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Original Article

Effects of rhein on intestinal transmission, colonic electromyography and expression of aquaporin-3 by colonic epithelium cells in constipated mice

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Abstract: Constipation is one of the most common gastrointestinal diseases in the world. This study was to investigate the effects of rhein on intestinal transmission and colonic electromyography and expression of aquaporin-3 (AQP3) in the colonic mucosa of mice with constipation. The mouse model of constipation was established using the compound diphenoxylate. The first defecation time, the number of stools in the initial 6 hours and the promoting rate of eosin were measured as the bowel transit function. The BL-420F system was used to compare changes in the myoelectrical signals in the colons of the mice. Immunohistochemical analysis was used to detect the expression of AQP3 in the colonic mucosa of mice. Rhein had an obvious effect on the first defecation time and the number of red stool in the initial 6 hours. The first defecation time is reduced, and the number of red stools in 6 hours and the promoting rate of the small intestine were significantly increased after the treatment of rhein. In the rhein group, the slow-wave frequency and slow-wave amplitude of colonic myoelectrical activity were increased, and the mean optical density of AQP3 in the colonic mucosa and the area of positive expression were decreased. In conclusion, rhein can improve motor function and colonic electromyography of constipation mice, and reduce expression of AQP3 in the colonic mucosa, thereby relieving the symptoms of constipation effectively.

Keywords: Constipation, rhein, mice, promoting rate, myoelectrical, colon, aquaporin-3

Introduction

Constipation is a common clinical symptom, with a prevalence of 19% in the general population and 30-40% in those over the age of 65 years [1]. The main symptoms of constipation are generally dry and hard stools, an extension of the defecation interval, straining during defecation, sensation of incomplete evacuation, abdominal distension, abdominal pain [2, 3], and/or three or fewer defecations per week [3]. The overall effect is to exert a deleterious influence on health. Constipation patients with sleep disorders may increase the risk of emotional disorders, such as anxiety and depression [4, 5]. If people only defecate every 3-4 days, they are more at risk for many diseases such as colon cancer, hemorrhoids, irritable bowel syndrome [6, 7]. Constipation also causes symptoms including acne, facial pigmentation and skin rashes as stool remains in the intestines for extended periods of time. Constipation patients can experience neurasthenia symptoms such as irritability and insomnia. For women, chronic constipation causing long-term pelvic muscle stimulation can often result in dysmenorrhea and hysterectomy [8]. Based on these studies [3, 8, 9], constipation increases the risk of anorectal diseases, such as hemorrhoids, anal fissure, rectal prolapse, irritable bowel syndrome, colonic diverticulum and colorectal cancer. Chronic constipation is closely linked to neurological and cardiovascular diseases, such as Alzheimer's disease [5], multiple sclerosis, Parkinson's disease and myocardial infarction [3, 8, 10]. With the changes in diet structure and psychological and social factors in recent years, constipation has undergone a clear upward trend, seriously affecting human health and quality of life, which increases the psychological and economic burden [4, 11-16]. Therefore this issue deserves more

attention. Studies have shown that most of the patients generally use irritant drugs to promote defecation [12, 17, 18]. Nevertheless, approximately 50% of patients with constipation are not satisfied with the current treatment, mainly due to lack of efficacy and side effects of irritant laxatives [2]. Therefore, the search for safer and more effective clinical therapeutic agents with few side effects is of great significance.

