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Original article

Effect of curcumin on glucose and lipid metabolism, FFAs and TNF-α in serum of type 2 diabetes mellitus rat models

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ABSTRACT

Objective: To investigate how curcumin affects the glucose and lipid metabolism in type 2 diabetes mellitus (DM) rat models, and to explore its effect on the free fatty acid (FFA) and tumor necrosis factor α (TNF-α) in serum.
Methods: Successfully established type 2 DM rats were divided into three groups, i.e. the normal control group, model group and curcumin group, and received the medication for consecutive 8 weeks. Thereafter, we detected the level of fasting blood glucose (FBG), and the blood glucose at 30 min, 60 min and 120 min; besides, we also carried out the insulin tolerance tests to measure the levels of fasting serum insulin (FINS) and blood glucose at 40 min and 90 min; additionally, we also detected the levels of TC, TG, HDL-C, LDL-C, FFA and TNF-α in serum. The results were expected to discover the mechanism of curcumin in decreasing the blood glucose level in DM rats.
Results: Compared with the model group, AUCs of FBG, blood glucose at 30 min, 60 min and 120 min, and glucose were decreased in varying degrees in the curcumin group, and the differences had statistical significance ($p < .05$). After subcutaneous injection of insulin, we found that the blood glucose at 40 min and 90 min in the curcumin group was decreased, while AUC of glucose level was also decreased ($p < .05$ or .01). Eight weeks after medication, compared with the rats in the normal group, the levels of HDL-C, LDL-C, TC and TG in rats of the model group and the curcumin group were obviously increased ($p < .05$). In comparison with the model group, the level of LDL-C in rats of the curcumin group was also decreased significantly ($p < .05$). In comparison with the normal control group in the same period, we found that the content of FFAs and TNF-α in serum of rats of the model group were elevated significantly, and the differences had statistical significance ($p < .05$ or .01); the levels in the curcumin group were significantly decreased in comparison with the model group in the same period, and the difference had statistical significance ($p < .05$ or .01).
Conclusion: Treatment with curcumin can significantly improve the metabolic disorder of glucose and lipid, enhance the sensitivity to the insulin, and ameliorate the resistance to insulin of the type II DM rats. Meanwhile, this treatment method can also significantly decrease the level of FFA and TNF-α in serum of type II DM rats. Thus, we inferred that the mechanism of curcumin to improve the insulin resistance might be correlated with the decreases of FFA and TNF-α in serum.

0. Introduction

Diabetes mellitus (DM) is one of the major chronic non-infectious diseases in the world, and, with a continuous increase in number of patients, the prevalence of DM is also constantly aggravated; thus, DM has brought a much more severe burden to human beings. According to the *Global Report on Diabetes* of World Health Organization (WHO) in 2016, there were 422 million (or 8.5% of the population) in the world suffering from the DM, and DM as well as its complication has been affecting the life quality of human beings, and become an important public health problem.

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Thus, research and development of Traditional Chinese Medicine (TCM) for effective treatment of DM and its complications are of great application significance (Srinivasan, 1972). Curcumin, a kind of diketone compounds extracted from the Curcuma Longa, is quite safe with extensive pharmacological effects, such as anti-inflammation, anti-oxidation, decreasing the blood glucose, immune regulation and anti-proliferation (Suresh Babu and Srinivasan, 1997; Arun and Nalini, 2002). A variety of biological effects of curcumin are quite conducive to ameliorating the progression of DM, and thus, curcumin is a kind of natural drug with great potential (Arun and Nalini, 2002). According to the study, it is reported that curcumin can not only protect the pancreatic β cell and increase the sensitivity to insulin (Onozato et al., 2002), but also manifest positive significance for treatment of DM complication.

Although the glucose-lowering effect of curcumin has been reported, we have not found any studies reporting the effect of curcumin on the blood glucose levels of type 2 DM rats in different stage of disease, or the effect on the postprandial blood sugar and levels of FFAs and TNF-α in serum. Thus, this study aimed to explore the effect of curcumin, as the active ingredient, on the postprandial blood sugar and the levels of FFAs and TNF-α in different stage of disease in the DM rat models, so as to figure out the characteristics of curcumin in decreasing the blood sugar and the specific mechanism.

