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EXPERIMENTAL STUDY

Effect of a combination of calorie-restriction therapy and Lingguizhugan decoction on levels of fasting blood lipid and inflammatory cytokines in a high-fat diet induced hyperlipidemia rat model

Wang Yuanyuan, Jin Minghua, Zhang Lina, Li Suhua, Zhai Jiayu, Shen Yongzhi, Chen Chunyu, Qin Jian

Wang Yuanyuan, Jin Minghua, Zhang Lina, Li Suhua, Zhai Jiayu, Shen Yongzhi, Chen Chunyu, Qin Jian, Department of Traditional Chinese Medicine, First Affiliated Hospital, Sun Yat-Sen University, Guangzhou 510080, China
Correspondence to: Professor Qin Jian, Department of Traditional Chinese Medicine, First Affiliated Hospital, Sun Yat-Sen University, Guangzhou 510080, China. himybox@yeah.net
Telephone: +86-18902233983; +86-13760831583
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Abstract

OBJECTIVE: To combine calorie-restriction therapy (CRT) with Traditional Chinese Medicine (TCM) using Lingguizhugan Decoction and note the effects on expression of inflammatory cytokines [tumor necrosis factor (TNF)-α, high-sensitivity C-reactive protein (hs-CRP), interleukin (IL)-6] in high-fat-diet induced hyperlipidemia in rats.

METHODS: Forty rats were divided randomly and equally into four groups: control group A (regular feeding for 5 weeks); model group B (high-fat diet for 5 weeks), calorie-limited group C (high-fat diet for 5 weeks) and TCM calorie-limit group D (high-fat diet for 5 weeks). After modeling, groups C and D were fasted for 3 days: group C with simple fasting, and group D with TCM fasting. The motion as well as changes in color, body weight, food intake, plasma lipids [low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol, total cholesterol (TC) and triglyceride (TG) along with TNF-α, hs-CRP and IL-6] were measured before and after intervention.

RESULTS: Modeled rats were established after five weeks. After 3 days of fasting, compared with group B, blood lipid levels (TG, TC, LDL-C) of groups C and D decreased dramatically. Those of group D decreased more obviously than those of group C. However, the values of TNF-α, hs-CRP and IL-6 showed no obvious difference between each of the three treatment groups.

CONCLUSION: CRT can reduce fasting blood lipid levels in rats, but not by reducing the levels of inflammatory cytokines.

Key words: Hyperlipidemias; Tumor necrosis factor-alpha; Interleukin-6; Lingguizhugan decoction; Calorie-restriction therapy

INTRODUCTION

Hyperlipidemia is an independent risk for atherosclerosis and is the pathologic basis of cardiovascular and cerebrovascular diseases. In recent years, there has been an improvement in people's living standards. Consumption of high-calorie, high-fat diets and less exercise have led to a higher incidence of obesity, hypertension, diabetes mellitus (DM), hyperlipidemia, coronary heart disease and cerebrovascular diseases.[1,2] Strategies for controlling diets and improving lifestyle have become a necessary means for the treatment of obesity and related metabolic disorders.

Calorie-restriction therapy (CRT) has been demonstrated to decrease body weight, improve insulin resistance, and regulate immune disorders. Studies have also shown that CRT is effective against metabolic diseases.[1,3] Through long-term clinical experiments,[4] our research

team found that pure CRT had lipid-lowering effects in hyperlipidemic patients but, during treatment, many patients felt hungry and weak, and that their tongues were significantly thicker after treatment.

We added Lingguizhugan Decoction (LGZGD) to CRT to create Traditional Chinese Medicine (TCM)-CRT. This new "hybrid" therapy not only lowered lipid profiles in blood, it also reduced the prevalence of metabolic-related factors and their side effects. We hypothesized that the mechanism of action of these effects may involve vascular inflammatory cytokines, and wished to test our theory in animals, hence the study we describe here.

MATERIALS AND METHODS

Ethical approval of the study protocol

The study protocol was approved by the Ethics Committee of the First Affiliated Hospital (Sun Yat-Sen University, Guangzhou, China).

Animals and diets

Male Wistar rats [specific pathogen-free; 8 weeks; (245 ± 8) g] were purchased from the Experimental Animal Center of Guangdong Province [certificate SCXK (Guangdong) 2009-0011].