Rhubarb is a traditional Chinese herbal medicine with purgative effect and broad clinical applications for solid heat constipation, stagnant abdominal pain and jaundice [19, 20]. Rhein (4,5-dihydroxyanthraquinone-2-carboxylicacid, RH), a anthraquinone glycoside, is the major bioactive component of rhubarb and the main component with purgative activity, which is a single anthracene nuclear class 1, 8-dihydroxy anthraquinone derivative [19]. Studies found that rhein has protective effects on the liver, ameliorating liver fibrosis, as well as preventing and treating diabetic nephropathy (DN), and having anti-tumor, anti-inflammatory, antioxidation, immune suppression, and diuretic effects [19]. In traditional Chinese medicine (TCM), the intestinal bacterial product of rhein, rhein anthrone, has purgative activity and can protect the gastric mucosa and clean the internal environment, as well as having lipid-lowering, weight-reducing, and anti-aging effects [21]. Our previous studies have shown that rhein improves the symptoms of constipation. However, the efficacy and safety and mechanisms of rhein in constipation are not clearly at present. Understanding the effects of rhein on colonic electromyography and expression of aquaporin-3 (AQP3) in constipation will contribute to providing safer and more effective therapeutic agents for constipation.

In this study, the first defecation time, feces grain number and the character of stool, small intestinal propulsion rate, colonic electromyography and expression of AQP3 are performed. The aims of this study are to find a new and effective TCM for treating constipation and demonstrate the mechanism of rhein in constipation.

Materials and methods

Chemicals and reagents

Diphenoxylate compound tablets were acquired from Jiangsu Pharmaceutical Company Limited (batch number: 0805221, 2.5 g each); rhein was acquired from Beijing Ding Changsheng Biotechnology Co. Ltd.; eosin was from Shanghai Yongye Biological Technology Company (CAS no. 17372-87-1); rabbit polyclonal antibodies against mouse AQP3 and rabbit sp detection kit were from Beijing Ding Changsheng Biotechnology Ltd; phosphate buffer solution (PBS, 0.01 M, pH = 7.2-7.4); and DAB chromogenic reagent kit ZLI-9018 were from Chinese Fir Jinqiao Biological Technology Co. Ltd. Beijing (batch number: K1352222E).

Reagent preparation

Two tablets of diphenoxylate were crushed and mixed into a suspension in distilled water to a volume of 5 mL, at a dosage of 0.1 mL/10 g body weight. Fifty milligrams of rhein was accurately weighed, added to 10 mL of distilled water, mixed evenly, and then administered to mice of the rhein group by gavage treatment, at a dosage of 0.1 mL/10 g body weight. A solution of 2 g eosin dissolved in 10 mL distilled water was administered with the mixed suspension, at a dose of 0.1 mL/10 g.

Animal experiments

Ninety healthy mice, weighing 22-30 g, in equal numbers of males and females, were provided by Xinxiang Medical College animal center.

Experimental groups: Thirty mice were randomly divided into three groups: control group, constipation group, and rhein-treated constipation (rhein) group, n = 10/group, which were then fed adaptively for one week.

Mice were fasted for 24 h but were allowed to drink water, and then the constipation and rhein groups were treated with 0.1 ml/10 g compound diphenoxylate suspension by gavage to create the constipation mouse model. The control group was administered distilled water, at a dosage of 0.1 mL/10 g. Mice were observed daily, and excrement morphology, as well as the condition of the hair and the weight of the mice was recorded. Based on the observation of mouse stools and the changes in weight, a decision was made to increase or decrease the dose of diphenoxylate appropriately. After successful establishment of constipation model, the constipation and rhein groups were administered compound diphenoxylate suspension (0.1 ml/10 g) and rhein (0.1 ml/10 g) [22], respectively, by gastric gavage, while the control

group was administered distilled water (0.1 ml/10 g). Then, after 0.5 h each group was given eosin at a dose of 0.1 mL/10 g. The interval that the mice excreted the first red stool, traits of the excrement and the number of red stool in the initial 6 hours after administration were recorded.

Mice were treated as described above, and then 25 min after administering eosin, 30 mice were immediately sacrificed by dislocation of the cervical spine. The abdomen was opened and the mesenteries were separated, and then the intestine from the pylorus to the ileocecal junction was removed. The removed intestine was placed in a glass which was gently extended into a straight line. The total length of the small intestine and eosin propulsion length (from the pylorus to the eosin frontier) were measured to determine the eosin advancing rate. The eosin propulsion rate was calculated as: eosin propulsion rate = eosin propulsion length (cm)/the total length of the small intestine (cm) × 100%.