1. Materials

1.1. Reagent

Total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) (Shanghai High-track Medical Equipment Co., Ltd.); citric acid and sodium citrate (Beijing Taibo Chemical Co., Ltd.); buffer for paraffin-embedding, washing, dilution, substrate (pH = 5.0), tetramethyl benzidine (TMB), and ABTS (Guangzhou Ribobio Co., Ltd.); curcumin (99%; Sinopharm Chemical Reagent Co., Ltd.); streptozotocin (STZ; Sigma, US).

1.2. Instruments

Roche glucometer (ACCU-CHEK, Roche, Germany); automatic biochemical analyzer (AU2700, Olympus, Japan); electronic scale (Mettler-Toledo, Switzerland).

1.3. Experiment animals

Male specified-pathogens free (SPF) SD rats (weight between 220 and 240 g) were provided by Henan Experiment Animal Center [SCKK (Yu) 2010–0002].

2. Methods

2.1. Establishment of animal models (Shome et al., 2016; Lee et al., 2003)

In 60 SPF SD male rats, 15 were selected randomly as the normal control and received the normal feeding procedure, while remaining rats were fed with the high-fat and -glucose food (cholesterol 1%, yolk powder 3%, lard oil 10%, sucrose 20% and basic food 66%). After 4 weeks of feeding, intraperitoneal injection of STZ (1%; 25 mg/kg) prepared by the citric acid and sodium citrate buffer. Thereafter, high-fat and -glucose feeding procedure lasted for another 4 weeks followed by measurement of the random blood glucose with the glucometer, and random blood glucose $\geq$16.7 mmol L^{-1} suggested that the model was established successfully.

2.2. Drug intervention

In this study, models were successfully established in 30 rats, and these rats were randomly divided into two groups, i.e. the model group (n = 15) and the curcumin group (n = 15), and received the intragastric administration at a dose of 250 mg/kg. Rats in the curcumin group took curcumin, while rats in the normal control group and the model group took normal saline in equivalent volume instead. Intragastric administration was performed once per day, and lasted for 8 weeks. During this period, rats in each group underwent regular feeding.

2.3. Observation indexes and measurement methods

During drug administration, we observed the responses, status and death of rats in each group closely. At the 4th and 8th weeks, blood samples were collected from the caudal vein for detecting the FBG level and the blood glucose levels at 30 min, 60 min and 120 min with the glucometer; as for insulin tolerance test, we detected the FINS level and the blood glucose at 40 min and 90 min; after the last time of drug administration, we collected the blood sample from the arteria cruralis to measure the levels of TC, TG, HDL-C and LDL-C in serum with the automatic biochemical analyzer; in addition, before drug administration and after the last time of drug administration, we assayed the levels of FFA and TNF-α in blood with the automatic biochemical analyzer.

2.4. Statistical analysis

Experiment data were presented as mean ± standard deviation ($\bar{x} \pm s$) and SPSS 17.0 was applied for statistical analysis. As for intergroup comparison, t test was adopted. $p < .05$ suggested that the difference had statistical significance (see Table 1).

3. Results

3.1. General condition of rats

3.1.1. Changes in glucose tolerance

From the results of this study, we found that in the same period, the FBG level in the curcumin group was significantly lower than that in the model group, and the difference had statistical significance ($p < .05$); the blood glucose at 30 min in the curcumin group was also lower than those in the model group and the normal control group, but when it came to the 4th week, the differences had statistical significance ($p < .05$); the blood glucose at 60 and 120 min was also lower than those in the other two groups, but these differences only had statistical significance until the 4th week ($p < .05$); at the 8th week, we found that the differences with the model group in the same period also showed statistical significance ($p < .05$). Compared with the normal control group in the

Table 1
General condition of rats in all groups.

Group	Diet and activity	Mental status	Skin and fur
Normal control group	Normal	Fine	Lustrous
Model group	Decreased	Dispirited	Reluster and messy
Curcumin group	Decreased in varying degree	Fair	Reluster

same period, we found that the AUCs of glucose in each group were all significantly increased, and the difference had statistical significance ($p < .05$). In comparison between the curcumin group and the normal control group, model group in the same period, we found that the AUCs of glucose were decreased in the curcumin group, and the differences had statistical significance ($p < .05$). All these results suggested that curcumin can lower the blood glucose level in type II DM rat models (Table 2).

