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RESEARCH ARTICLE

Tangnaikang improves insulin resistance and β-cell apoptosis by ameliorating metabolic inflammation in SHR.Cg-Lepr^{cp}/NDmcr rats

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Abstract

OBJECTIVE: To investigate the effects of Tangnaikang (TNK), a mixture of five herbal plant extracts, on inflammation-mediated insulin resistance and β-cell apoptosis in SHR.Cg-Lepr^{cp}/NDmcr (SHR-cp) rats.

METHODS: Seven-week-old SHR-cp rats were randomly divided into a control (CON) group and a TNK (3.24 g/kg) group. Wistar-Kyoto rats at the same age were used as the normal control group. After 7 weeks of continuous intragastric administration of TNK, the glucose metabolic status and insulin sensitivity of the rats were evaluated by assessing fasting serum glucose (FBG), the oral glucose tolerance test (OGTT), fasting serum insulin (FINS), and the insulin sensitivity index (ISI). Serum tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), C-reactive protein (CRP) and adiponectin were measured *via* enzyme-linked immunosorbent assays. Macrophage infiltration and apoptosis in adipose tissues were detected through F4/80 immunohistochemistry and the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. Islet morphology and β-cell apoptosis were investigated using double immunofluorescence staining and the TUNEL assay. The expression of cytokine genes in adipose tissue, the liver, and the pancreas was detected in real-time polymerase chain reaction assays. The expression and phosphorylation levels of insulin signaling-, inflammation-, and β-cell survival-related proteins in the liver and pancreas of SHR-cp rats were detected by western blotting.

RESULTS: TNK (3.24 g/kg) treatment significantly decreased body weight, FBG and FINS; improved impaired glucose tolerance; increased the ISI; reduced serum levels of TNF-α, CRP and IL-6; and increased serum adiponectin. The mRNA expression of inflammatory markers was markedly reduced in the liver, pancreas, and adipose tissue. F4/80- and TUNEL-positive cells in adipose tissues were decreased, as was the number of TUNEL-positive

β-cells. The phosphorylation of c-Jun N-terminal kinase and that of insulin receptor substrate-1 at serines 307 and 1101 was significantly decreased. In the pancreas, the expression of nuclear factor kappa-light chain-enhancer of activated B cells-p65 was significantly decreased, and phosphoinositide 3-kinase and IRS-2 were significantly increased.

CONCLUSION: TNK was able to improve insulin resistance and β-cell apoptosis in SHR-cp rats, which might be associated with its ability to relieve the overall and local metabolic inflammatory responses observed in SHR-cp rats.

Keywords: Metabolic inflammation; Insulin resistance; Islets of langerhans; Apoptosis; Rats, inbred SHR; Tangnaikang

INTRODUCTION

The interaction between insulin resistance and metabolic inflammation promotes the occurrence and development of type 2 diabetes and represents a major pathological basis for the development of many metabolic disorders, such as metabolic syndrome, atherosclerosis, and coronary heart disease. Metabolic inflammation is a bridge connecting obesity and insulin resistance.[1] Adipose tissue expansion induced by excessive energy intake causes the release of adipose inflammatory factors, such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α, to activate inflammation pathways that interfere with the transduction of insulin signals and induce insulin resistance and β-cell apoptosis. In addition, macrophage aggregation and infiltration in adipose tissue in obese individuals further aggravate the inflammatory responses in adipose tissue, causing a vicious cycle.[2-4] Therefore, anti-inflammatory therapy has become a focus of current research on the prevention and treatment of insulin resistance-related diseases.

The Chinese medicine Tangnaikang (TNK) is a mixture of extracts from five herbal plants: Xiakucao (*Spica Prunellae Vulgaris*), Fanshiliuye (*Psidium guajava*), Nüzhenzi (*Fructus Ligustri Lucidi*), Sanbaicao (*Herba Saururi*), Renshen (*Radix Ginseng*). Over the years, these herbs have been widely used for the treatment of type 2 diabetes. Modern pharmacological studies have shown that the various components of TNK all exhibit different degrees of anti-diabetic and anti-inflammatory functions.[5-10] TNK has been found to be effective for improving abnormal glucose metabolism in patients with impaired fasting glucose (IFG) or glucose intolerance (IGT).[11] TNK can reduce blood glucose, improve insulin resistance, and reduce TNF-α and IL-6 in the serum and adipose tissue of diabetic animals,[12,13] suggesting that TNK may improve insulin resistance and metabolic inflammation. However, the specific mechanism of its action is still not clear. Therefore, in this study, spontaneous insulin-resistance diabetic SHR. Cg-Lepr^{cp}/NDmcr (SHR-cp) rats were used to further investigate the effects and mechanism of action of TNK in inflammation-mediated insulin resistance and β-cell apoptosis.

MATERIALS AND METHODS

Preparation of TNK

TNK is composed of iakucao (*Spica Prunellae Vulgaris*), Fanshiliuye (*Psidium Guajava*), Nüzhenzi (*Fructus Ligustri Lucidi*), Sanbaicao (*Herba Saururi*), Renshen (*Radix Ginseng*), prepared in a proportion of 4∶4∶2∶2∶1. All herbal drugs were purchased from the Shenyang Pharmaceutical Group Corporation (Shenyang, China) and authenticated according to the Pharmacopoeia of the People's Republic of China (2000 ed). The first four plants were mixed and extracted twice by refluxing with 75% (v/v) ethanol (1∶8 w/v) for 1 h, and the obtained solution was concentrated to an ethanol extract, with a recovery rate of 13.83%. The resultant residue was decocted twice with water (1∶6 w/v) for 1 h, and this solution was then concentrated to a water extract, with a recovery rate of 5.49%. Renshen (Radix Ginseng) was dried by baking at 60 ℃ for 4 h and subsequently crushed to yield a fine powder [(150 ± 7) μm]. Finally, all components were homogenously mixed in the following weight ratio: ethanol extract/water extract/Radix Ginseng fine power/excipients (25/10/15/10), to produce dried granules. The entire process was performed by the Sichuan Medco Pharmaceutical Limited Corporation (Deyang, China). Spectrophotometry analysis showed that the contents of total flavonoids, triterpenoid saponin and triterpene acids in the ethanol extract were 4.23%, 3.67%, and 1.99%, respectively, whereas for the water extract, the content of total polysaccharides was 5.83%. High-performance liquid chromatography (HPLC) analysis revealed that the concentrations of Rg1, Re, Rb1 and rosmarinic acid in TNK were 0.09%, 0.12%, 0.10%, and 0.55%, respectively.[14]

Animals

Male SHR-cp rats (SLC, Inc., Shizuoka, Japan) at an age of 7 weeks, with a body weight of 195-217 g, were used in the experiments, along with 7-week-old male Wistar Kyoto (WKY) rats (SLC, Inc., Shizuoka, Japan), with a body weight of 148-170 g. The animals were housed in specific pathogen free animal rooms at Mukogawa Women's University. All animal protocols conformed to the Guidelines for the Care and Use of Laboratory Animals approved by the Animal Care and Use Committee of Mukogawa Women's University.