Effect of rhein on the colonic electromyographic activity of constipated mice

Changes in colonic electromyography were examined in the mice of the three groups. After a 24 h fast, the mice were anesthetized by intraperitoneal injection of 20% ethylcarbamate (0.3 mL per mouse). The abdomen was opened by a midline incision of 1.5 cm. A pair of brass wire electrodes (positioned 0.5 cm apart with the two electrodes inserted into the seromuscular layer) were placed in the segment near the colon (approximately 1 cm from the cecum) and were connected to the BL-420F biological function experiment system. After 30 min, the frequency and amplitude of colonic slow waves were recorded. Sensitivity settings: gain of 2 mV, time constant of 0.001 s, high frequency filtering of 20 Hz. The numerical data were continuously recorded for 45 minutes and recordings were repeated three times.

Colonic electromyographic activity parameter analysis

The results of each group for a time period of 3 minutes were analyzed, and the mean amplitude, mean frequency, standard deviation and variation coefficient were calculated.

Slow wave frequency coefficient of variation (%) = standard deviation of the slow wave frequency/mean frequency of slow wave × 100%.

Slow wave amplitude coefficient of variation (%) = standard deviation of the slow wave amplitude/mean amplitude of slow wave × 100%.

Western blot analysis

Colon tissues were removed and the colon mucosa cells were extracted and lysed immediately. Cells were then centrifuged at 12000 rpm for 10 minutes at 4°C. Western blot was carried out as described previously [23]. The primary antibody is rabbit anti-Aquaporin 3 (1:1000 Abcom) and the secondary antibody is horseradish peroxidase-conjugated goat anti-rabbit IgG (1:2500; Zymed).

Immunohistochemical staining

After a 24 h fast, the mice were anesthetized by intraperitoneal injection of 20% ethylcarbamate (0.3 mL per mouse). The colon was immediately removed and fixed in 4% paraformaldehyde solution, dehydrated, paraffin-embedded, and cut into sections of 7 μm thickness. Following conventional dewaxing and rehydration, immunohistochemical staining was performed using rabbit anti-mouse AQP3 antibody (1/200). The expression of AQP3 was analyzed by light microscopy at a magnification of 400 times. Cells containing brown granules were considered positive cells. Six non-overlapping fields of view were recorded and the average optical density value and positive expression rate per area were analyzed by Image pro-Plus 6.0 statistical software.

Statistical analysis

SPSS12.0 was used for statistical analysis of all data. Image pro-Plus 6.0 was used to analyze the immunostaining images. The results were expressed as mean ± SD. The first defecation time, the number of stools in the initial 6 hours, the promoting rate of eosin, colonic electromyography and the expression of AQP3 were analyzed with one-way analysis of variance, followed by Student's t-test for pairs of values. Value differences were considered statistically significant at $P < 0.05$.

Results

Changes in fecal characteristics

Compared with the constipation group, the feces of the control group and the rhein group mice were moister, softer and longer, while in

Table 1. Effects of rhein on the defecation characteristics of mice (mean ± SD)

Group	N	Time of first appearance of red stool (min)	6 h defecation: grain number	Fecal characteristics
Control	10	153.50 ± 15.84#	39.50 ± 2.80#	wet and soft
Constipation	10	280.40 ± 20.20	19.60 ± 3.04	Hard and small
Rhein	10	169.10 ± 19.74#	35.80 ± 2.56#	Soft with increased grain length

Note: Compared with constipation group, #$P < 0.01$.

Table 2. Small intestinal propulsion in mice (mean ± SD) for determination of the rate of change

Group	N	Small intestine length (cm)	Small intestine propulsion length (cm)	Small intestine propulsion rate %
Control	10	66.58 ± 4.89	40.40 ± 4.33	60.73 ± 5.39#
Constipation	10	62.17 ± 2.88	18.84 ± 3.40	30.40 ± 5.91
Rhein	10	65.45 ± 3.13	39.30 ± 2.66	60.22 ± 5.65#

Note: Compared with constipation group, #$P < 0.01$.

