JTCM 中医杂志 Journal of Traditional Chinese Medicine

Online Submissions:http://www.journaltcm.com
info@journaltcm.com

J Tradit Chin Med 2012 December 15; 32(4): 627-630
ISSN 0255-2922

BASIC INVESTIGATION

Effects of Danqidihuang Granules on glucolipid metabolism in insulin-resistant rats

Minghua Jin, Yuanyuan Wang, Bin Ou, Jun Meng, Bin Ke, Jian Qin

Minghua Jin, Yuanyuan Wang, Jun Meng, Bin Ke, Jian Qin, Department of Traditional Chinese Medicine, First Affiliated Hospital, Sun Yat-Sen University, Guangzhou 510080, China

Bin Ou, Department of Traditional Chinese Medicine, Taihe Hospital of Baiyun District in Guangzhou, Guangzhou 510540, China

Correspondence to: **Prof. Jian Qin**, Department of Traditional Chinese Medicine, First Affiliated Hospital, Sun Yat-Sen University, Guangzhou 510080, China. himybox@yeah.net

Telephone: +86-18620808898

Accepted: May 17, 2012

Abstract

OBJECTIVE: To explore whether the insulin resistance (IR) model could be established through feeding Sprague-Dawley (SD) rats high-sugar and high-fat diets and to further observe the preventive and treatment effects of different doses of Danqidihuang Granules in rats.

METHODS: Thirty-two SD rats were divided randomly into control group A (given regular feed), model group B (food high in sugar and fat), intervention group C (food high in sugar and fat as well as regular doses of Danqidihuang Granules), and intervention group D (food high in sugar and fat as well as double doses of Danqidihuang Granules). The interventions were for 8 weeks. Motion, change in color, body weight, and food intake, as well as plasma lipids (including low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), total cholesterol (TC) and triglyceride (TG) , fasting blood glucose (FBG), fasting insulin (FINs) levels, insulin sensitivity index (ISI), and insulin resistance index (HOMO-IR) were observed.

RESULTS: At the end of the second week of the experiment, the appetite and activities of rats in groups B, C and D decreased significantly compared with group A. The fur of the rats in those three groups was curly. After the fourth week, the activities, food intake and color of rats in group B were worse than those in groups C and D, but there were no significant differences in weight (P>0.05). Compared with group A, LDL-C, TC, FBG and HOMO-IR in model group B were increased significantly (P<0.05), whereas the FINs and ISI increased obviously (P<0.05). The levels of LDL-C and TC in group D was decreased obviously compared with those in group C, and HOMO-IR in group D was less than that in group B (P<0.05).

CONCLUSION: Danqidihuang Granules helped to prevent and improved the insulin resistance of rats.

Key words: Insulin resistance; Dose-response relationship, drug; Diabetes mellitus, Experimental; Danqidihuang Granules

INTRODUCTION

Insulin resistance (IR) is a condition in which insulin-effective organs are insensitive to glucose metabolism as well as the physiological function and performance of the liver, muscles and adipose tissue.[1,2] It is an independent risk factor for diabetes mellitus, hyperten-

sion, hyperlipemia, and cardiovascular diseases.[3,4] Therefore, researching how to improve insulin resistance to prevent and reduce the development of cardiovascular diseases is important.

Danqidihuang Granules are composed of Liuweidihuang tablets. These tablets consist of Shudihuang (prepared Radix rehmanniae), Shanzhuyu (Fructus corni), Shanyao (Rhizoma dioscoreae), Zexie (Rhizoma alismatis), Mudanpi (Cortex moutan), Fuling (Poria cocos), Salvia miltiorrhiza and Radix notoginseng. Liuweidihuang tablets can be used to improve the IR seen in diabetes mellitus, and Salvia miltiorrhiza, and Radix notoginseng can be used to improve the IR observed in coronary heart diseases. [5]

The present study was based on research[1,5] and combined with clinical experiences to create a model of insulin resistance in rats. We also studied if Danqidihuang Granules had superposition effects on IR or even had a role in improving it.

MATERIALS AND METHODS

Experimental animals

Thirty-two healthy male and female specific pathogen-free Sprague-Dawley (SD) rats [(130 ± 10)] g were purchased from Sun Yat-Sen University Laboratory Animal Center (certification number 0041083; Guangzhou, China).

Drugs, reagents and instruments

Qi Di Huang Granules consisting of Shudihuang (prepared Radix Rehmanniae), Shanzhuyu (Fructus Corni), Shanyao (Rhizoma Dioscoreae), Zexie (Rhizoma Alismatis), Mudanpi (Cortex Moutan), Fuling (Poria Cocos), Danshen (Salvia Miltiorrhiza), and Sanqi (Radix Notoginseng) in the proportion 8∶4∶4∶3∶3∶3∶4∶2 were obtained from the 999 Medicine Group Company (Shenzhen, China).

