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The Effect of Chaibanliuhe decoction on GAS, MTL and SS in Rat Models with Chronic Renal Failure*

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ABSTRACT Objective: To observe the effect of Chaibanliuhe decoction on the levels of Gastric secrete element(GAS), Gastric dynamic element(MTL), somatostatin(SS) in rat chronic renal failure(CRF) model, and to explore its mechanism. **Methods:** The model of CRF in rats were established by the method of 5/6 nephrectomy. Rats were randomly divided into control group, model control group, high Chaibanliuhe decoction group, low Chaibanliuhe decoction group and Niaoduqing control group. The levels of Scr, BUN, GAS, MTL and SS in serum were detected, and the pathological changes of Gastric mucosa were observed by hematoxylin-eosin (HE) staining. **Results:** Compared with model control group, Scr, BUN, GAS and MTL decreased while SS increased significantly in every administration group. The levels of Scr, BUN, GAS and MTL in the high Chaibanliuhe decoction group were lower than that in the low Chaibanliuhe decoction group and Niaoduqing control group while the level of SS was higher. Compared with that in the Niaoduqing control group, GAS and MTL decreased while SS increased in the low Chaibanliuhe decoction group. There was no significant difference between low Chaibanliuhe decoction group and Niaoduqing control group with the levels of Scr and BUN. **Conclusion:** Chaibanliuhe decoction can improve the gastrointestinal symptoms of CRF in rat model by decreasing the levels of GAS and MTL and increasing the level of SS.

Key words: Chaibanliuhe decoction; CRF rats; GAS; MTL; SS

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Introduction

Gastrointestinal symptoms are the common clinical manifestations of chronic renal failure patients , appear early in the course of the disease, and seriously affect the life quality of patients. Some researches have shown that the changes in the level of many gastrointestinal hormones such as GAS, MTL and SS and the pathological damage in mucous membrane are the foundation of the CRF gastrointestinal symptoms. According to the Traditional Chinese medicine theory of "kidney-stomach related", it is considered that the pathogenesis of chronic renal failure combined with gastrointestinal symptoms is related to the disorder of water metabolism which is caused by kidney deficiency. The disorder of water metabolism can induce endogenous turbid dampness, which can further choke Sanjiao and Shaoyang and cause the obstruction of cardinalate, finally leading to the movement disorder of spleen and stomach. In the treatment, we should pay attention to harmonizing Shaoyang and dredging Sanjiao[1,2]. So in this study, we integrate Xiaochaihu decoction, Banxiaxiexin decoction and Liuweidihuan decoction into Chaibanliuhe decoction to investigate its mechanism on improving the disorder of gastrointestinal functions in CRF by observing the level changes of gastrointestinal hormones and the pathology of gastric antrum before and after treatment.

1 Experimental Materials

1.1 Animals

Eighty male wistar rats of pathogen-free aged 6-8 months, with weight ranging from 240 to 280g, were provided by Qingdao Coastal Institute for Drug Control, certificate of qualified animals: scxk (LU) 20080002.

1.2 Drugs

Chaibanliuhe decoction: the medicine of chaihu, huangqin, jiangbanxia, huanglian, shengjiang, taizishen, baizhu, shengdi, shanyao, shanyurou, tufuling, xianlingpi and danshen is decoction distill and concentrate, provided by traditional Chinese medicine hospital of Qingdao. Niaoduqing granules: producted in Guangzhou pharmaceutical company.

2 Experimental methods

2.1 The method of establishing model and experimental groups

Sixteen rats were randomly selected as control group and the rest 64 rats were used to establish the CRF models by the method of 5/6 nephrectomy. After the CRF models were established successfully, the 64 rats were divided into 4 groups (model control group, high Chaibanliuhe decoction group, low Chaibanliuhe decoction group and Niaoduqing control group) randomly with each group of 16 rats. To establish the disease model, rats were anesthetized by intraperitoneal injection with 10% chloral hydrate (4mL/kg) and fixed on the operating table with supine position. The 2/3 weight of left kidney was removed, and the rats had a rest

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for 1w before we cut the whole right kidney off. The rats of control group were operated as the above but without kidney removed.