Table 3. Colonic slow wave frequency, variation coefficient of frequency and the amplitude and the variation coefficient of amplitude (mean ± SD) in the three groups of mice

Group	N	Frequency (Hz)	Variation coefficient of frequency (%)	Amplitude (mV)	Variation coefficient of amplitude (%)
Control	6	49.67 ± 0.52#	1.04 ± 0#	0.15 ± 0.01*	10.00 ± 2.61#
Constipation	6	43.00 ± 5.18	9.36 ± 3.19	0.07 ± 0.03	33.49 ± 6.90
Rhein	6	49.33 ± 0.52#	0.97 ± 0.13#	0.11 ± 0.01*	7.76 ± 0.18#

Note: Compared with constipation group, *$P < 0.01$; #$P < 0.05$.

the constipation group, the feces of mice were drier, harder, and shorter. In the rhein group, the results showed an obvious increase in defecation, with an increase in grain number within 6 h, while the interval that the mice excreted the first red stool was significantly reduced (**Table 1**).

Impact of the change in rate of small intestinal propulsion

The rate of small intestinal propulsion in rhein group was increased obviously than that in constipation group, showing that the effect of rhein on small intestine propulsion is significant in constipated mice (**Table 2**).

Influence of colonic electromyography changes in mice

In all three groups of mice, the colonic slow wave curve approximated a sine wave. In the control group, the frequency was 49.67 ± 0.52, while in the constipation group the frequency was 43.00 ± 5.18, which was significantly slower compared with the control group ($P < 0.05$). In contrast, in the rhein group, the frequency was 49.33 ± 0.52, showing a significant increase compared with the constipation group ($P < 0.05$). The variation coefficient of frequency in the control group was 1.04 ± 0%, while in the constipation group it showed a highly significant increase to 9.36 ± 3.19% ($P < 0.05$). In contrast, the variation coefficient of frequency in the rhein group was 0.97 ± 0.13%, showing a significant decrease compared with the constipation group ($P < 0.05$). The amplitude of the control group (0.15 ± 0.01 mV) and the rhein group (0.11 ± 0.01 mV) were both significantly different to the constipation group (0.07 ± 0.03 mV), ($P < 0.01$). The amplitude variation coefficient of the control group was 10.00 ± 2.61%, while that of the constipation group was 33.49 ± 6.90%, showing a significant increase compared with the control group, ($P < 0.05$). The amplitude variation coefficient of the rhein group was 7.76 ± 0.18%, and, compared with the constipation group, the colon slow wave amplitude variation coefficient decreased significantly in the rhein group, ($P < 0.05$). The detailed results are shown in **Table 3** and **Figure 1**.

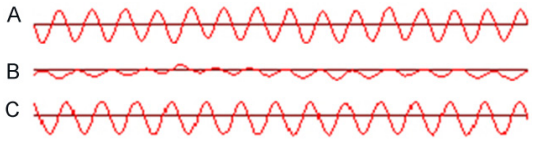

Figure 1. Colonic myoelectrical activity in the three groups of mice. Representative results are shown. Similar results were obtained in six distinct experiments. (A: Control; B: Constipation; C: Rhein).

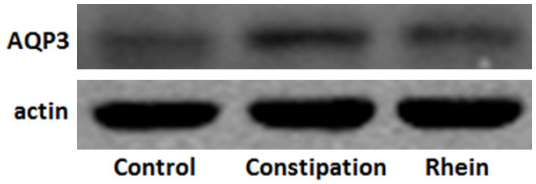

Figure 2. Western blot analysis for expression of AQP3 in colonic epithelial cells of the three groups. Total protein (30 ug) was electrophoresed and subjected to immunoblot analysis with rabbit anti-Aquaporin 3, detected a single band at 31 kDa.

