

Effect of puerarin on type II diabetes mellitus with orthopaedic footwear

Qinyuan Yu¹, Wenzhong Han², Yixi Zhu¹ and Hua Zhai^{1*}

¹Shanghai Sunshine Rehabilitation Center (Yangzhi Affiliated Rehabilitation Hospital of Tongji University), Shanghai, China

²Shanghai Songjiang Central Hospital, Shanghai, China

Abstract: In recent years, the antioxidant efficacy of puerarin has been recognized. However, there is less research on Puerarin used in diabetes. This paper analyzes the effect of puerarin on type II diabetes mellitus induced by streptozotocin combined with orthopaedic footwear. In this study, 80 Sprague Dawley (SD) rats were fed with high fat and high sucrose diet for one month, and 1% streptozotocin (STZ) was used to induce type II diabetes mellitus. After 6 weeks aerobic exercise and puerarin intervention in rats, the effect of aerobic exercise and puerarin intervention on antioxidant ability in diabetic rats was investigated. The results showed that aerobic exercise and puerarin intervention can improve the insulin resistance in rats. Meanwhile, the annual incidence of foot ulcers in diabetic patients is 2%, while orthopaedic footwear can reduce the probability of diabetic foot ulcers. In general, exercise and puerarin intervention can really play a role in the prevention and treatment of diabetes, such as improving the metabolic status of diabetic patients and reducing their dependence on drugs.

Keywords: Puerarin, type II diabetes mellitus, insulin resistance, Streptozotocin STZ.

INTRODUCTION

The diabetic patients in China increased at a rate of 1%, the number more than 30 million, that China has become the world's second largest country of diabetes (Kang *et al.*, 2003). Diabetic foot refers to diabetic patients due to diabetic neuropathy and different degree of peripheral vascular disease with foot ulcer of lower extremity infection, ulcer formation or deep tissue destruction (Ostojic *et al.*, 2015). Hospitalized patients with diabetes, about 7% have foot ulcers (Piro *et al.*, 2002). Because the diabetic foot feels absent, it is vulnerable to injury. Sandals or slippers are also avoided because it causes more areas of toes or feet to be exposed to loss of protection (Leng *et al.*, 2004).

Diabetes mellitus is mainly divided into insulin dependent type I diabetes mellitus and non-insulin dependent diabetes mellitus type II diabetes mellitus (Pacez *et al.*, 2014). The pathogenesis of diabetes is not fully understood and involves many factors. Research shows that the gene mutation, diabetes and autoimmune (islet cell antibodies), insulin resistance (or lower affinity receptors), physical activity, obesity, mental stress, trauma, stress and some drugs (thiazide diuretics, glucocorticoid, phenytoin, certain contraceptives) reduced sensitivity of the impaired high blood glucose, and insulin resistance induced by sugar (Shuldiner *et al.*, 2004; Seshiah *et al.*, 2002). Sustained high blood glucose caused the non-enzymatic glycation of protein oxidation, strengthen and abnormal lipid metabolism is the main reason for the increase in diabetes patients produce

reactive oxygen species ROS, and some antioxidant enzyme activities were also significantly lower in patients with diabetes, the body of oxidative stress in certain degree. Oxidative stress will decrease the sensitivity of peripheral tissues to insulin, glucose utilization decreased; in addition, oxidative stress can also aggravate the apoptosis of pancreatic β cells, islet cell number decreased, decreased insulin synthesis and secretion, the abnormal glucose metabolism further intensified, so the oxidative stress plays an important role in the pathological development of diabetes (Santos *et al.*, 2003).

Facing the increasingly serious trend of diabetes, diabetes treatment at home and abroad for a multi-faceted research, reveal the pathogenesis of diabetes from drug development to the molecular level, from the use of health care treatment daily to psychotherapy, all around the core issue of diabetes cure (Tang *et al.*, 2017). As one of the therapies, exercise therapy plays an irreplaceable role in the treatment of diabetes. The domestic and foreign research that, through long time aerobic endurance training, can improve the sensitivity of insulin receptor in patients with diabetes, the increased use of peripheral glucose, improve glucose tolerance, heart and lung function, improve blood lipid level (Tural *et al.*, 2015; Takahashi, 2017).

