa

The colonic tissues of rats were collected by about 1 cm at day 7, 14 and 21 respectively. After separating the muscular layer and serosal layer, they were fixed in the Ussing chamber. 5 mL sodium chloride solution (0.9%) was added at the side of mucosa, while 5 mL Kreb buffer at the side of serosa. The whole device was kept at 37 °C and the medium on both sides was filled with the mixture of 5% CO_2 and 95% O_2; the Ussing chamber technique was employed to detect and record the transepithelial electrical resistance (TER) and short-circuit current (SC) of colonic mucosa of rats.

2.3.4. Detection of expression of tight junction protein

The quantum dots labeling technique was employed to detect the expression level of claudin-1 and claudin-2 in the colonic tissues: the colonic tissues was sliced into pieces, then deparaffined and hydrated normally with the antigen retrieval. It was washed with TBST buffer 3 times and then incubated in the wet box at 37 °C for 30 min; the claudin-1 and claudin-2 polyclonal antibodies were added respectively and then it was incubated at 37 °C for 3 h; it was washed with TBST buffer 3 times and then incubated in the wet box at 37 °C for 30 min; the goat anti-mouse IgG secondary antibody was added and it was incubated in the wet box at 37 °C for 30 min; it was then washed with TBST buffer 3 times and incubated in the wet box at 37 °C for 30 min. The streptomycin labeled quantum dots were added and it was mounted with 50% glycerin; the quantum dots were excited using different wavelengths under the fluorescence microscope. It appeared to be positive when the cells with the red-orange fluorescent particles occurred. The area and fluorescence intensity of positive cells were measured, while the fluorescence

intensity was calculated, namely the fluorescence intensity referring to the expression of protein. The image pro plus 6.0 was employed to calculate the average fluorescence intensity of each group.

2.4. Statistical analysis

The SPSS19.0 was used for the statistical analysis. The clinical data of patients was expressed by mean ± sd. The t test was adopted for the comparison between two groups, while the analysis of variance for the comparison among groups and SNK-q test for the multiple comparison. $P < 0.05$ referred to the significant difference.

3. Results

3.1. Rat model of inflammatory bowel disease

After being treated with the dextran sulfate sodium, rats in the model group had the symptoms of diarrhea with the purulent blood and positive occult blood at day 3–5 and they all had the rectal bleeding at day 5–6; rats appeared to be thin with matt fur, tired of moving, significant loss of diet and water drinking and weight loss; at day 14, the weight was reduced to the lowest level and then it began to increase diet and water drinking with the gradual relief of clinical symptom. DAI score of rats in the model group was increased from day 2 and it reached to the highest level at day 12 and then began to decrease gradually; two of them still had the positive occult blood even at day 21.

3.2. Colonic tissue morphology of rats in both groups

The colonic tissues of rats in the control group had the clear skin layers and complete mucosal structure; rats in the model group had the inflammatory response at the distal colon and the mucosal edema at day 7, which indicated the infiltration of eosinophils and neutrophils, but only limited to the submucosa; rats in the model group had the aggravated inflammatory response at day 14 under the microscope, serious necrosis in the colonic epithelial cells with the crypt disorder and abscess, while the inflammatory cells expanded to the muscular layer; at day 21, the colonic tissues had a great number of crypt disorder with the infiltration of lymphocytes, as well as abundant lymphatic follicles around the inflammatory tissue.

3.3. Changes of activity of myeloperoxidase and WBC in different time intervals for rats in both groups

The activity of myeloperoxidase and WBC at day 7, 14 and 21 of the model group were all significantly higher than that of the control group at the same time ($P < 0.05$); while the activity of myeloperoxidase and WBC of the model group reached to the highest level at day 14 and then began to decrease gradually ($P < 0.05$). There was no significant difference in the activity of myeloperoxidase and WBC of the control group before and after treatment ($P > 0.05$), as shown in Table 1.

3.4. Changes of TER and SC in different time intervals for rats in both groups

There was no significant difference in TER and SC in different time intervals for rats in the control group ($P > 0.05$). TER for rats in the model group reached to the lowest level at day 14 and then it began to increase gradually; while SC reached to the highest level at day 14 and then decreased gradually; TER at day 7, 14 and 21 for rats in the model group was significantly lower than that in the control group, while SC was significantly higher than that in the control group ($P < 0.05$), as shown in Table 2.