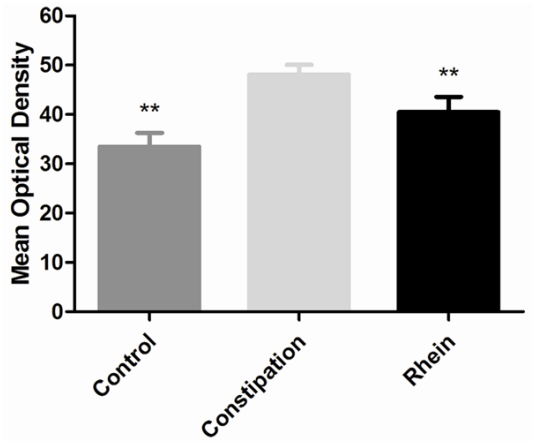

Figure 3. The average optical density of AQP3 expression in colonic epithelial cells of the three groups. Bars represent the mean ± SD. $^{**}P < 0.01$ VS. constipation group.

AQP3 expression in colonic mucosa

The AQP3 expression levels in the colonic mucosa were detected by Western blot and immunohistochemical technique. As shown in **Figure 2**, rhein decreased the expression of AQP3 in colonic mucosa cells compared with the constipation group. Our study also showed that AQP3 was expressed in the control group, with a mean optical density of 33.454 ± 2.826, while in the constipation group it was 48.089 ± 1.987, showing a significant difference in expression between the two groups ($P < 0.01$). In the rhein group, the mean optical density was 40.455 ± 3.118, which was significantly reduced compared to the constipation group ($P < 0.01$). In the control group the positive expression rate of area was 23.185 ± 1.514, showing a significant difference ($P < 0.01$) compared with the constipation group (33.502 ± 1.651). In the rhein group, the positive expression rate of area was 28.141 ± 1.669, showing a significant decrease compared with the constipation group ($P < 0.01$). The results of the average optical density of AQP3 in the colonic mucosa and the positive expression rate of area and images of typical stained areas are shown in **Figures 3-5**.

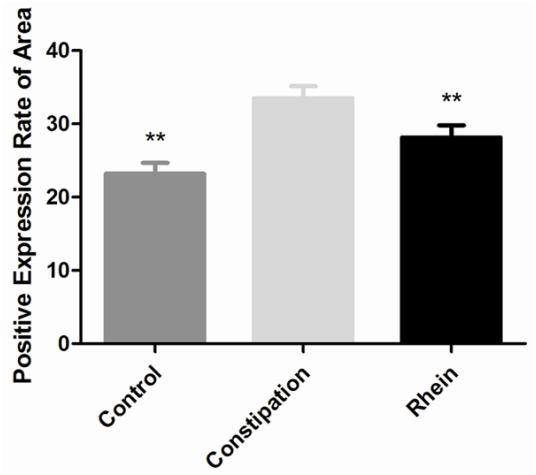

Figure 4. Positive expression rate per area in the three groups of mice. Bars represent the mean ± SD. $^{**}P < 0.01$ VS. constipation group.

Discussion

Constipation is a clinically common disorder with complex symptoms. Its main sign is reduced defecation, dry and hard stools. Patients with constipation are difficult and laborious to defecate. Constipated patients often suffer from abdominal pain, discomfort, insomnia, frequent dreams, depression, anxiety and other mental disorders. Its etiology is complex, and can be divided into organic and functional aspects. Nowadays, many people are affected particularly by changes in lifestyle, nature of work and time changes and they may also have eating disorders or mental illness. The incidence of constipation increases with age, and primarily occurs among the elderly [1, 6]. However, nowadays the onset of disease is