Puerarin is the root part of *Radix pueraria* (Leguminous plant), main components of isoflavones, clinical practice proved that it has the effect of reducing blood sugar, reducing triglyceride and increase high density lipoprotein, free radical scavenging and improving the microcirculation. It has been widely used in the treatment of diabetes and its complications, but its specific

*Corresponding author: e-mail: zhaihua16 @163.com

mechanism of action is unknown (Udagawa *et al.*, 2012). As a traditional herbal medicine, *Radix puerariae* has been recognized for its antioxidant activity in recent years. Puerarin natural antioxidants are becoming more and more popular because of their safety, non-toxic and other advantages. However, the research of puerarin used in diabetes is rare, but the study of puerarin and exercise combined with prevention and treatment of diabetes and its complications is also rarely reported (Wollheim, 2000). This paper through high fat and high glucose diet of 80 SD (Sprague Dawley) rats were fed for one month after the 1% streptozotocin (STZ) induced by type II diabetes. Rats after 6 weeks of aerobic exercise, then we made puerarin intervention of aerobic exercise and test the puerarin intervention on diabetes rat anti oxygen ability influence.

MATERIALS AND METHODS

Experimental material

In this study, we selected 80 Sprague-Dawley (SD) male clean rats of 8 weeks old. The average weight was 206.3 ±24.2g, single cage feeding, room temperature 20, relative humidity 50%, 1 days light: the dark time was 12h:12h. Free intake of drinking water, Streptozotocin (STZ) purchased from Sigma company, with citric acid buffer (pH 4.0), with a concentration of 1% STZ solution.

Experimental method

80 healthy male SD rats were randomly divided into two groups: 20 as normal control group and 60 as diabetes group. The normal control group was fed with normal diet, and the diabetic modeling group was fed with high fat diet. After 4 weeks of feeding, 12h was fasted, and then the intraperitoneal injection of streptozotocin (STZ) 45mg/kg was performed according to the literature method (Leng *et al.*, 2004). The normal control group was injected with 0.1mmol/L and pH4.0 citrate buffer. 72h after tail vein blood, blood glucose was measured with glucose meter gauge, determination of urine glucose test strip. If the blood glucose value is more than 16.7mmol/L, Glucose ++ ~ + + + +, identified as diabetic rats. In the process of modeling, 8 rats had fasting blood glucose below 11mmol/L, which did not meet the requirements and eliminated. The experimental rats were 52 rats and fed with normal diet. Then we randomly selected 12 rats, the 12 modeling group of diabetic rats and normal control group 10 rats were killed, heart blood glucose, insulin and free radical metabolism.

The remaining rats in the diabetes modeling group were randomly divided into five groups: DM control group, DM exercise intervention group, DM puerarin intervention group, DM exercise + puerarin intervention group, normal control groups, 10 rats in each group, total as 5 groups. The animal experiment program has been approved by the experimental animal ethics committee, which conforms to the principles of animal protection,

animal welfare and ethics, and conforms to the relevant provisions of the national laboratory animal welfare ethics, No.WDKZPF/16SQ.

Intervention method

Exercise intervention: treadmill training, speed of 15m/min, treadmill slope of 5 degrees (equivalent to 50% Vmax), each training 60min, the next morning training, and continuous training for 6 weeks. Puerarin intervention: Puerarin was injected with 500mg/kg weight, and intragastric administration was performed at around 5 p.m. every day for 6 weeks.