3.2. Changes in insulin tolerance

In the experiment of insulin tolerance test, the results showed that in the same period, the FBG level in the curcumin group was significantly lower than that in the model group, and the difference had statistical significance ($p < .05$). After subcutaneous injection of insulin, we found that the blood glucose at 40 min and 90 min in the curcumin group was decreased, while AUC of glucose level was also decreased ($p < .05$ or .01). These results suggested that curcumin can ameliorate the decrease in insulin tolerance and increase the sensitivity to insulin in type II DM rat models (Table 3).

3.3. Changes in TC, TG, HDL-C and LDL-C in serum

From Table 4, we can see that after 8 weeks of medication, compared with the rats in the normal group, the levels of HDL-C, LDL-C, TC and TG in rats of the model group and the curcumin group were obviously increased ($p < .05$). In comparison with the model group, the level of LDL-C in rats of the curcumin group was also decreased significantly ($p < .05$), suggesting that curcumin can decrease the LDL-C level in type II DM rats induced by high-fat food and STZ.

3.4. Changes in FFA and TNF-α in serum

In comparison with the normal control group in the same period, we found that the content of FFAs and TNF-α in serum of rats of the model group were elevated significantly, and the differences had statistical significance ($p < .05$ or .01); the levels in the curcumin group were significantly decreased in comparison with the model group in the same period, and the difference had statistical significance ($p < .05$ or .01; Table 5). This suggested that curcumin can decrease the levels of FFA and TNF-α in type II DM rats in varying degrees.

4. Discussion

TCM studies have indicated that curcumin is a major active ingredient with the pharmacological effects of anti-oxidation, anti-inflammation and anti-canceration (Masuda, 1999; Weisberg et al., 2016). However, there remain few studies reporting the role of curcumin in treatment of DM, and the effect of curcumin on

Table 2
Changes in the glucose tolerance of rats at the 4th and 8th weeks after drug administration ($\bar{x} \pm s$).

Group	Time	Case (n)	Blood glucose				
			0 min	30 min	60 min	120 min	AUC
Normal control group	4th week	15	5.73 ± 0.52	7.41 ± 0.69	7.98 ± 0.98	7.69 ± 0.82	22.38 ± 1.12
	8th week	15	5.8 ± 0.7	8.52 ± 0.67	8.41 ± 0.79	7.73 ± 1.23	24.13 ± 1.98
Model group	4th week	15	7.83 ± 2.67*	13.73 ± 1.17*	14.25 ± 2.63*	13.78 ± 2.52*	32.65 ± 2.78*
	8th week	15	11.99 ± 1.52**	15.78 ± 2.69*	16.18 ± 3.28*	15.92 ± 3.96*	35.96 ± 4.21*
Curcumin group	4th week	15	4.83 ± 1.81#	10.07 ± 3.61*,#	11.76 ± 2.96*,#	10.91 ± 3.02*,#	28.99 ± 3.92*,#
	8th week	15	6.96 ± 0.76#	11.03 ± 3.51*	12.89 ± 2.99*,#	11.77 ± 4.12*,#	29.97 ± 3.89*,#

* $p < .05$ vs. normal control group.
\# $p < .05$ vs. model group.

Table 3
Changes in the insulin tolerance of rats at the 4th and 8th weeks after medication.

Group	Time	Case (n)	Blood glucose			
			0 min	40 min	90 min	AUC
Normal control group	4th week	15	6.39 ± 0.97	4.23 ± 1.09	4.02 ± 0.93	9.27 ± 1.18
	8th week	15	5.97 ± 1.12	4.11 ± 0.99	4.52 ± 0.69	9.33 ± 1.09
Model group	4th week	15	8.99 ± 2.63*	7.93 ± 1.56**	7.17 ± 1.89**	12.98 ± 2.32**
	8th week	15	12.01 ± 1.98**	10.99 ± 1.28**	10.81 ± 1.85**	14.92 ± 1.69*
Curcumin group	4th week	15	5.02 ± 1.58#	4.19 ± 0.98##	4.07 ± 1.77#	9.32 ± 1.88#
	8th week	15	6.89 ± 1.73#	4.03 ± 1.51#	3.89 ± 1.99#	9.23 ± 2.08#

* $p < .05$ vs. normal control group;
** $p < .01$ vs. normal control group;
\# $p < .05$ vs. model group;
\## $p < .01$ vs. model group.

Table 4
Changes in TC, TG, HDL-C and LDL-C in serum of rats in all groups after 8 weeks of medication.