Assay kits for high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), triglyceride (TG), total cholesterol (TC) and glucose enzymes were purchased from Wako (Tokyo, Japan). A 7600 Automatic Biochemistry Analyzer was from Hitachi (Tokyo, Japan).

High sugar and fat ingredients

High sugar and fat ingredients comprised 10.0% lard, 20% sucrose, 2.5% cholesterol, 1.0% bile salts, and 66.5% conventional feed. It was prepared by Guangdong Animal Medical Centre (Guangzhou, China).

Grouping and feeding of experimental animals

Thirty-two SD rats were assigned randomly to control group A, model group B, intervention group C and intervention group D. Control group A was given conventional feed without other interfering factors. Model group B was given high sugar and fat feed but no other interfering factors. Intervention group C was fed high sugar and fat as well as regular doses of Danqidihuang Granules. Intervention group D was fed high sugar and fat feed and twofold doses of Danqidihuang Granules.

We divided SD rats into eight cages. All groups underwent intervention for 8 weeks. An electronic balance was used to calculate the average daily food intake before feeding. Rats in groups C and D underwent gastric lavage with Danqidihuang Granules at 10 am every day. Rats in groups A and B were given clean water. Room temperature was maintained at 22°C-27°C; humidity was maintained at 50%.

Doses of Danqidihuang Granules

The doses of Danqidihuang Granules were based on the Chinese Pharmacopoeia.[6] We determined the daily doses for adults (according to an average body weight of 60 kg): Shudihuang 24 g; Shanzhuyu 12 g; Shanyao 12 g; Zexie, 9 g; Mudanpi 9 g; Fuling 9 g; Danshen 12 g; and Sanqi 6 g.

The rat/human dose conversion formula was: Db= Da(Rb/Ra)*POWER (Wa/Wb,1/3)*

Where D is the daily dose per unit kilogram, R is the animal size coefficient, W is animal weight, a and b mean after or before the experiment. Daily regular doses were determined after conversion. Turbid liquor was confected with daily doses of Danqidihuang Granules and physiological (0.9%) saline. The volume of gastric lavage for each rat was 2 mL of liquor. The specific quantity and actual weight of rats were calculated according to the conversion formula.

Observation and evaluation

The motion, color changes, change in weight, and change in food intake in rats were recorded. After 8 weeks of intervention, rats were fasted for 12 h and blood samples taken from the abdominal aorta after general anesthesia using ether. Fasting blood glucose and four indices of blood lipids were tested, and radioimmunoassay detection conducted to measure fasting insulin levels. The insulin sensitivity index (ISI) was calculated according to the formula set by Li Guang-wei:[7]

ISI = LN [1/(FBG×FINs)] (LN= Napierian Logarithm)

In addition, the insulin resistance index (HOMO-IR) was calculated as an auxiliary measure of insulin sensitivity (HOMO-IR=FBG × FINs/22.5).

Statistical analyses

SPSS ver14.0 software (IBM, Armonk, NY, USA) was used for statistical analyses. The independent sample *t*-test was applied to compare differences between two groups and single-factor analysis of variance used to compare between more than two groups. The method of significant difference was adopted to show the result as mean ± standard difference. The two-sided test was used for all of the hypotheses. $P \leq 0.05$ was considered

significant, and $P \leq 0.01$ highly significant.

RESULTS

General conditions

No deaths were observed in groups A, C and D, but 2 rats died in group B. One started to fast without an obvious reason in the middle of the experiment, then started to look peeky gradually and then died. Another death for no obvious reason occurred in the seventh week (before the end of the experiment); we took blood samples from the rat for analyses. Thirty-one rats were assessed in total.

In the first 2 weeks, the food intake of model rats (groups B, C and D) and control rats (group A) were approximately equal, but rats in model group B and intervention groups C and D were more active and sprightly. From the second week to the end of the experiment, the food intake, as well as the activities of rats in groups B, C, and D, were significantly reduced compared with those in control group A. The fur of the rats in those three groups also became unkempt and matted gradually. Defecations in those rats were also relatively scarce compared with those in group A.

In the first 3 weeks, there was no evident difference in food intake, activity and color between the rats in groups B, C, and D. However, after the fourth week, the activities and food intake, as well as the colors of model rats were worse than those in the drug intervention groups C and D.

Body weight

The average weight of each rat increased during the study. After 2 weeks, the weight changes in the experimental rats became stable. There were no significant differences in body weight ($P>0.05$) between the intervention, control and model groups.

Changes in blood lipid levels in the groups

The result show in Table 1 and Table 2.