Collection of blood samples

In one week after the injection of STZ slaughtered diabetic rats and 10 normal control group rats; and 6 weeks after intervention in diabetic rats killed the remaining model and 10 normal control group B rats. Blood samples were collected, 12h was fasted before the specimens were collected, and free drinking water was obtained. The specific methods of collecting are: first, the SD rats are stunned with ether, then the heart of the chest is opened quickly, the blood is taken, the serum is separated and placed in the -20°C refrigerator for examination. During the entire experimental period, fasting 12h was given every Saturday evening and recorded and recorded by the morning blood glucose meter. In this experiment, the method of radioimmunoassay was used to determine the insulin.

Statistical processing

The experimental data were expressed by mean addition and subtraction standard deviation ($\bar{X} \pm SD$), and then SPSS12.0 statistical software was used for statistical analysis. The difference between the groups was tested by independent sample t test, the difference was significant $P < 0.05$, the difference was very significant, $P < 0.01$.

RESULTS

Establishment of rat model of type II diabetes mellitus

After 4 weeks feeding with high fat and high sugar diet and ordinary diet, the body weight of rats in high fat and high glucose group was obviously higher than that of normal diet group. Before modeling DM model rats and normal control rats fasting blood glucose had no significant difference ($P > 0.05$), while the body weight is significantly higher than the control group, indicating that DM model group rats had appeared obese (table 1). One week after the injection of STZ, the fasting blood glucose and insulin of the rats in the modeling group and the normal control group showed a very significant difference ($P < 0.01$), as shown in table 2. This is a good illustration of the fact that rats fed high fat and high sugar with a further injection of STZ significantly reduced their insulin levels, increased their blood sugar levels, and presented a typical symptom of diabetes. Before modeling, the

Table 1: Mean fasting plasma glucose and body weight before modeling

Group	Blood sugar (Mmol/l)	Weight (g)
DM modeling group	8.13±1.25	323.5±49.2
Normal control group	8.11±1.21	314.3±48.6

Table 2: Changes of fasting blood glucose and insulin after modeling

Group	Blood sugar (Mmol/l)	Insulin (Miu/ml)
DM modeling group	18.04±2.85	8.95±2.52
Normal control group	8.21±1.32	17.36±4.27

Table 3: Exercise combined with puerarin intervention

Group	Blood sugar (Mmol/l)	Insulin (Miu/ml)	Insulin Sensitive Index (ISI)
DM control group	21.46±4.36	6.83±3.15	-8.12±0.26
DM exercise intervention group	12.17±4.28	14.23±5.17	-6.53±0.87
DM puerarin intervention group	18.05±3.37	8.39±3.24	-6.26±0.48
DM exercise + puerarin intervention group	9.23±1.48	15.12±5.42	-6.04±0.72

Table 4: The effects of T-AOC, SOD and MDA on DM rats in each group

Group	T-AOC	SOD(U/ml)	MDA(nmol/ml)
DM control group	3.96±0.85	94.65±21.66	11.46±2.35
DM exercise intervention group	7.24±2.17	116.24±5.73	10.17±1.09
DM puerarin intervention group	7.05±1.25	103.17±14.25	8.54±1.26
DM exercise + puerarin intervention group	10.23±3.14	125.14±16.56	5.87±1.35

Table 5: Changes of CAT, GSH and GSH-PX in DM rats in each group

Group	CAT(U/ml)	SOD(U/ml)	MDA(nmol/ml)
DM control group	0.57±0.32	172.53±22.65	312.45±12.65
DM exercise intervention group	1.21±0.52	225.48±23.26	328.17±12.53
DM puerarin intervention group	1.42±0.41	187.13±21.59	315.29±18.72
DM exercise + puerarin intervention group	2.40±1.01	225.16±11.43	348.20±4.13

animals in each group responded sensitively, with smooth hair, normal diet and quick movement. STZ 3-4d after modeling, the rats drinking and eating, polyuria, emaciation, gradually dispirited. The modeling group of symptoms significantly, and gradually appear yellow, sparse hair, dry ear, tail erosion, bleeding, and dry necrosis, abdominal distention, stool pond, and more time, form significant weight loss, slow walking from Susan, slow reaction and other symptoms. Based on the above indexes, the establishment of II diabetic rat model was successful.

