Pharmacodynamic Experiment of Dachengqi Decoction and Separated Decoction on Incomplete Intestinal Obstruction in Rats

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Abstract [**Objectives**] To investigate the effect and mechanism of Dachengqi Decoction and separated decoction on incomplete intestinal obstruction in rats. [**Methods**] 80 healthy SD rats were selected to establish incomplete intestinal obstruction model by silk ligation. The dosage was 20 mL/kg for 3 d, and the damage index of ileocecal mucosa was analyzed; the morphology of ileocecal mucosa was observed by HE staining; the serum levels of IL-1α, IL-1β, IL-6, IL-18, Ach, NO, ET, IL-1, TNF-α and ultra-micro Na^+-K^+-ATPase were detected by ELISA. [**Results**] Compared with the model group, the mucosal damage index of Dachengqi Decoction and each separated decoction group decreased significantly ($P<0.05$); compared with the normal group and sham operation group, the serum level of IL-1, IL-6, TNF-α and other factors in the model group increased significantly ($P<0.05$); compared with the model group, the serum IL-1, IL-6 and TNF-α secretion levels of rats in Dachengqi Decoction group and separated decoction group decreased ($P<0.01$). [**Conclusions**] Dachengqi Decoction and each separated decoction can effectively improve intestinal tissue pathological damage in the incomplete intestinal obstruction model rats, and reduce the inflammatory reaction in the rat body.

Key words Dachengqi Decoction, Separated decoction, Incomplete intestinal obstruction, Inflammatory factor

1 Introduction

Dachengqi Decoction comes from *Treatise on Febrile Diseases*. It is a representative prescription for diarrhea. It is composed of rhubarb, bark of magnolia, citron fruit and Glauber's salt. In the prescription, rhubarb purges heat and relaxes the bowels, cleanses the gastrointestinal tract, and is considered to be the sovereign drug[1-2]. Glauber's salt assists rhubarb in purging heat and relaxing the bowels, can soften hardness and moisten dryness, so it is chosen as the minister drug. The two medicines work with each other, and the power of drastically purgating heat is very strong; accumulation of internal resistance will lead to impassability of visceral qi, so bark of magnolia and citron fruit are used to promote the circulation of qi and remove stasis, and assist Glauber's salt and rhubarb in accelerating the disappearance of heat accumulation, and they act as assistant drug and courier drug. Intestinal obstruction refers to the movement disorder of the intestinal wall muscles caused by any reason, the intestinal contents are unable to pass through, unable to run, and stay in the intestinal lumen[3-4]. Intestinal obstruction is a common disease in surgery, and it is also a common complication accompanied by various abdominal diseases. Its etiology is complex, the condition is changeable, and the development is rapid[5]. Intestinal obstruction includes complete, incomplete and partial intestinal obstruction. Different types of intestinal obstruction can be transformed under certain conditions. Incomplete and partial intestinal obstruction can develop into complete intestinal obstruction due to inflammation and edema or untimely treatment, causing intestinal wall congestion, edema, necrosis, perforation, and even infection and hypovolemic shock, endangering human health and endangering life[6].

The indications of Dachengqi Decoction are consistent with the clinical manifestations of incomplete intestinal obstruction, and a large number of researches and clinical studies have shown that it has a good therapeutic effect on incomplete intestinal obstruction[7]. The purpose of this study was to verify the efficacy of Dachengqi Decoction on incomplete intestinal obstruction.

2 Materials and methods

2.1 Materials

2.1.1 Drugs and reagents. Interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-18 (IL-18), interleukin-1α (IL-1α), interleukin-1β (IL-1β), Acetylcholine (Ach), nitric oxide (NO), endotoxin (ET) kit, tumor necrosis factor (TNF-α), ultra-micro Na^+-K^+-ATPase, Coomassie brilliant blue protein kit, were all purchased from Nanjing Jiancheng Institute of Bioengineering.

Rhubarb, citron fruit, bark of magnolia and Glauber's salt were purchased from Guangxi Xianzhu Technology Co., Ltd., and were all authenticated by Zhong Wen, chief pharmacist of traditional Chinese medicine with the Guangxi International Zhuang

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2.1.2 Laboratory animals. SPF SD rats were purchased from the Experimental Animal Center of Guangxi Medical University, weighing (180 ± 20) g, with equal numbers of both sexes, production license number: SCXK Gui 2014-0002, animal quality certificate number: 0001757. Rats were kept in the laboratory animal room, with humidity (70 ± 2)%, room temperature (25 ± 2) ℃, the animals were routinely reared, eating and drinking freely.

