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Effect of sanhuanghuoxue decoction on the treatment of chronic renal failure

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Abstract

The aim of this study was to investigate the efficacy of sanhuanghuoxue decoction in the treatment of chronic renal failure by examining the changes in serum creatinine and blood urine nitrogen using rat models. Compared with the control group, which was treated by coated aldehyde oxystarch, the group treated by sanhuanghuoxue decoction showed remarkable decrease in serum creatinine and blood urine nitrogen. The difference was found statistically significant.

Introduction

Chronic renal failure is a condition characterized by a gradual loss of kidney function over time due to damage to the renal parenchyma. The multi-factorial etiology of the disease limits early diagnosis and treatment options, contributing to a high risk of chronic renal failure mortality. While the existing medical treatments have failed to deliver significant clinical outcomes, it is imperative to explore new treatments to effectively reduce kidney damage, to recover kidney function, and in long-term to achieve reduction of mortality from chronic renal failure.

Sanhuanghuoxue decoction is composed of 16 types of Chinese herbal medicines, including shengdahuang, yinhuang, huangshi and sanqifen (Yang et al., 2012). Shengdahuang is an effective medicine for catharsis and purgative, heat-clearing and detoxication. It has bactericidal and anti-inflammatory activity, astringes to arrest bleeding, and promotes blood circulation to dissolve stasis.

The characteristic properties of the medicine include hemostasis without creating stasis, purging the kidney while eliminating damp-heat pathogens. The incorporation of yinhuang to shengdahuang can greatly improve the efficacy of shengdahuang. Sanqifen is effective in hematic tonofication and anti-anemia. It opens up the narrowed or blocked renal artery, improves the microcirculating system, and facilitates the removal of waste products. The singular advantage of Chinese medicine is that, the combined usage of small amounts of selected types of herbs can significantly boost the efficacy of the medication, resulting in the removal of damp-heat pathogens in kidney and accumulated wastes, and promoting Qi to invigorate the circulation of blood. Thus, it has the function of enhancing blood circulation to dispel blood stasis, heat-clearing and detoxication to achieve balanced "Yin" and "Yang", improving total immune function against the attacks, enhancing body resistance and accelerating the removal of metabolic waste products, hence, curing the chronic renal failure.

The objective of this research is to examine the mecha-

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nism and efficacy of sanhuanghuoxue decoction on the treatment of chronic renal failure by monitoring the changes of serum creatinine and blood urine nitrogen in disease and control groups of rats.

Materials and Methods

Animal

In this study, we chose to use 60 healthy Wistar rats (male: female 1:1, weight ranging 180-200 g), provided by the Center of Laboratory Animal Resources in Jilin University.

Medicine

One gram of effective dose of sanhuanghuoxue decoction was equivalent to 7 g of raw medicine. Raw sanhuanghuoxue decoction was prepared by combining 27% shengdahuang (w/w), 27% yinhuang, 9% sanqifen and 37% huangzhi. All were purchased from Beijing Tongrentang. Adenine ($C_5H_5N_5$) was purchased from Xinjingke Biotechnology Inc. (lot# 20130126). Coated aldehyde oxystarch was purchased from Tianjin Pacific Chemicals Inc. (lot# H12021136).

Reagents and Instruments

Homogenizer kit and urea nitrogen kit were purchased from Shanghai Lugu Inc. (lot# 130516 and 121108). A CL-770 clinical spectrophotometer (Shimadzu Corporation; Kyoto, Japan) was used for biochemical analysis. A viscometer from Chengdu Instruments Inc. was used to measure blood viscosity. Rat metabolism cages, a centrifuge and an incubator were also used in this study.

Animal models

Rats were randomly divided into normal/control group, prevention group, disease group, sanhuanghuoxue decoction-treated group and coated aldehyde oxystarch-treated group. They were free to take food. Rats with chronic renal failure were prepared following the protocol by Yokozawa, et al. (1986). Rats in prevention, disease, sanhuanghuoxue decoction-treated and coated aldehyde oxystarch-treated groups were administered adenine by intragastric administration (Guo et al., 2001) at a dose of 150 mg/kg, once per day for 21 days. Rats in the prevention group were also given sanhuanghuoxue decoction twice per day, 0.5 g each time for 21 days. After treated with adenine for 21 days, rats in the sanhuanghuoxue decoction-treated group were treated with sanhuanghuoxue decoction 0.5 g/time twice per day for consecutive 30 days. Rats in the coated aldehyde oxystarch-treated group were treated with coated aldehyde oxystarch 2 mL per day for consecutive 30 days; rats in the disease group were treated with water only for consecutive 30 days. Rats in the control group were treated with water only during the entire 51 days of experimental period.