Changes of blood glucose and insulin after intervention

It can be seen from table 3, after the intervention, compared with DM control group, DM group and DM exercise intervention exercise plus puerarin intervention group fasting blood glucose level decreased very significant ($P<0.01$), while the DM puerarin intervention group fasting blood glucose level slightly decreased, there was significant difference ($P<0.05$). DM + puerarin group fasting insulin levels have been increased very significant

($P<0.01$), elevated DM exercise intervention group fasting insulin levels had significantly ($P<0.05$), and the changes of DM puerarin group and fasting serum insulin levels are not significant ($P>0.05$). Elevated DM exercise intervention group and DM intervention of Puerarin group and insulin sensitivity index there was significant ($P < 0.05$), while the DM sports + puerarin group and insulin sensitivity index increased significantly, there was significant difference.

It can be seen from table 4, after the intervention, compared with DM control group, DM intervention group and DM intervention of Puerarin in rats of T-AOC group increased significantly ($P<0.05$), while the DM sports + puerarin group T-AOC rats had increased very significant ($P<0.01$). The activities of SOD in the DM intervention group were significantly higher than those in the DM control group ($P<0.05$). DM exercise intervention group and DM puerarin intervention group had a significant decrease in MDA ($P<0.05$), while DM exercise + puerarin intervention group had a very significant difference in

MDA concentration ($P < 0.01$). The changes of catalase (CAT), glutathione (GSH) and glutathione peroxidase (GSH-PX) in the DM rats of each group can be seen in table 5, after the intervention, compared with DM control group, DM intervention group CAT activity have a very significant change ($P < 0.01$); DM DM exercise group and exercise plus puerarin intervention group GSH level was significantly increased ($P < 0.05$), while DM puerarin group slightly increased, no statistical significance ($P > 0.05$); DM intervention group and DM intervention of Puerarin group the level of GSH-PX increased significantly ($P < 0.05$), while the DM sports + puerarin group had very significant difference ($P < 0.01$).

DISCUSSION

The animal model of streptozotocin hyperglycemia was used in this study. In the experimental study of mechanism of action of drugs in the pathological model of choice is very important, the design should first follow the pathological model of similarity, reproducibility, reliability, adaptability, controllability and easy principle, and the evaluation of the pathological model is mainly from the model, model of symptoms and the stability of the model (Wollheim *et al.*, 2000). At present, the animal models of diabetes mainly include three types: experimental diabetic animal model, spontaneous diabetic animal model and transgenic animal model. Although the animal model and transgenic animal models are more ideal and have higher application value, they are still not widely used because of the limitation of objective conditions (Zhao *et al.*, 2016). For a long time, the use of various methods to damage the pancreas or pancreatic beta cells, resulting in insulin deficiency, or the use of various antagonists against insulin, can lead to experimental diabetes or experimental hyperglycemia. Such as surgery, immune mediated, viral infection, hormone induction, and the formation of chemical damage, diabetes, etc., are the classic methods used in pharmacological research (Leng *et al.*, 2004; Ostojic *et al.*, 2015). Among them, the most commonly used is the chemical damage caused by diabetes animal model, commonly used drugs are mainly streptozotocin and four oxygen pyrimidine. We have found through the literature, type II diabetes ideal animal model should maximize the simulation of the natural course of human type II diabetes, and has the characteristics of long survival time, convenient feeding and low cost and easy popularization (Tang *et al.*, 2017).