2.1.3 Instruments. UV-1800 ultraviolet spectrophotometer (Shimadzu, Japan), 20110793 full-wavelength microplate reader (BIOTEK-Epoch, USA), cryogenic centrifuge (Shanghai Anting Scientific Instrument Factory, China), PRACTUM224-KN SOP electronic analysis balance [Sartorius Scientific Instruments (Beijing) Co., Ltd.].

2.2 Method

2.2.1 Drug preparation. Rhubarb, citron fruit, bark of magnolia, and Glauber's salt were taken according to the prescription ratio, extracted and finally concentrated to a liquid medicine concentration of 0.3∶1 (containing 0.3 g/mL of crude drug), and placed in a refrigerator at 2 – 8 ℃ for storage. The medicine solution was prepared according to the same method, the medicine solution was concentrated to a concentration of 0.5∶1 (containing 0.5 g/mL of crude drug), and stored in a refrigerator at 2 – 8 ℃ for later use.

2.2.2 Animal modeling. Rats were anesthetized with 10% chloral hydrate (300 mg/kg), skin-prepared, disinfected with iodine, deiodinated with alcohol, and laparotomy was performed on 1/3 of the abdominal line to expose the ileocecal area. A central venous catheter with a diameter of 3.2 mm was placed side by side with the ileal intestinal tube, and a circular needle and silk thread were used to penetrate the mesentery 1 cm away from the ileocecal region to bypass the intestinal tube and bind with the central venous catheter. Finally, the central venous catheter was withdrawn to cause incomplete intestinal obstruction, and then the abdominal wall was sutured layer by layer[8]. In the process of modeling, gentle manipulation must be adopted to reduce the irritation to the rat intestine. The aseptic operation procedure should be strictly followed during the whole operation process, and the rats after operation should return to the conventional feeding conditions. For the sham operation group, the modeling steps were the same as normal modeling, but only a thread was used to penetrate the rat's mesentery without ligation during the operation, and no treatment was performed in the normal control group. In the preparatory stage, the rats in the model group, sham operation group and Dachengqi decoction group all had the possibility of death.

2.2.3 Animal grouping and administration. 80 rats were taken, weighing (180 ± 20) g. After being adapted for 3 d in the animal room, the rats were randomly divided into normal control group, model group, sham operation group, positive control group, rhubarb group, rhubarb-Glauber's salt group, rhubarb-Glauber's salt-bark of magnia group and Dachengqi Decotion group, with 10 rats in each group. Rhubarb group, rhubarb-Glauber's salt group, rhubarb-Glauber's salt-bark of magnolia group and Dachengqi Decoction group contained 5, 10, 15 and 20 g/kg of crude medicine, respectively. The dosage of Dachengqi Decoction was 20 g per kilogram of rat body per day, and the dosage by gavage was 20 mL per kg of body. The dosage of Dachengqi Decoction was the clinical equivalent dosage, and it was converted into the experimental equivalent dosage by the body type coefficient standard; normal control group, model group, sham operation group were given the same dosage of normal saline, and positive control group (Phenolphthalein tablet, 60 mg/kg) was set.

2.2.4 Model replication and evaluation criteria. Referring to the *Chinese Medicine Pharmacological Research Methodology* edited by Chen Qi, sham operation was used to replicate the rat model of incomplete intestinal obstruction. The following symptoms of incomplete intestinal obstruction in rats are considered successful in modeling: abdominal distension, abdominal pain, stopping venting and defecation, drinking water, amount of feces, urine output, stool dryness, mental state and activity, *etc.*

2.2.5 Sample collection. Each experimental group was administered the drug 0.5 h before modeling and for 4 consecutive days after modeling, and the blood was collected from the abdominal aorta of the rats. Sera were collected and used for the detection of experimental animal indexes. Then the colon and ileocecal region of rats in each group were preserved in 10% paraformaldehyde and fixed, and prepared with histopathological sections.

2.2.6 Index measurement. The determination of related inflammatory factors in serum (acetacholine Ach, NO, IL-1α, IL-1β, IL-18, IL-6, ET, IL-1, TNF-α), ileocecal mucosa damage index, ileocecal mucosa tissue staining HE microscopic examination, relevant biochemical indexes of colon and liver tissue, Na^+-K^+-ATPase activity of cells in the colonic wall, and Na^+-K^+-ATPase in liver were all performed in strict accordance with the instructions of the kit.