Measurements

Metabolism cages were used to collect 24 hours urine. Twenty four hours urinary protein quantification was carried out using Coomassie staining method. Retinol-binding protein was evaluated by ELISA. Blood samples were collected from vein near the rat eye. After centrifugation, serum was collected for analyzing the amounts of blood urine nitrogen, serum creatinine, triglycerides (TG), total cholesterol (TC) and plasma fibrinogen using the total automatic clinical spectrophotometer. SPSS 7.0 software was applied for statistical analysis of the data. All data were expressed in the form of mean ± standard derivation and the statistical significance was analyzed using Fisher's exact test in quadruple tabular form. Differences were considered statistically significant at $p<0.05$.

Table I

Changes of rat weight with time after adenine treatment

Groups	Before adenine treatment (g)	After adenine treatment (g)		
		Day 7	Day 21	Day 35
Normal/control	186.7 ± 1.6 (60)	221.5 ± 2.7 (60)	339.3 ± 2.4 (60)	389.3 ± 2.3 (60)
Sanhuanghuoxue decoction-treated	187.9 ± 2.0 (60)	201.4 ± 3.9 (60)	308.6 ± 2.7 (59)	377.3 ± 2.1 (59)
Coated aldehyde oxystarch-treated	186.4 ± 1.2 (60)	196.3 ± 1.9 (60)	297.4 ± 2.6 (59)	345.2 ± 2.1 (58)
Disease group	186.1 ± 1.8 (60)	179.5 ± 1.7 (59)	266.1 ± 2.2 (57)	328.2 ± 2.1 (56)

Data are mean ± SD; The numbers in the parenthesis represent the number of rats alive after sampling and excluding the number of death

Table II

Effect of sanhuanghuoxue decoction treatment on interstitial nephritis and renal failure

Group	Retinol binding protein (g)	Urinary protein (g)	Blood urea nitrogen (mmol/L)	Serum creatinine (μmol / L)
Normal/control	0.1 ± 0.0	13.8 ± 2.1	5.7 ± 0.2	45.0 ± 6.1
Coated aldehyde oxystarch-treated	7.7 ± 0.8*	106 . 2 ± 11.5*	16.5 ± 4.3*	114.1 ± 8.5*
Disease	8.9 ± 1.1*◇	1.57 ± 15.5*◇	28.1 ± 1.0*◇	185.3 ± 24.1*◇
Prevention	4.8 ± 1.2*△☆	43.3 ± 5.9*△☆	9.7 ± 2.2*△☆	76.7 ± 9.54*△☆
Sanhuanghuoxue decoction-treated	7.0 ± 1. 7*#%	88.3 ± 14.4*#%	14.0 ± 0.7*#%	103.6 ± 19.3*#%

Comparisons between other groups and the normal control group, *p<0.01; between the prevention group and the coated aldehyde oxystarch-treated group: Δp<0.01; between the sanhuanghuoxue decoction-treated group and the disease group: #p<0.01; between the coated aldehyde oxystarch-treated group and the disease group: ◇between ; 10.0<p the prevention group and the sanhuanghuoxue decoction-treated group: ★ p<0.01; between the sanhuanghuoxue decoction-treated and the coated aldehyde oxystarch-treated groups: %p<0.05, $p<0.01

Results

General observation.

Rats in the normal control group showed shinny hairs, alert eyes, quick reactions, good appetite, regular defecation, and increasing weight (Table I). They discharged 15 to 20 mL of urine per day, light yellow in color. For the rest groups, after 21 days treatment with adenine, rats in the disease group, coated aldehyde oxystarch-treated group and sanhuanghuoxue decoction-treated group were less energetic, and showed slower reaction, loss of appetite, slower increase of weight (some even lost weight). They discharged 8 to 13 mL of urine per day, dark yellow in color. Rats in the prevention group behaved similar to those in the control group, and discharged urine at 14 to 18 mL per day. By day 51 of the experiment, rats in the disease group became much weaker, reacted much slower and were sleepy most time with worse appetite. They lost weight significantly and tended to stay together. Their eyes were unconscious and the hairs were loose and dull. Compared to rats in the coated aldehyde oxystarch-treated group, rats in the prevention group and the sanhuanghuoxue decoction-treated group were more active, reacted more alert, showed shinny hairs and faster increase in weight. Rats in disease group, coated aldehyde oxystarch-treated group and sanhuanghuoxue decoction-treated group drank and urinated more than rats in normal and prevention groups. In particular, the disease group rats drank and urinated frequently, displaying the symptom of kidney tubule dysfunction. During the experiment, the number of death was two in the coated aldehyde oxystarch-treated group, four in the disease group and one in the sanhuanghuoxue decoction-treated group.