2.2 Administration methods

All rats were administered with the method of oral gavage after four weeks the method established, and the gavage continued for 8w. High and low Chaibanliuhe decoction group were administered with Chaibanliuhe decoction 2ml [crude drug 4.34g/(kg·d) and 2.17 g/ (kg·d)]. Dose conversion is according to the method that rat dose is 7 times amount of people in same surface area[3]. Niaoduqing control group were administered with niaoduqing granules 2ml [crude drug 2.0g/(kg·d)]. Control group and model group were administered with 2ml normal saline.

2.3 Targets

To estimate whether the CRF models were established successfully, the levels of Scr and BUN in serum were detected at the end of the fourth week after the operation. All rats were anesthetized by intraperitoneal injection with 10% chloral hydrate (3mL/kg). Then 2ml blood was collected by severing tails of rats and detected by AU640 automatic biochemical analyzer. After eight weeks of administration, the levels of Scr, BUN, GAS, MTL and SS in serum were determined, and the pathological changes of Gastric mucosa were analysed. All rat ventricular were punctured for 6 ml blood. 2 ml blood is used to detect the levels of Scr and BUN; 2 ml blood was added into drying tube to centrifuge for 10 min with 2500rpm in 4℃ to detect the level of GAS; 2 ml blood was blended with 30 μL 500 u aprotinin and 30 μL 10% EDTA to detect the levels of MTL and SS. The levels of GAS, MTL and SS were determined by ABC-ELISA. The stomach of each rat was cut along the greater curvature side. Tissue at antrum or suspected lesion was cut down quickly and be sheared as blocks with the size of 1.0cm × 0.5cm. The tissue cut down was fixed at 10% neutral formaldehyde for 24 hours. After that the tissue was dealt regularly by HE staining and observed under light microscope.

2.4 Statistical analysis

Values are represented as means± standard deviation ($\bar{x}$± s). The data was analysed by SPSS17.0 software and performed by normal test and f test firstly. Two-group comparison was analysed by t-test, and multi-group comparison was analysed by one-way analysis of variance. The difference was considered of statistical significance with $P < 0.05$.

3 Results

3.1 Animal deaths

During the establishment of CRF model, there were 6 rats died in total, 1 in both high Chaibanliuhe decoction group and Niaoduqing control group, 2 in both model control group and low Chaibanliuhe decoction group. In the process of blood collection from severed tails one rat in control group died. And during the period of gavage, there were 9 rats died in total , 1 in high Chaibanliuhe decoction group, 2 in low Chaibanliuhe decoction group, 3 in both model control group and Niaoduqing control group.

3.2 Result of the level of Scr and BUN after the CRF model established

Compared with that in the control group, Scr and BUN increased significantly in control group and every administration group ($P < 0.05$). There was no significant difference between dose groups (Table 1).

Table 1 Results of the level of Scr and BUN

Groups	n	Scr(umol/L)	BUN(mmol/L)
control group	16	50.36± 3.85	3.78± 1.04
model control group	14	62.30± 6.85★	6.71± 2.47★
high chaibanliuhe group	15	61.01± 4.57★	7.13± 1.39★
low chaibanliuhe group	14	59.97± 6.23★	6.29± 1.38★
Niaoduqing group	15	62.02± 5.45★	7.69± 2.01★

Note: Compared with control group ★ $P < 0.01$

3.3 Result of Chaibanliuhe decoction influenced Scr and BUN in CRF rats

Compared with that in the model control group, Scr and BUN decreased significantly in every administration groups; the decrease of Scr and BUN in high Chaibanliuhe decoction group was more pronounced than that in low Chaibanliuhe decoction group ($P<0.05$) and Niaoduqing control group ($P<0.05$); in low Chaibanliuhe decoction group the level of BUN decreased significantly compared with Niaoduqing control group($P <0.05$)(Table 2).