2.2.7 Statistical analysis. SPSS 23.0 software was used for statistical analysis. The experimental data were all expressed in $\bar{x} \pm s$, and one-way ANOVA was used. If the variance was homogeneous, the *LSD* method was used for group comparisons, and if the variance was not homogeneous, the Dunnett's T3 method was used for group comparisons. $P < 0.05$ means the difference is statistically significant.

3 Results and analysis

3.1 Changes of intestinal mucosa in ileocecal region of rats in different groups In the experiment, it was observed that the ileocecal intestinal cavity of SD rats in the model group was dilated, congested, the peristaltic function was decreased or even lost, and the color seemed dull. In addition, inflammatory mucus was attached to the intestinal wall of rats, and these characteristics together indicated severe pathological damage in the ileocecal region of rats, suggesting the need for drug therapy. There were no obvious abnormalities in the ileocecal intestinal mucosa of SD rats in

the normal and sham operation groups. In the experimental group, the intestinal mucosa of the ileocecal region of SD rats was less damaged. When comparing the Dachengqi Decoction group and its separated decoction groups with the model group, it could be found that the morphological changes of the ileocecal intestinal mucosa of SD rats were not significant.

3.2 Microscopic observation and comparison of ileocecal intestinal mucosa of rats in different groups The intestinal mucosa in the ileocecal region of SD rats in the normal and sham operation groups had no significant changes, and the structure was normal. The intestinal mucosa of the ileocecal part of SD rats in the model group showed exudation, the intestinal mucosa epithelial cells were swollen, the cell boundaries were unclear, the interstitial tissue had obvious edema, and a large number of inflammatory cells infiltrated the intestinal mucosa of ileocecal part. In some parts, necrosis and shedding appeared in epithelial cells. Compared with the model group, the swelling degree of intestinal mucosal epithelial cells of SD rats in the experimental group of Dachengqi Decoction and separated decoction was reduced, the infiltration of inflammatory cells was significantly reduced, edema and exudation were not obvious, and necrosis and shedding of intestinal mucosal epithelial cells in the ileocecal region of rats were not found. The SD rats in Dachengqi Decoction group suffered the lightest intestinal mucosa damage in the ileocecal region.

3.3 Comparison of damage index of ileocecal mucosa in different groups In the experiment, the ileocecal mucosa of model group SD rats showed the characteristics of crypt destruction, mucosal edema and inflammatory cell infiltration and other inflammatory damage. Compared with the normal group and sham operation group, the ileocecal region of rats in the model group and the separated decoction experimental group showed intestinal dilation and expansion, and the ileocecal mucosa of SD rats in the model group also showed intestinal adhesion and obstruction and other atrophy phenomena. Compared with the normal group and sham operation group, the damage index of the ileocecal mucosa in the model group significantly increased ($P<0.01$), which indicated that the experimental model was built successfully. Compared with the model group, the damage index of ileocecal mucosa of rats in the Dachengqi Decoction and separated decoction experimental groups decreased significantly ($P<0.05$) (Table 1).

Table 1 Comparison of damage index of ileocecal mucosa and serum levels of Ach and NO in rats in different groups ($\bar{x}\pm s$, $n=10$)

Group	Mucosal damage index/points	Ach // pmol/L	NO // μmol/L
Normal control	$1.35\pm0.62^{\triangle\#\#}$	$43.30\pm6.18^{\triangle\triangle\#\#}$	$2.38\pm0.62^{\triangle\triangle\#\#}$
Sham operation	$4.06\pm1.15^{**\#}$	$61.18\pm6.50^{**\#\#}$	$3.29\pm1.02^{**\#\#}$
Model	$8.51\pm2.01^{**\triangle\triangle}$	$88.35\pm7.16^{**\triangle\triangle}$	$4.87\pm1.35^{**\triangle\triangle}$
Positive control	$5.39\pm1.62^{*\#}$	$40.17\pm4.28^{**\triangle\triangle\#\#}$	$2.41\pm0.80^{**\triangle\triangle\#\#}$
Rhubarb (sovereign)	$3.23\pm0.86^{*\#\#}$	$64.40\pm5.21^{**\#\#}$	$3.95\pm0.96^{**\#\#}$
Rhubarb-Glauber's salt (sovereign and minister)	$3.58\pm1.29^{*\#\#}$	$60.93\pm6.30^{**\#\#}$	$3.62\pm1.04^{**\#\#}$
Rhubarb-Glauber's salt-bark of magnolia (sovereign, minister, assistant)	$4.42\pm1.38^{*\#\#}$	$55.13\pm5.12^{*\#\#}$	$2.81\pm0.83^{*\#\#}$
Dachengqi Decoction (sovereign, minister, assistant, courier)	$5.21\pm1.91^{*\#}$	$48.09\pm4.01^{\triangle\#\#}$	$2.53\pm0.57^{\triangle\#\#}$