3.5. Average fluorescence intensity of claudin-1 and claudin-2 in different time intervals for rats in both groups

There was no significant difference in the average fluorescence intensity of claudin-1 and claudin-2 in different time intervals for rats in the control group ($P > 0.05$). The claudin-1 and claudin-2 for rats in the model group reached to the highest level at day 14 and then decreased gradually; the claudin-1 and claudin-2 at day 7, 14 and 21 for rats in the model group

Table 1
Changes of activity of myeloperoxidase and WBC in different time intervals for rats in both groups.

Group	Amount	Activity of myeloperoxidase (U/g)			F	P	WBC ($\times 10^9$/L)			F	P
		Day 7	Day 14	Day 21			Day 7	Day 14	Day 21		
Model group	6	4.57 ± 0.59	5.29 ± 0.61	3.17 ± 0.31	25.631	0.000	17.60 ± 3.40	27.10 ± 5.40	12.90 ± 2.10	20.873	0.000
Control group	6	0.57 ± 0.11	0.60 ± 0.14	0.58 ± 0.09	0.106	0.901	4.10 ± 0.80	3.90 ± 0.60	4.20 ± 1.10	0.193	0.829
t		16.325	18.356	19.654			9.467	10.459	8.989		
P		0.000	0.000	0.000			0.000	0.000	0.000		

Table 2
Changes of TER and SC in different time intervals for rats in both groups.

Group	Amount	TER (Ω/cm^2)			F	P	SC (μA/cm^2)			F	P
		Day 7	Day 14	Day 21			Day 7	Day 14	Day 21		
Model group	6	24.5 ± 6.1	17.9 ± 5.9	28.4 ± 7.3	4.046	0.039	153.1 ± 21.5	194.4 ± 37.2	143.0 ± 18.9	6.059	0.012
Control group	6	46.8 ± 13.1	47.4 ± 12.9	47.1 ± 10.8	0.004	0.996	30.9 ± 7.4	31.4 ± 8.7	31.1 ± 7.8	0.006	0.994
t		3.627	5.094	3.514			13.164	10.451	13.406		
P		0.002	0.000	0.003			0.000	0.000	0.000		

Table 3
Average fluorescence intensity of claudin-1 and claudin-2 in different time intervals for rats in both groups.

Group	Amount	Claudin-1			*F*	*P*	Claudin-2			*F*	*P*
		Day 7	Day 14	Day 21			Day 7	Day 14	Day 21		
Model group	6	58.3 ± 13.6	79.4 ± 14.8	53.1 ± 12.7	6.177	0.011	67.4 ± 15.30	84.3 ± 17.9	59.4 ± 13.5	3.948	0.042
Control group	6	43.5 ± 10.3	42.2 ± 11.6	41.8 ± 7.9	0.051	0.951	39.7 ± 8.4	41.2 ± 9.7	40.4 ± 8.4	0.043	0.958
t		2.125	4.846	1.851			3.887	5.185	2.927		
P		0.030	0.000	0.047			0.002	0.000	0.008		

was significantly higher than that in the control group ($P < 0.05$), as shown in Table 3.

4. Discussion

According to foreign researches [7,8], the colonic mucosa played a key role in the pathogenesis of inflammatory bowel disease. The normal colonic mucosa has the tight junction permeability and its integrity of function can maintain the normal histomorphology of colonic mucosa to prevent the invasion of bacteria. The permeability for patients with the acute inflammatory bowel disease may always be increased because of the injured colonic mucosa and thus the integrity of intestinal epithelium will be destroyed. Petit *et al.* [9] reported that as long as the loss of the tight junction of intestinal epithelial cells, the permeability of intercellular space would be increased to make the endotoxin and pathogenic bacteria enter into the intestine and systemic circulation through the tight junction. In this study, after the successful modeling, rats in the model group had the significant loss of weight and increase of DAI score. The weight reached to the lowest level at day 14 and then it began to increase; while DAI score reached to the highest level at day 12 and then decreased gradually, which indicated that rats with the inflammatory bowel disease entered in the chronic recovery stage after experiencing the acute mucosa injury stage. Besides, the activity of myeloperoxidase and WBC for rats in the model group all reached to the highest level at day 14 and then began to decrease, which proved that rats in the model group experienced the processes of acute inflammation stage and inflammation subsiding, while rats entered in the chronic recovery stage during the later period. Rao Yanxia *et al.* [10] reported that in case of inflammation in the colon, autoimmune of the organism would up-regulate the expression of tight junction protein and thus recover the function of mucosal barrier, which was in accordance with the findings of this study.