Treatments on interstitial nephritis and renal failure

By day 21 of the experiment, retinol-binding protein, 24 hours urinary protein, blood urine nitrogen and serum creatinine of rats in control group were remarkably lower than those in other groups. The differences were statistically significant (p<0.01). Values of these parameters for rats in the prevention group were lower than those in the coated aldehyde oxystarch-treated group, sanhuanghuoxue decoction-treated group and disease group, and the differences were significant (p<0.05 or 0.01). By day 51 of the experiment, values of the above parameters for rats in both the coated aldehyde oxystarch-treated group and the sanhuanghuoxue decoction-treated group were lower than those in the disease group. The differences were significant (p<0.05 or 0.01). On the other hand, the values for rats in the prevention group was significantly lower than those in the sanhuang-huoxue decoction group (p<0.05 or 0.01). Significant difference was also found between the coated aldehyde oxystarch-treated group and the sanhuanghuoxue decoction group. Retinol binding protein was a sensitive to indicate the extent of damage of renal tubule function (Table II).

Treatments on plasma lipids and hemorheology

Plasma lipids and hemorheology reflect the hemoperfusion of kidney tissue microcirculation. From the experimental results, the hemorheology index was higher when the renal failure got more severe. As shown in Table III, parameters including total blood viscosity, plasma viscosity, volume of packd red blood cells, plasma fibrinogen (FI), triglycerides (TG) and total cholesterol (TC) were evaluated. The total blood viscosity for rats in the disease group was higher than those in the coated aldehyde oxystarch-treated group, and the values in the disease group and the coated aldehyde oxystarch-treated group were higher than those in the prevention group and the sanhuanghuoxue decoction-treated group (p<0.05 or 0.01). The value for the sanhuanghuoxue decoction-treated group was higher than those for the prevention group. For plasma lipids, the amounts of triglycerides and total cholesterol were abnormal in the diseased and the sanhuang-huoxue decoction-treated groups. The total cholesterol

Table III

Effect of sanhuanghuoxue decoction on blood viscosity and plasma lipids

Group	Total blood viscosity (mPa.s)		Plasma viscosity (mPa.s)	HCT%	Fibrinogen (g/L)	TH (mmol/L)	TC (mmol/L)
	High shear	Low shear					
Normal/control	3.8-0.3	6.9-0.5	2.7-0.3	42.8-2.8 —	3.5 ± 0.2	1.3-0.2	4.3-0.5
Coated aldehyde oxystarch-treated	6.3-0.5*	10.7-0.7*	3.5-0.4*	40.1-3.0 —	6.1 ± 1.0*	1.7-0.3*	6.1-0.3*
Disease	7.5-0.5*◇	11.3-0.7*◇	4.1-0.4*◇	36.3-9.8*	7.6 ± 1.0*◇	1.8-0.4*	7.0-0.5*◇
Prevention	4.5-0.3*△	9.3-0.6*	3.1-0.3△★	41.0-1.8 —	3.7 ± 0.6 —△	1.5-0.2 —	4.8-0.3*△
Sanhuanghuoxue decoction-treated	5.1-0.4* #☆%	9.3-0.5* #☆%	3.4-0.5*#☆%	40.4-2.0 —	4.8 ± 0.8*#$	1.7-0.1*	5.5-0.6**#☆$

Comparisons between other groups and the normal control group, *p<0.01; between the prevention and the coated aldehyde oxystarch-treated groups: Δp<0.01, ★ p<0.05; between the sanhuanghuoxue decoction-treated the disease groups: #p<0.01; between the coated aldehyde oxystarch-treated and the disease groups: ◇between ;10.0< the prevention and the sanhuanghuoxue decoction-treated groups: ★ p<0.01; between the sanhuanghuoxue decoction-treated and the coated aldehyde oxystarch-treated groups: %p<0.05, $p<0.01; between the other groups and the normal control group, p>0.05

was abnormal in the prevention group. However, the total cholesterol in the disease group was significantly higher than that in the prevention and the sanhuanghuoxue decoction-treated groups (p<0.05 or 0.01), and was also higher than that in coated aldehyde oxystarch-treated group. On the other hand, the total cholesterol in the sanhuang-huoxue decoction-treated group was higher than that in the prevention group (p<0.01). There was a significant difference between values of the sanhuanghuoxue decoction-treated and coated aldehyde oxystarch-treated groups (p<0.05 or 0.01). The volume of packed red blood cells was essentially the same among different groups (p>0.05), except that of the normal control group and the disease group.