NOTE Compared with normal control group, $^{*}P<0.05$, $^{**}P<0.01$; compared with sham operation group, $^{\triangle}P<0.05$, $^{\triangle\triangle}P<0.01$; compared with model group, $^{\#}P<0.05$, $^{\#\#}P<0.01$. The same below.

3.4 Comparison of the content of Ach and NO in different groups Compared with the normal control group, the serum levels of Ach and NO in SD rats significantly increased ($P<0.05$); compared with the model group, the serum levels of Ach and NO in rats in Dachengqi Decoction and separated decoction group significantly decreased ($P<0.05$); compared with the sham operation group, the levels of Ach and NO in SD rats in Dachengqi Decoction and separated decoction group were significantly reduced ($P<0.05$). The results are shown in Table 1.

3.5 Comparison of serum levels of IL-1α, IL-1β, IL-6, IL-18 in different groups The levels of IL-1α, IL-1β, IL-6, IL-18 and eNOS in SD rats of model group were significantly higher than those of normal control group and sham operation group ($P<0.01$); compared with the model group, the serum levels of IL-1α, IL-1β, IL-6, IL-18 and eNOS in the SD rats in Dachengqi Decoction and separated decoction group decreased to different degrees ($P<0.01$) (Table 2).

Table 2 Comparison of serum IL-1α, IL-1β, IL-6, IL-18 expression levels in different groups ($\bar{x}\pm s$, $n=10$, pg/mL)

Group	IL-1α	IL-1β	IL-6	IL-18
Normal control	$6.48\pm1.75^{\triangle\#\#}$	$8.74\pm2.12^{\triangle\#\#}$	$8.81\pm1.06^{\triangle\#\#}$	$10.43\pm1.24^{\triangle\#\#}$
Sham operation	$11.54\pm1.10^{*\#\#}$	$12.65\pm1.83^{*\#\#}$	$12.95\pm1.08^{*\#\#}$	$13.21\pm1.64^{*\#\#}$
Model	$43.86\pm5.13^{**\triangle\triangle}$	$37.02\pm5.21^{**\triangle\triangle}$	$32.89\pm3.82^{**\triangle\triangle}$	$35.08\pm3.16^{**\triangle\triangle}$
Positive control	$15.68\pm2.62^{*\triangle\#\#}$	$15.49\pm2.30^{*\triangle\#\#}$	$14.92\pm2.01^{*\triangle\#\#}$	$14.78\pm1.69^{*\#\#}$
Rhubarb (sovereign)	$38.21\pm0.86^{**\triangle\triangle\#}$	$35.06\pm1.82^{**\triangle\triangle}$	$30.08\pm1.63^{**\triangle\triangle}$	$31.18\pm3.02^{**\triangle\triangle\#}$
Rhubarb-Glauber's salt (sovereign and minister)	$34.67\pm2.25^{**\triangle\triangle\#}$	$30.81\pm3.28^{**\triangle\triangle\#}$	$27.05\pm2.54^{**\triangle\triangle\#}$	$28.58\pm3.21^{**\triangle\triangle\#}$
Rhubarb-Glauber's salt-bark of magnolia (sovereign, minister, assistant)	$24.52\pm2.31^{**\triangle\triangle\#\#}$	$23.98\pm2.42^{**\triangle\triangle\#}$	$23.01\pm2.42^{**\triangle\triangle\#}$	$23.85\pm2.12^{**\triangle\triangle\#}$
Dachengqi Decoction (sovereign, minister, assistant, courier)	$16.23\pm2.51^{*\triangle\#\#}$	$16.62\pm2.25^{*\triangle\#\#}$	$15.84\pm2.12^{*\triangle\#\#}$	$15.83\pm1.72^{*\triangle\#\#}$

3.6 Comparison of serum levels of ET, TNF-α and IL-1 in different groups Compared with normal control group and sham operation group, the content of ET and TNF-α in SD model group was significantly changed ($P<0.01$). Compared with the model control group, the content of ET and TNF-α in serum of rats in Dachengqi Decoction and separated decoction group and positive group changed significantly, and the difference was statistically significant (Table 3).