DISCUSSION

According to the theories of Traditional Chinese Medicine, the kidney governs water; if more water is present, it can become phlegm. The origin and essence of phlegm are in the kidney. Renal insufficiency (or disorders of the physiological function of the kidney) could weaken the function of the viscera. These actions result in loss of the transporting function of the spleen, eventually leading to the accumulation of phlegm and body fat. Therefore, prevention of obesity and related diseases such as IR could be treated by regulating the kidney and spleen.[8]

Danqidihuang Granules eliminate phlegm by strengthening the kidney. Danqidihuang Granules are use to

Table 1 Comparison of the blood lipid levels in the groups (mmol/L, $\bar{x} \pm s$)

Group	*n*	LDL-C	HDL-C	TC	TG
A	8	1.00±0.42	0.17±0.05	1.57±0.18	1.01±0.26
B	7	1.25±0.33	0.28±0.10[a]	1.86±0.27[c]	1.97±0.60[a]
C	8	1.37±0.56	0.19±0.04[b]	1.57±0.11[d]	1.43±0.46[d]
D	8	1.20±0.52	0.13±0.02[b]	1.17±0.34[b]	1.02±0.53[b]

Notes: A: control group, given conventional feed without other interfering factors. B: model group, given high sugar and fat feed but no other interfering factors. C: intervention group, fed high sugar and fat as well as regular doses of Danqidihuang granules. D: intervention group, fed high sugar and fat feed and twofold doses of Danqidihuang granules; LDL-C: low-density lipoprotein-cholesterol; HDL-C: high-density lipoprotein-cholesterol; TC: total cholesterol; TG: triglyceride; Compared with control group A, [c]$P<0.05$, [a]$P<0.01$. Compared with model group B, [d]$P<0.05$, [b]$P<0.01$.Changes in fasting plasma glucose and insulin, ISI and HOMO-IR.

Table 2 Comparison of the levels of fasting plasma glucose, insulin, ISI an HOMO-IR in different groups ($\bar{x} \pm s$)

Group	*n*	FBG (mmol/L)	FINs (mIU/mL)	ISI	HOMO-IR
A	8	4.19±0.63	0.35±0.06	1.75±1.51	0.09±0.09
B	7	5.31±1.06[a]	0.46±0.22[a]	0.52±0.31[c]	0.11±0.07
C	8	6.87±1.80[b]	0.15±0.08[b]	1.11±0.43	0.06±0.03[d]
D	8	2.90±1.08[b]	0.13±0.06[b]	3.28±1.41[b]	0.02±0.02[b]

Notes: A: control group, given conventional feed without other interfering factors. B: model group, given high sugar and fat feed but no other interfering factors. C: intervention group, fed high sugar and fat as well as regular doses of Danqidihuang granules. D: intervention group, fed high sugar and fat feed and twofold doses of Danqidihuang granules; FBG: fasting blood glucose; FIN: fasting insulin; ISI: insulin sensitivity index; HOMO-IR:insulin resistance index; Compared with control group A, [a]$P<0.05$, [c]$P<0.01$. Compared with the model group, [d]$P<0.05$, [b]$P<0.01$.

tone the kidney, eliminate phlegm, and promote blood circulation by removing blood stasis. We use these Granules to treat coronary heart diseases, hypertension and diabetes complicated by IR. Danqidihuang Granules nourish the spleen, liver and kidney, and can remove dampness, eliminate phlegm and clear deficient heat. The Granules can also promote blood circulation, enrich the blood and stop bleeding. No obvious side effects of the Granules have been found, and they may be used in the long-term prevention of IR.

Research has shown that Liuweidihuang tablets can decrease the levels of sugar and lipids in blood, and are widely used in the treatment of DM. One study suggested that Liuweidihuang tablets could clearly reduce the level of blood sugar in DM rats, effectively control levels of blood lipids and relieve IR.[5,9] Qian et al.[10] found that Liuweidihuang tablets could significantly reduce glucose levels, serum levels of triglycerides, cholesterol and free fatty acids, plasma levels of insulin, β-cell apoptosis, and the number of pancreatic islands in type-2 DM in Otsuka-Long-Evans-Tokushima Fatty (OLETF) rats. The tablets could prevent DM effectively. The preventive roles were unrelated to reducing the weight of the rats, and the tablets helped to maintain the normal structure of pancreatic islands of OLETF rats to alter dyslipidemia and improve IR effectively.[11-13]

The present study provided a better animal model of disease than the above-mentioned studies and simulated the pathophysiology of human IR effectively.[14] To improve the research methods for the prevention of cardiovascular diseases, SD rats were used instead of OLETF rats and Danqidihuang Granules substituted for Liuweidihuang tablets for IR experiments.