Discussion

After taking adenine 150 mg/kg/day, rats absorbed them to blood through alimentary canal, and converted adenine to 2,8-dimethyladenine by xanthine oxidase. After filtration by glomerule, it forms needle-like crystalline precipitates in the kidney tubule, then blocks and presses the tubule causing the damage of the epithelial cells in nephric tubule, and consequently the granulomatous inflammation in renal interstitial tissue. This induces the appearance of foreign body granuloma in renal interstitial tissue, neutrophil graneulocyte, monocytes, infiltration of lymphocytes, fibrous hyperplasia (Augustin and Lutz, 1991), the appearance of fiberosisin in renal interstium, and invokes failure of kidney function. Meanwhile, it causes the pathological changes such as hyperlipemia, proteinuria. Current studies suggest that when the chronic pathological changes happen in renal interstium, neutrophil granulocytes, monocytes and lymphocytes infiltrated in tubulointerstitial nephritis, secreting a large amount of cytokines which stimulate the proliferation of fibroblast and the synthesis of collagen. This leads to fiberizing renal interstium and narrowing the blood capillary of renal tubules, which result in the increase of vascular resistance and decease of blood flow to the kidney, and eventually cause the damage of renal function (Neilson et al., 1994; Strtz and Neilson, 1994). At the same time, both damaged and regenerative epithelial cells in nephric tubule synthesize and secret a large amount of tumor necrosis factor (TNFa), which also stimulates the proliferation of fibroblast and synthesis of collagen and consequently fiberizing renal interstium (Huang, 2005; Li and Zou, 1998). Addi-tionally, adenine can induce the xanthine oxidase mediated augmentation of radicals which attack the membranes to cause the damage of renal parenchyma. According to the research in recent years, hyperlipemia and myxemia are associated. It also plays an important role in the continuous worsening of kidney function. Hence, decreasing hyperlipemia and blood viscosity is expected to rectify microcirculation and delay the fiberization of renal interstium. In this study, rats administered with adenine were found exhausted and inactive, reaction slow, getting weak, lost weight, unconscious eyes, depressed, sleepy, staying together, loose and dull hairs, losing hairs and bad appetite. They drank and urinated frequently showing the symptoms of failure of kidney tubule.

The principle of herb medicine for the treatment of chronic renal dysfunction is not solely based on the invigoration of Qi and tonification of blood, strengthening the spleen and kidney and promoting blood circulation to dispel blood stasis. We chose shengdahuang, huangzhi, sanqi, and yinhunag as the therapeutic components in this research.

There were significant differences in retinol binding protein, urinary protein, blood urine nitrogen, serum creatinine when compared with the normal control group. These values dropped in prevention, coated alde -hyde oxystarch-treated and sanhuanghuoxue decoction-treated groups when compared to values in disease group, with the drop in prevention group the most significant. The values for the sanhuanghuoxue decoction-treated group are lower than that for the coated aldehyde oxystarch-treated group, implying that coated aldehyde oxystarch-treated is effective on improving the kidney function, however is less effective than sanhuanghuoxue decoction.

The total blood viscosity of the sanhuanghuoxue decoction-treated rats is significantly different from that or the control group ($p<0.01$). Values of these parameters dropped in prevention, coated aldehyde oxystarch-treated and sanhuanghuoxue decoction-treated groups, with the drop in the prevention group the most significant. Plasma fibrinogen is a key factor that affects the total blood viscosity. Compared with the normal group, no significant difference in plasma fibrinogen was observed in the prevention group ($p>0.05$). A significant decrease was found in the coated aldehyde oxystarch-treated group and the sanhuanghuoxue decoction-treated group when compared with the disease group ($p<0.05$ or 0.01). Apparently, sanhuanghuoxue decoction treatment caused a bigger drop than coated aldehyde oxystarch-treated did, suggesting that sanhuanghuoxue decoction is more effective in improving the blood viscosity of rats with chronic renal failure. Normally, the higher the blood viscosity index is, the higher the volume of packed red blood cells. In our case, compared with the normal group, the volume of packed red blood cells became significantly lower in the disease group ($p<0.01$), but the difference is insignificant for any other groups ($p>0.05$). It suggests that the rats with chronic renal failure likely had anemia. For TG and TC, all other groups showed significant differences ($p<0.05$ or 0.01) with respect to the normal control group, except for TG in the prevention group ($p>0.05$), suggesting that sanhuang-huoxue decoction can remarkably improve the rats' hyperlipemia. The results imply that rats in the sanhuanghuoxue decoction-treated group were overall in a healthier condition than in the coated aldehyde oxystarch-treated group.

Conclusion

Sanhuanghuoxue decoction is capable of reducing the markers of chronic renal failure.

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Conflict of Interest

The authors declare no conflict of interest.

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