Table 3 Comparison of serum levels of ET and TNF-α in different groups ($\bar{x}\pm s$, $n=10$)

Group	ET//Eu/L	TNF-α//ng/L	IL-1//ng/L
Normal control	0.15±0.08	30.02±2.10	8.39±1.21
Sham operation	0.12±0.051	69.74±3.63	12.91±2.03
Model	0.23±0.06$^{*\triangle}$	38.92±3.50$^{*\triangle}$	31.85±4.05$^{*\triangle}$
Positive control	0.080±0.002$^{\#\#}$	24.54±2.17$^{\#\#}$	14.87±2.11$^{\#\#}$
Rhubarb (sovereign)	0.142±0.025$^{\#\#}$	58.61±2.98$^{\#\#}$	18.64±3.42$^{\#\#}$
Rhubarb-Glauber's salt (sovereign and minister)	0.131±0.011$^{\#\#}$	52.83±3.22$^{\#\#}$	17.20±2.53$^{\#\#}$
Rhubarb-Glauber's salt-bark of magnolia (sovereign, minister, assistant)	0.105±0.006$^{\#\#}$	45.07±2.26$^{\#\#}$	15.63±2.21$^{\#\#}$
Dachengqi Decoction (sovereign, minister, assistant, courier)	0.092±0.005$^{\#\#}$	36.62±1.56$^{\#\#}$	14.02±3.10$^{\#\#}$

3.7 Effect on Na^+-K^+-ATPase activity of cells in the colonic wall and hepatocytes Compared with the normal control group, the Na^+-K^+-ATPase activity of cells in the colonic wall in model group, positive group, Dachengqi Decoction and separated decoction group significantly decreased ($P<0.05$); the ATPase activity of hepatocytes in model group, rhubarb group and rhubarb-Glauber's salt group significantly decreased ($P<0.05$). Compared with the model control group, the activity of ATPase in cells in the colonic wall of rats in rhubarb group, rhubarb-Glauber's salt group and positive control group increased significantly ($P<0.05$); the ATPase activity of hepatocytes in positive control group, rhubarb-Glauber's salt-bark of magnolia group and Dachengqi Decoction group significantly increased ($P<0.05$) (Table 4).

Table 4 Effects on Na^+-K^+-ATPase activity of cells in the colonic wall and hepatocytes ($\bar{x}\pm s$, $n=10$)

Group	ATPase activity of cells in the colonic wall U·mg/prot	ATPase activity of hepatocytes U·mg/prot
Normal control	3.11±0.62$^{\triangle\#\#}$	2.08±1.12$^{\#}$
Sham operation	2.16±1.03$^{\#}$	1.84±1.60
Model	0.94±0.16$^{**\triangle\triangle}$	1.59±1.51$^{*\triangle}$
Positive control	1.66±0.30$^{*\#}$	1.93±2.12$^{\#}$
Rhubarb (sovereign)	1.51±0.31$^{*\#}$	1.51±0.96$^{*\triangle}$
Rhubarb-Glauber's salt (sovereign and minister)	1.43±0.28$^{*\#}$	1.63±1.08$^{*\triangle}$
Rhubarb-Glauber's salt-bark of magnolia (sovereign, minister, assistant)	1.29±0.23$^{*\triangle}$	1.75±1.20$^{\#}$
Dachengqi Decoction (sovereign, minister, assistant, courier)	1.21±0.25$^{*\triangle}$	1.82±1.12$^{\#}$

4 Discussion

Dachengqi decoction and each separated decoction group can improve the inflammatory pathological injury to varying degrees. The serum levels of Ach, NO, IL-1α, IL-1β, IL-6, IL-18, ET, TNF-α and IL-1 in the model control group were significantly higher than those in the normal control group ($P<0.05$). It was found that the release of inflammatory factors in the model control group significantly increased, Dachengqi Decotion and each separated decotion group inhibited the release of Ach, NO, IL-1α, IL-1β, IL-6, IL-18, ET, TNF-α and IL-1 inflammatory factors in SD rats, and the content of inflammatory factors in the serum of SD rats was reduced, thereby alleviating the organ toxicity of SD rats, intestinal dilation, fever and other disease symptoms caused by it.

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