Compared with model group B and control group A, blood cholesterol, TC and FBG of the rats in group B were increased significantly (P<0.05). It was suggested that we had created stable models of high sugar and fat in rats.[15] In addition, the fasting insulin levels of rats in group B were increased more than those in group A (P< 0.05). This finding was contrary to a decrease in the blood insulin levels seen in IR. Nonetheless, compared with groups B, C or D, the HOMO-IR of rats in groups C and D showed an ascending trend, and the ISI declined. It indecated the successful models of insulin resistance with insulin secretion shortage, not just a single animal model of insulin resistance. We confirmed that Danqidihuang Granules decreased the blood levels of lipids and glucose, reduced TC, elevated LDL-C, and improved the metabolism of glycolipids in rats. This study also suggested that the mean ISI in the drug intervention groups was higher than in model groups. The HOMO-IR was decreased obviously. The present study showed that Danqidihuang Granules could increase insulin sensitivity and reduce IR. The results also revealed that double-dose groups could improve IR better than regular-dose groups. The study partly demonstrated that the improving effects had a concentration-response relationship.

In brief, Danqidihuang Granules could enhance the physique, appetitite and activity of rats. They could also reduce blood glucose and blood fat, as well as improve IR. The mechanisms of IR are complicated and not clear.[16,17] More clinical and experimental studies are needed for further clarification.

REFERENCES

1 **Wang SH** , Wang WJ. Pathogenesis of insulin resistance and prevention and treatment strategy of integrated traditional Chinese and Western Medicine. J of Chin integr med 2004; 2(1): 14-16.

2 **Zhou JG**. Clinical intervention and experimental research progress of TCM on insulin resistance. Zhong Hua Zhong Yi Yao 2011; 26(10): 2332-2335.

3 **Guo JJ**, Lv L, Guo YP. Research progress on insulin resistance of type 2 diabetes in TCM. Zhong Xi Yi Jie He Xin Nao Xue Guan Bing 2009; 7(5): 588-589.

4 **Liu L**, Zhang N. Clinical research progress for insulin resistance of hypertension in Traditional Chinese medicine. Zhong Yi Yao Xin Xi 2009; 26(6): 123-125.

5 **Jin ZS**, Yang SQ, Pan YQ, Gao Y. On experimental liu wei di huang pills insulin sensitiity to diabetic rats. Gansu Zhong Yi Xue Yuan Xue Bao 2008; 25(1): 9-11.

6 **Yan Li**, Song HL, Cui LP, He HM. Pharmacopoeia of the People's Republic of China (Enlarged edition of China Pharmacopoeia committee). Zhong Guo Yi Yao Ke Ji Chu Ban She 2010; (8): 396-398.

7 **Li GW**. Evaluation of insulin sensitivity and its application in clinical research. Zhong Hua Nei Fen Mi Dai Xie 2000; 16(3): 295-300.

8 **Chen YQ**, Dong FL, Wang YF, Yan QL. Therapeutic thought on spleen in insulin resistance with glucolipid metabolism disorders. J Tradit Chin Med 2010; 30(1): 79-81.

9 **Cai KF**, Wang YL. Eliminating phlegm and removing blood stasis in insulin resistance. Zhong Hua Zhong Yi Yao 2005; 20(9): 542-543.

10 Qian Y, Li J, Xue YM, Zhu B, Pan YH, Zhang Y. Liuweidihuang Pills for idiopathic type 2 diabetic rats plasma adiponectin levels influences. J of n.a. Med Univ 2008; 28 (1): 34-36.

11 **Zhen H**, Ge YR. Research progress on IR improvement and acting mechanisms of active ingredients of traditional Chinese medicine. Zhong Guo Zhong Yao 2010; 35(4): 531-535.

12 **Chen P**, Xu XJ, Shi DH. Effects of Danshen Dripping Pill on glycolipid metabolism in insulin resistant rats. Zhong Cheng Yao 2008; 30(4): 489-493.

13 **Reimer MK**, Ahren B. Altered β-cell distribution of pdx-1 and GLUT-2 after a short-term challenge with a high-fat diet in C57BL/6J mice. Diabetes 2002; 51: S138-143.

14 **Korach-Andre M**, Gao J, Gounarides JS, et al. Relationship between visceral adiposity and intramyocellular lipid content in two models of insulin resistance. Am J Physiol Endocrinol Metab 2005; 288: E106-116.

15 **Tian AP**, Guo SS, Chen YT, Shen ZF. Experimental research of high heat feed induced insulin resistance animal models. Zhong Guo Yao Xue 2006; 41(11): 827-831.

16 **Vazquez LA**, Pazos F, Amado JA, et al. Effects of changes in body weight and insulin resistance on inflammation and endothelial function in morbid obesity after bariatric surgery. J Clin Endocrinol Metab 2005; 90(1): 316-322.

17 **Liu W**, Li J, Shen B, et al. Research progresses of insulin resistance animal models. Zhong Yi Yao Xin Xi 2008; 15(6): 9-11.