Table 2 Results of chaibanliuhe decoction influenced Scr and BUN in CRF rats

Groups	n	Scr(umol/L)	BUN(mmol/L)
control group	15	53.34± 5.48	4.16± 1.97
model control group	11	93.21± 7.49	15.28± 3.21
high chaibanliuhe group	14	70.61± 6.46▲●◆	9.94± 1.92▲●◆
low chaibanliuhe group	12	76.87± 4.96▲	11.43± 2.82▲◆
Niaoduqing group	12	80.42± 5.45▲	13.07± 2.77▲

Note: Compared with model control group ▲ $P<0.05$; compared with low Chaibanliuhe decoction group ● $P<0.05$;
compared with Niaoduqing control group ◆ $P<0.05$

3.4 Result of Chaibanliuhe decoction influenced GAS, MTL and SS in CRF rats

Compared with that in the model control group, GAS and MTL decreased while SS increased in every administration group, especially in high Chaibanliuhe decoction group; Compared with that in the Niaoduqing control group, GAS and MTL decreased while SS increased in both high (P<0.01) and low (P<0.05) Chaibanliuhe decoction group, and the difference in high Chanbanliuhe decoction group was more significant than that in low Chaibanliuhe decoction (Table 3).

Table 3 Results of Chaibanliuhe decoction influenced GAS, MTL and SS in CRF rats

Groups	n	GAS(pg/ml)	MTL(pg/ml)	SS(pg/ml)
control group	15	35.43± 15.47	80.29± 16.64	17.89± 3.13
model control group	11	62.97± 8.99	123.82± 10.24	4.99± 1.17
high chaibanliuhe group	14	42.15± 10.38▲●◆	93.47± 9.17▲●◆	12.10± 1.90▲●◆
low chaibanliuhe group	12	50.22± 11.69▲◆	101.58± 11.91▲◆	9.03± 2.29▲◆
Niaoduqing group	12	59.14± 12.06▲	117.29± 10.76▲	6.74± 2.73▲

Note: Compared with model control group ▲P<0.05; compared with low Chaibanliuhe decoction group ● P<0.05; compared with Niaoduqing control group ◆P<0.05.

3.5 Result of Chaibanliuhe decoction affect the pathology of Gastric mucosa

The pathological changes of gastric antrum were observed by staining HE under light microscope. In control group, there was no mucosal congestion or edema; and the size of glands was normal without atrophy; only a small amount of lymphocytic invasion in mucous layer could be observed (Fig 1A). In model group, the gastric mucosa with obvious congestion and edema can be observed; the glands decreased with remarkable atrophy; and mucosa, submucosa and muscular layer were infiltrated with a large number of lymphocytes (Fig 1B). Compared with model group, the damage of gastric antrum was relieved in all administration groups, especially in high and low Chaibanliuhe decoction groups. In high Chaibanliuhe decoction group, there was no congestion but slight edema on gastric mucosa; and the glands showed slight atrophy with a normal number; a large number of lymphocyte invasion in mucous layer could be observed (Fig 1C). In the low Chaibanliuhe decoction group, slight congestion and edema gastric mucosa can be observed; the atrophy of glands was more serious than high Chaibanliuhe decoction, but the number of glands was normal; the mucosa and submucosa layer were infiltrated with a large number of lymphocytes (Fig 1D). In the Niaoduqing control group, gastric mucosa showed congestion and edema; the glands decreased slightly and complicated with atrophy; and a large number of lymphocyte invasion in mucosa and submucosa layer could be observed (Fig 1E).