TER was the common physiological index for the evaluation of transportation rate [11,12] and the detection of TER can indirectly reflect the permeability of barrier. Zhang Jianbin *et al.* [13] proved the negative correlation between the cell permeability and TER, namely the increased permeability of cells would lead to the decrease of TER, vice versa. Besides, compared with other indices, TER has the strong specificity. SC is the total of all kinds of transmembrane transport of ions, which can reflect the absorption of ions and nutrients by the epithelial cells [14]. The increased permeability of cells will lead to the significant increase of transportation rate and total amount of all kinds of ions, showing the increase of SC. According to the results of this study, TER at day 7, 14 and 21 for the rats in the model group was significantly lower than that in the control group, while SC was significantly higher than that in the control group, which indicated the increased permeability of colonic mucosa and decreased tight junction of intestinal epithelium of rats with the inflammatory bowel disease. Wang Bo *et al.* [15] induced the rats with the inflammatory bowel disease using the bradykinin and found that the bradykinin could induce the secretion of Cl^- to affect the absorption of Na^+ and water and thus result in the changes of TER and SC. The further results showed that TER for rats in the model group reached to the lowest level at 14 d and then it began to be increased; while SC reached to the highest level at day 14 and then it began to be decreased, which proved that when being in the chronic recovery stage after the stage of acute inflammation, the permeability of intestinal epithelial cells was recovered gradually.

The previous researches had proved that the abnormal expression of claudin was related to the decreased function of colonic mucosa barrier [16,17], but its specific mechanism has not been clear yet. As the important molecular structure of junction cells, the claudin is the general name of a wide class of proteins. By now, there have been 23 claudin proteins at least, where the claudin-1 and claudin-2 are closely related to the inflammatory bowel disease [18,19]. Though the claudin-1 and claudin-2 all belong to the claudin family, but they have the different functions. The claudin-1 is mainly to close the colonic mucosa and its upstream regulatory molecule is β-catenin. The claudin-2 is also named as the pore-forming protein [20] and its increased expression is one of causes of diarrhea. Besides, many cytokines are involved in the regulation of claudin-2. According to the results of this study, the claudin-1 and claudin-2 at 7 d, 14 d and 21 d for rats in the model group were all significantly higher than that in the control group, which indicated that the abnormal expression of tight junction protein was the main mechanism to cause the reduced function of colonic mucosa barrier and increased permeability. Rao Yanxia *et al.* [21] also detected the abnormal changes of claudin during the active stage of inflammatory bowel disease. The claudin-1 and claudin-2 for rats in the model group reached to the highest level at day 14 and then it began to decrease, which indicated that expression of claudin protein was significantly increased in the stage of acute inflammation. After being in the chronic recovery stage, the expression of claudin was decreased gradually, which pointed out that the claudin played a key role in the pathogenesis and prognosis of inflammatory bowel disease.

In conclusion, the inflammatory bowel disease is in the chronic recovery stage after the acute stage. The increased permeability of colonic mucosa and increased expression of tight junction protein of intestinal epithelium are closely related to the occurrence and development of the disease. The tight junction protein plays an important role in the pathogenesis of injured colonic mucosa barrier of inflammatory bowel disease. The limitation of this study is that the relationship between the expression of tight junction protein and disease activity was not

analyzed. Besides, the cause for the tight junction protein to increase the permeability of colonic mucosa has not been clear yet, which will be discussed in the further studies.

Conflict of interest statement

We declare that we have no conflict of interest.

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