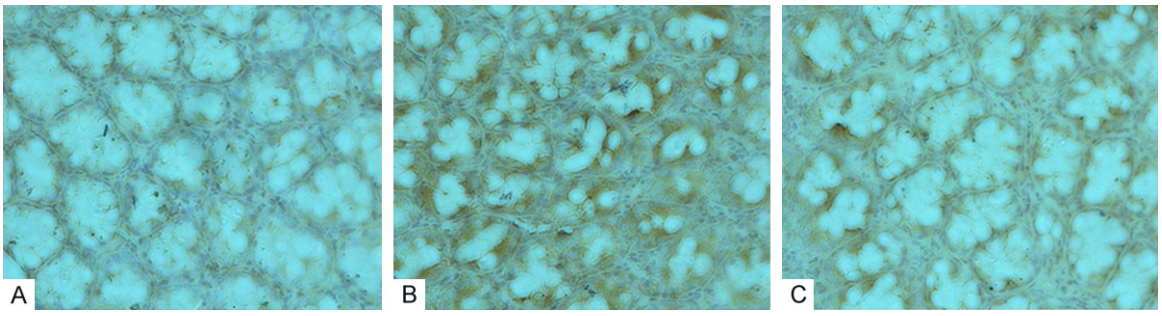

Figure 5. Immunohistochemical localization of the main sites of AQP3 expression in the colonic mucosa. Brown particles indicate sites of AQP3 expression. Representative results are shown. Similar results were obtained in six distinct experiments. (A: Control; B: Constipation; C: Rhein (SP × 400)).

apparently occurring at an earlier age. Constipation can do a great deal of harm. Constipation warning signs include blood in the stool, black stool, anemia, fever, abdominal pain, and family history of cancer. Dry and hard stool can accumulate in the bowel of a patient with constipation, causing damage to the intestinal mucosa and causing hemafecia, anal fissures and hemorrhoids. It can also cause abdominal pain, abdominal distension, indigestion, dizziness, headache, fatigue and other symptoms. Additionally, constipation increases the risk of cancer, cardiovascular disease, diabetes, immune disorders, skin disease, premature aging, rheumatism, parasitic disease, and disorders of the female menstrual cycle and menstrual cramps. Constipation can cause a lot of complications and can be a serious life-threatening condition. Consequently, it is particularly important to look for positive, effective drugs with few side effects to relieve constipation. At present, Chinese patent medicines are welcomed by more people because of their reliable curative effect, reduced risk of side effects and convenience.

Rhein belongs to the hydroxyl anthraquinone derivative group of medicines and is brown acicular crystal. It is one of the main highly active components of plants of the polygonaceae family, which include medicinal rhubarb, Palmate rhubarb (*Rheum palmatum*) or Tangute rhubarb (*Rheum palmatum var. tanguticum*). Rhubarb is one of the most widely used Chinese medicinal herbs in China. It is famous for causing diarrhea and reducing fevers, and is widely used clinically. Rhein is one of the main active substances in rhubarb. The rhein biotransformation product rhein anthrone, produced by intestinal bacteria, has the effect of causing diarrhea and can reduce the colonic absorption of sodium and chloride ions, and increase the secretion of potassium ions. At present, rhein is the only type of new drug that has been accepted by the national Ministry of Science and Technology as one of the “fifteen” key state science and technology projects, and as a result rhein has become a hot spot of research at home and abroad. Rhein is the best raw material that can be used in the preparation of health care products to reduce fat and lose weight, clean the internal environment, prevent gastric cancer, protect the gastric mucosa, protect against the effects of aging and so on. The fields in which it is involved are gradually expanding.

In our experiments, after successfully establishing a constipation model, we used rhein as a gavage treatment in the rhein group. After a week, the interval of the first red stool, the number of stools in the first 6 hours, fecal characteristics, the promoting rate of eosin, colonic myoelectrical activity and the expression of AQP3 in colonic mucosa were measured. The effects of rhein on the intestinal transmission function, colonic electromyography and the expression of AQP3 in the colonic mucosa of constipated mice were discussed.