All rats lived in their own cage. They were placed in a room with a 12-h light-dark cycle and had free access to food and water. The basic diet and high-fat diet were purchased from Guangdong Province Animal Experiments (Guangdong, China). The high-fat diet contained 55% more fat than a normal diet (15% lard, 21% sucrose, 9% egg-yolk powder, 0.5% bile salts).

Drugs and reagents

LGZGD comprising Fuling (*Poria*), Guizhi (*Ramulus Cinnamomi*), Baizhu (*Rhizoma Atractylodis Macrocephalae*), and stir-frying with liquid adjuvant Gancao (*Radix Glycyrrhizae*) in the proportion 4∶3∶2∶2 was obtained from 999 Medicine Group (Shenzhen, China).

Assay kits for tumor necrosis factor (TNF)-and interleukin (IL)-6 were obtained from Ray Biotech (Atlanta, GA, USA). An assay kit for high-sensitivity C-reactive protein was purchased from Millipore (Bedford, MA, USA). A Microplate Reader (Sunrise ™; Tecan, Männedorf, Switzerland), an ultrapure water system for trace organic analyses (Yee Young Enterprises, Nanjing, China) and a Thermostatic Incubator (Jiangnan Instrument Factory, Ningbo, China) were also purchased.

Doses of LGZGD

Doses of LGZGD were based on those stated in the Chinese Pharmacopoeia.[5] We determined the daily doses for adults according to a mean adult body weight of 60 kg as: Fuling (*Poria*) 12 g, Guizhi (*Ramulus Cinnamomi*) 9 g, Baizhu (*Rhizoma Atractylodis Macrocephalae*) 6 g, and stir-frying with liquid adjuvant Gancao (*Radix Glycyrrhizae*) 6 g.

Grouping and feeding of the rats

First, rats were allowed to adapt to a normal diet for one week. Rats were then divided into four groups (A-D) of ten in a 1∶1∶1∶1 ratio randomly using Matlab software. Group-A rats were fed a normal diet and the other three groups were fed a high-fat diet for five weeks. We evaluated the modeling effects at the fifth weekend.

After modeling, groups A and B were administered the original diet for three days. Group C underwent simple restriction of calorie intake for three days (i.e., no food, free access to water, and gavage using physiologic (0.0%) saline (9 mg/100 g) every day). Group D underwent calorie restriction and LGZGD administration for three days [no food, free access to water, and gavage with LGZGD (9 mg/100 g) every day].

Measurements

General condition of rats before and after treatment: motion as well as changes in color, weight, and food intake of rats was recorded. We measured the blood lipid profiles of all rats at the fifth weekend as well as before and after restriction of calorie intake. After restriction of calorie intake and an overnight fast, 3 mL of blood was obtained from the tail vein. Blood was centrifuged at 1000 × *g* for 10 min at room temperature. Serum was separated from plasma and sent (as 1-mL samples) to Affiliated Hospital Laboratory for determination of levels of triglycerides (TG), total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C) and hs-CRP. The remaining 2-mL samples were sent to Sun Yat-Sen Center for measurement of levels of inflammatory factors.

TNF-α and IL-6

According to a series of standard concentrations and the corresponding values of optical density at 450 nm (OD_{450}) we created a TNF-α standard curve using Origin 7.5 software (OriginLab, Northampton, MA, USA) to obtain the equation:

$Y = 2.1637 + (0.025742\text{-}2.16372)/(1 + (X/68212)\ 0.82886)$,

where Y is OD_{450} value and X is the TNF-α standard concentration.

We followed an identical procedure to an equation for IL-6:

$Y = 5.0013 + (\ \text{-}\ 0.012815\text{-}5.0013)/(1 + (X/36451)\ 0.93208)$,

where Y is OD_{450} value and X is the IL-6 standard concentration.

Using the standard curves, we could obtain the values of the samples in pg/mL at an absorbance of 450 nm.

Statistical analyses

SPSS v18.0 (IBM, Armonk, NY, USA) was used for statistical analyses. Measurement data for mean ± standard deviation ($\bar{x} \pm s$), and the Student's *t*-test was used to compare between the two groups. Multiple sets of single-factor analysis of variance were used to compare multiple groups. $P \leq 0.05$ was considered significant.