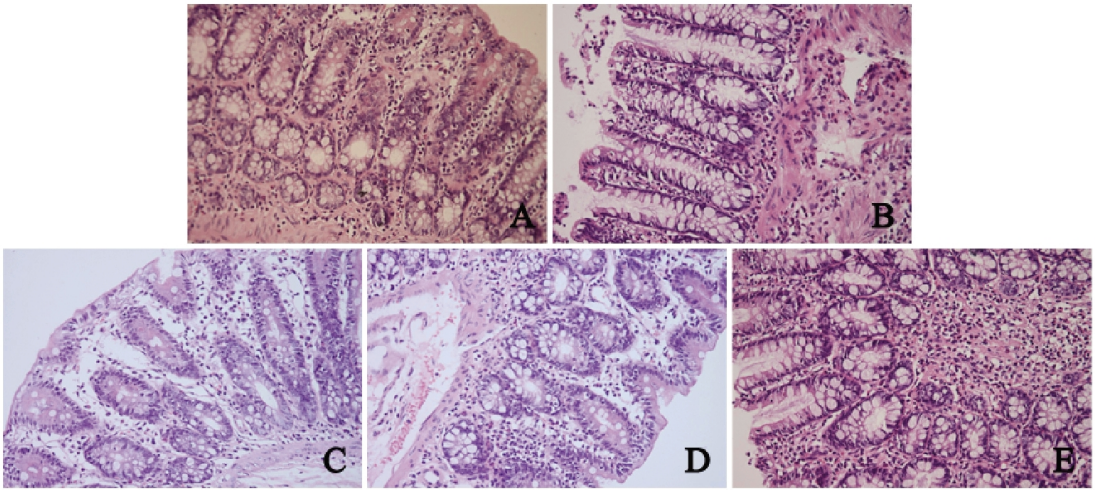

Fig. 1 Pathological changes of gastric antrum in the groups(HE,10× 40): A: control group;B:model group; C: high Chaibanliuhe decoction group; D: low Chaibanliuhe decoction group; E: Niaoduqing control group

4 Discussion

Based on the long-term clinical observation, we think the chronic renal failure is related to Shaoyang disease, and the obstruction of Shaoyang and cardinalate as well as Sanjiao full of turbid dampness is one of the most important pathogenesis of CRF [4]. There are many clinical features such as nausea, hiccups, mouth stick and stay and abdominal distension etc. In the progression of CRF, the deferment of disease, the disorder or slip of visceral functions, the disturbance or deficiency of Yin and Yang, qi and blood, and actual situation mixture are very common. In the treatment, we should follow the principle of compromise, and pay attention to the dredge of Sanjiao and the mediation of Shaoyang in the period of tonifying kidney [5]. So we integrated Xiaochaihu

decoction, Banxiaxiexie decoction and Liuweidihuan decoction into Chaibanliuhe decoction to treat CRF combined with gastrointestinal symptom, and achieved significant effect. Studies of Ye Yong etc have found that Xiaochaihu decoction can promote the gastrointestinal motility [6]. Liu Xiaoxia etc found that Banxiaxiexie decoction can decrease the level of bioactive peptide and P substance while increasing the level of SS in rats, and promote gastrointestinal emptying[7].

The accumulation of poison is an important reason for the appetite decreases in CRF patients. The accumulation of Nitrogen metabolites such as Scr and BUN can cause gastrointestinal symptoms like nausea, vomiting and appetite decreases etc. The results of this study suggest that Chaibanliuhe decoction can decrease the level of Scr and BUN in CRF rats, and compared with model group and Niaoduqing control group the difference was of statistical significance ($P < 0.05$). The results indicate that Chaibanliuhe decoction can improve the renal function and reduce the gastrointestinal symptoms by preventing the accumulation of Nitrogen metabolites.