The results show that, compared with the control group, the time to first defecation of the constipation group was obviously prolonged, the number of stools in the initial 6 hours of the constipation group obviously reduced, the promoting rate of eosin in the constipation group obviously decreased, and mice in the constipation group excreted dry and tiny stools, with curled and lackluster hair and weight loss. The results demonstrate that the constipation

model was successful. Compared with the control group, the time to first defecation in the rhein group was obviously reduced, and the number of stools in the initial 6 hours and the promoting rate of eosin in the rhein group obviously increased, and the mice in the rhein group excreted soft and moist stools with soft and shiny hair. These results show that rhein has a significant enhancement of intestinal transit function, lubricating the bowel and promoting defecation.

Slow wave potential is also known as basic electrical rhythm. It is the specific electrical activity of smooth muscle in the digestive tract and is formed by spontaneous depolarizations of the cellular pacemaker. Slow wave is local potential, deriving from the longitudinal muscle, which cannot directly lead to smooth muscle contraction. However, the action potential can only be produced on the basis of the potential of the slow wave, so slow wave is the starting potential of smooth muscle. This slow and rhythmic depolarization wave is produced on the basis of the resting potential and controls the rhythm of smooth muscle contraction, which is related to the activity of the cell membrane electric sodium pump. Colonic electromyography action potential arises from the slow wave potential, consistent with the smooth muscle contraction, which is the principal mechanism of promoting activity of the colon. Slow waves are relatively regular waves of periodic electrical activity and are always present whether or not the muscle contracts [24]. They can indirectly reflect the strength of the colon. Therefore, the colonic frequency and amplitude of a slow wave can be used as an objective electrophysiological index to reflect the function of the colon. The variation coefficient expresses the degree of dispersion which is used to reflect the frequency and amplitude in different periods within the same group and to measure the stability of the colonic activity rhythm in this experiment [25]. The results showed that the frequency and the amplitude of slow wave were decreased in the constipation group compared with the control group. These changes of frequency and amplitude can lead to difficulty in creating the action potential, thus subduing the smooth muscle contraction of the digestive tract, and depressing intestinal transmission. The stool accumulates in the intestinal tract and is not easy to excrete, which results in constipation. The coefficients of variation (CV) of the frequency and amplitude in constipation group were increased, with significant differences from the control group. The results suggest that the myoelectric activity of colon in constipation mice is disordered. Compared with constipation group, the frequency and amplitude of rhein group were increased, which suggests that rhein may promote to produce the action potential by raising the colonic electromyography frequency and amplitude, accelerating the rhythm of smooth muscle contraction, increasing intestinal peristalsis and transfer function, increasing defecation power, and accelerating the discharge of the stool from the colon. Our results showed that the colonic transmission function of the rhein group was enhanced. This may be related to the effect of the rhein intestinal bacterial product rhein anthrone in reducing colonic absorption of sodium and chloride ions and increasing the secretion of potassium ions. The colonic cell membrane sodium pump activity may also change as a result of the changes in colonic slow wave frequency and amplitude. Rhein has the effects of decreasing the slow wave frequency variation coefficient and slow wave amplitude variation coefficient in constipated mice. The results indicate that the stability of the colonic electrophysiological activity is increased, suggesting that rhein effectively improves the colonic peristalsis and promotes the stabilization of slow wave rhythm. Rhein effectively relieves constipation in the short term, but whether long-term usage will lead to contraction rhythm disorders due to the long-lasting increase of the number of colon contractions by rhein, and therefore further study is still required.