The research on the simulation of the natural history of classic type II diabetes rat model is divided into two stages, the first stage by high-fat high calorie diet fed SD rats for 4 weeks, body weight was more than the same week old rats were fed with common diet, simulate the occurrence and development of human insulin resistance in type II diabetes (Piro *et al.*, 2002). In the second stage,

the small dose of STZ was injected into the abdominal cavity to damage the pancreatic islet beta cells, reduce insulin secretion, increase blood sugar, and show the clinical symptoms of diabetes. At present, the exercise intervention mechanism of insulin resistance is mainly reflected in the effects of exercise on insulin receptor and insulin receptor level. The study found that endurance training increased by binding in hepatocytes, adipocytes and white muscle cell membrane insulin receptor, reduce high-fat feeding induced insulin resistance in obese rats, improve insulin sensitivity, reduce fat the content of diet combined with endurance exercise (Paccez *et al.*, 2014). It can reduce the content of body fat in obese rats to control level, which can improve the binding force of the insulin receptor, but also increased the activity of TPK receptors; the obese rats attenuate insulin resistance. Long-term regular exercise with small and medium intensity can increase the insulin binding capacity of the hepatic membrane and skeletal muscle cell membrane in rats, and increase the sensitivity of peripheral tissues to insulin (Seshiah *et al.*, 2002).

From the results of this study, it was found that after 4 weeks' feeding with high fat and high sugar diet, the body weight of rats was obviously obese. Compared with the normal control group, the rats had significant difference ($p < 0.05$). At the same time after STZ injection, blood glucose levels and normal control group have significant difference ($p < 0.01$), appeared the phenomenon of insulin resistance. But after 6 weeks of aerobic exercise and puerarin intervention, compared with DM control group, DM group and DM exercise intervention with Puerarin group the blood glucose levels in diabetic rats decreased significantly ($p < 0.05$), the intervention group of diabetic rats DM + Ge Gensu blood glucose decreased significantly ($p < 0.01$), compared with normal control, DM exercise plus puerarin intervention group blood glucose levels in diabetic rats had no significant difference, and the diabetic rats after exercise intervention, insulin values were obviously increased, especially DM exercise plus puerarin intervention group diabetic rats significantly increased insulin ($p < 0.01$), close to the normal value. Moreover, after the experiment, the insulin sensitive index (ISI) of all diabetic rats were significantly improved ($p < 0.05$), and the ISI in the diabetic rats with DM exercise + puerarin intervention group increased the most ($p < 0.01$). It is also well illustrated that aerobic exercise and puerarin intervention can improve insulin resistance in rats.

At the same time, the annual incidence of foot ulcers in diabetic patients is 2%, and inappropriate footwear is the leading cause of diabetic foot ulcers (Tang *et al.*, 2017). Neuropathy is not directly caused by ulcer, but peripheral neuropathy resulting in lower limb hypoesthesia, foot injury occurred in the absence of perception, caused by foot skin damage and ulcer and infection in diabetic

patients with lower extremity vascular disease, which leads to poor blood circulation, difficult to heal ulcer prone to infection (Tural *et al.*, 2015; Takahashi, 2017). There is evidence that appropriate screening and pre stroke measures can reduce the risk of diabetic foot ulcers. Clinicians can identify those patients at high risk of diabetic foot ulcers by monitoring screening, including the relevant medical history, examination has no structural abnormalities and foot deformity, particularly important is the single test evaluation of patients without loss of sensation of foot function. Other meaningful tests include measuring, assessing peripheral vascular disease, checking or fitting shoes, and assessing plantar pressure in patients where conditions permit.

CONCLUSION

From the point of view of exercise physiology, exercise therapy is an important means of treatment of diabetes mellitus and has been recognized. Puerarin from legumes exhibits the expansion of coronary artery and cerebral vessels, reduce myocardial oxygen consumption and blood circulation and improve microcirculation. In short, through exercise and Puerarin, really be able to play some role in prevention and treatment of diabetes, such as reducing some symptoms and improve the metabolic status of patients with diabetes, patients on drug dependence. However, the specific mechanism of its action needs further study.

ACKNOWLEDGEMENTS

The research was supported by Individualized design and application evaluation of orthopedic insoles for plantar fasciitis (Subject number: K2016030).

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