Gastrointestinal hormone, secreted by the endocrine cells and enteric nervous system neurons which are distributed in gastrointestinal tract, is a kind of bioactive peptides which have a hormone effect [8]. Gastrointestinal hormone is the important factor affecting the gastrointestinal dynamics and its content change will inevitably lead to the dysfunction of gastrointestinal [9]. Studies found that in CRF patients the level of gastrointestinal hormone have changed to varying degrees[10]. The decline in the excretion and degradation function of kidney can cause the disproportionate change of gastrointestinal hormone, further destroy physiological balance and weaken the function of self-regulation, finally leading to the dysfunction of gastrointestinal motility and pathological changes [11]. GAS is mainly secreted by G cells of the gastric antrum and intestine mucosa, and it can not only stimulate the secretion of stomach acid but also promote gastrointestinal movement[12]. GAS is mainly excreted in kidney and many studies have confirmed that the level of GAS can be elevated when the CRF established and the high GAS concentration is associated with the decrease of renal clearance[13].

In CRF, the increase of GAS and the decrease of gastric acid can slow the emptying of liquid and solid food in the stomach but enhance the stomach motor function, finally leading to gastrointestinal dysfunction[14]. The main physiological function of MTL is to affect the peristalsis of stomach and intestinal tract, and it is closely related to Interdigestive Myoelectric Complex (IMC) [15]and can stimulate the secretion of pepsin and pancreatic juice as well as the contraction function of gallbladder [16,17], and plays an role in promoting the digestion. The metabolism and removal of MTL are mainly processed in liver and kidney, so MTL in serum can be accumulated in body when renal function is damaged. The content of SS in gastric antrum is the highest in gastrointestinal mucosa.The experiment and clinical observation showed that SS could inhibit the exocrine function of gastrointestinal tract and pancreas [18] and the secretion of gastric acid and pepsin[19]. D cells in gastric antrum mucosa can secrete SS through paracrine way and inhibit the release of GAS by G cells, thus playing an important role in the protection of gastric mucosa[20]. It is speculated that the gastric mucosa lesion of CRF patients is related with the decrease of SS, the increase of GAS and gastric acid and the decline of glutathione peroxidase activity, which can lead to the lipid peroxidation damage of membrane[21].

The results of this study suggest that both the high and low Chaibanliuhe decoction group can increase the level of GAS and MTL while decreasing the level of SS, and the difference in high Chaibanliuhe decoction group is more significant than that in the low Chaibanliuhe decoction group. It indicated that Chaibanliuhe decoction can regulate the level of gastrointestinal hormone in CRF patients, and that may be one of the mechanisms by which Chaibanliuhe decoction improves the gastrointestinal symptoms in CRF patients. However, whether there are other mechanisms have to be confirmed by further studies.

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柴半六合汤对慢性肾衰竭模型大鼠血清胃泌素、胃动素及生长抑素的影响*

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摘要 目的:观察柴半六合汤对慢性肾衰竭(CRF)模型大鼠血清胃泌素(GAS)、胃动素(MTL)、生长抑素(SS)水平的影响,探讨其作用机制。**方法**:采用 5/6 肾切除的方法复制 CRF 大鼠模型,设立假手术组、模型组、柴半六合汤高剂量组、低剂量组、尿毒清对照组,检测血肌酐(Scr)、尿素氮(BUN)、胃泌素(GAS)、胃动素(MTL)及生长抑素(SS),取胃粘膜行 HE 染色,光镜下观察其组织病理变化。**结果**:用药各组大鼠治疗后 Scr 、BUN 低于模型组;柴半六合汤高剂量组大鼠 Scr 、BUN、GAS、MTL 低于低剂量组、SS 高于低剂量组;柴半六合汤高、低剂量组大鼠 GAS、MTL 低于尿毒清对照组、SS 高于尿毒清对照组。**结论**:柴半六合汤通过降低 CRF 模型大鼠 GAS、MTL 水平、SS 升高水平,改善胃肠道症状。

关键词:柴半六合汤;肾衰竭大鼠;胃动素;胃泌素;生长抑素
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