AQPs are a type of specific integrated membrane protein family with water-transporting function that are widely distributed in the human body and are continuously open. The transport of water through AQPs is much faster than the speed of simple diffusion, and consequently they play an extremely important role in trans-shipment and absorption of body fluids in vital tissues and organs, so the expression of aquaporins may be closely related to the physiological and pathological process of certain organs. AQP3, a subtype of the aquaporin family, is located on human chromosome 9p13 with 4 exons and 3 introns. AQP3 is composed of 292 amino acids with a molecular weight of 31544 Da. Meanwhile, AQP3 is predominantly expressed in mucosal epithelial cells of the

colon, ultimately regulating the water transport and fecal water content [26-28]. AQP3 is one of the most important functional molecules in water transport in the colon [27]. Its main function is to transfer water and solutes in the intestines from the basal side to the intercellular space, and reduce the moisture inside the colon [29]. Therefore, the intensity of expression of AQP3 in the colonic mucosa is closely related to fecal characteristics. Our experimental results show that in the constipation group compared with the control group, the average optical density value of AQP3 expression increases, and the area of positive expression increases, and that both these changes are very significant. This suggests that one of the causes of constipation is the enhanced expression of AQP3. With increased expression, the reabsorption of colonic water is increased, and colonic water is reduced. In the rhein group, compared with the constipation group, the average optical density value of AQP3 expression was reduced, and the area of positive expression also decreases. This shows that the intensity of AQP3 expression in the colonic mucosa is subdued after treatment with rhein. Thus it can be seen that one of the mechanisms involved in the treatment of constipation with rhein is to reduce colonic mucosal AQP3 expression, increase colonic water, soften the stool, lubricate intestinal contents, and reduce resistance. Therefore, rhein plays an important role in alleviating constipation.

Anthraquinones are known to induce diarrhea, and rhein belongs to the anthraquinone family. However, the mechanism of rhein in treating constipation is not fully understood. Vasoactive intestinal peptide (VIP) is a major inhibitory neurotransmitter in the digestive system and is present in the central nervous and the enteric nervous system, with the highest content in the duodenum and colon [30]. VIP has a strong role in promoting the secretion of colonic water and electrolytes, and has an inhibitory effect on contraction of the digestive tract smooth muscle. AQP3 is one of the target molecules of VIP action. VIP can change the content of aquaporins through the cell protein kinase A system , thereby adjusting the permeability of the membrane to water [31]. In the mouse constipation model, the content of VIP in the small intestine and colon is reduced [31, 32], suggesting that the development of constipation may be related to pathological changes in the VIP peptidergic nerves related to the content of VIP or the dysfunction of AQP3 expression. These changes may increase segmental peristalsis of the gut, which significantly decreases the propelling peristalsis observably, leading to constipation. This change in the frequency and amplitude of the slow waves may explain the weak colonic function of constipated mice. Studies have demonstrated that rhubarb increases the content of rat motilin (MTL) and substance P (SP), and decreases the content of the VIP [32]. We can therefore speculate that rhubarb may have effects on MTL, SP and VIP through the action of rhein to increase the secretion of gastrointestinal hormones and enhance the propulsive function of the colon. The colonic propulsion may be enhanced through the change in the colonic slow wave frequency and amplitude. In this experiment, the colonic mucosal expression of AQP3 was reduced in the rhein group. It can be speculated that rhein may regulate the content of VIP and downgrade the expression of AQP3 in the colonic mucosa. The apical membrane of the epithelial cells contains sodium channels, the basement membrane contains Na-K-ATPase, and thus we can speculate that rhein may modulate the content of sodium channel protein and Na-K-ATPase in the membrane. As a result, the speed of actively transporting sodium by the Na-K-ATPase from inside epithelial cells to the stroma of epithelial cells is reduced, so the deficit of sodium concentration in cells is reduced. It reduces intracavitary water and sodium into the epithelial cells through the top of the plasma membrane AQP3 and sodium channel protein. Consequently, the amount of water reuptake is reduced, and water within the intestinal lumen is increased. This has the effect of softening the stool, increasing the intestinal content and intestinal peristalsis, and reducing the resistance to defecation, so that it helps to relieve constipation. However, the effects of rhein on the other subtypes of aquaporins and on gastro-intestinal hormones need to be further studied.

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Disclosure of conflict of interest

None.

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