Group	Case (n)	HDL-C (mmol/L)	LDL-C (mmol/L)	TC (mmol/L)	TG (mmol/L)
Normal control group	15	0.93 ± 0.14	0.19 ± 0.07	1.21 ± 0.12	0.75 ± 0.24
Model group	15	1.52 ± 0.44*	4.09 ± 3.91*	6.61 ± 6.04*	1.25 ± 0.49*
Curcumin group	15	1.31 ± 0.71*	2.95 ± 6.42*,#	6.35 ± 10.31*	1.01 ± 0.47*

* $p < .05$ vs. normal control group.
\# $p < .05$ vs. model group.

Table 5
Changes in FFA and TNF-α in serum.

Group	Time	Case (n)	FFA (μmol/L)	TNF-α (ng/L)
Normal control group	4th week	15	143.98 ± 12.12	72.12 ± 11.45
	8th week	15	167.25 ± 9.89	73.98 ± 10.01
Model group	4th week	15	189.23 ± 11.56**	80.25 ± 8.25*
	8th week	15	195.56 ± 10.45**	79.50 ± 11.21*
Curcumin group	4th week	15	152.21 ± 5.96#	75.93 ± 9.12#
	8th week	15	149.99 ± 9.24##	74.82 ± 8.27#

* $p < .05$ vs. normal control group.
** $p < .01$ vs. normal control group.
\# $p < .05$ vs. model group.
\#\# $p < .01$ vs. model group.

glucose and lipid metabolism in type II DM as well as the molecular mechanism have been elusive.

In this study, with the type II DM rats as subjects, these rats were fed with high-fat food for 4 weeks followed by injection of STZ, and after the other 4 weeks of feeding with high-fat food, we screened out the rats with random blood glucose $\geq$16.7 mmol·L^{-1} as the subjects. Then, the subjects received the intragastric administration of curcumin, and the comparison between the curcumin group and the model group in the same period showed that the FBG, blood glucose at 30, 60 and 120 min and the AUC of glucose were decreased in varying degrees in the curcumin group, and the differences had statistical significance ($p < .05$). After subcutaneous injection of insulin, we found that the blood glucose at 40 min and 90 min in the curcumin group was decreased, while AUC of glucose level was also decreased ($p < .05$ or .01), suggesting that curcumin can decrease the blood glucose in type II DM models, and the effect usually appears after 4 weeks of medication.

Through measurement of TC, TG, HDL-C and LDL-C levels in each group, we found that eight weeks after medication, compared with the rats in the normal group, the levels of HDL-C, LDL-C, TC and TG in rats of the model group and the curcumin group were obviously increased ($p < .05$). In comparison with the model group, the level of LDL-C in rats of the curcumin group was also decreased significantly ($p < .05$), suggesting that curcumin can decrease the LDL-C level in the type II DM rat models induced by high-fat food and STZ.

Among the majority of patients with obesity, especially those with abdominal obesity, various factors can lead to an increase in visceral fat, among which the role of FFA is quite important (Ha and Lee, 2000). The mechanism might be (Vallianou et al., 2015; Weijing et al., 2014; Sophie, 2013): Under the homeostatic regulation, extra adipose tissues are prone to degradation and metabolism when the activity of LPL is increased, resulting in excessive release of FFA, and leading to or exacerbating the insulin resistance. TNF-α is a kind of cytokine in association with the insulin resistance and type II diabetes mellitus, and mainly secreted by mononuclear macrophage. It has been reported that TNF-α has a variety of functions, e.g. activating the leukocytes, mediating the endotoxic shock, regulating the inflammation and immune responses. TNF-α is highly expressed usually in the adipose cells of human beings or animals with obesity or insulin resistance (Zampetaki et al., 2010; Kong et al., 2011), and the mechanism might be that through enhancing the HSL activity and inhibiting the activity of LPL, TNF-α can promote the mobilization of fat and decrease the content of fatty acid, thereby accelerating lipid synthesis. In addition, there is a vicious cycle between the FFA and TNF-α (Karolina et al., 2011; Lebovitz et al., 2002), i.e. TNF-α can increase the level of FFA in plasma, but FFA can increase the expression of TNF-α through the autophagy-lysosome pathway. Thus, this viscious cycle promote and even exacerbate the insulin resistance (Charbonnel et al., 1999). This process might be realized through promoting the alterations in structure and function of mitochondria, oxidative stress and oxidation and overload of fatty acid.

From this study, we found that treatment with curcumin can ameliorate the disorder in glucose and lipid metabolism, increase the sensitivity to insulin and improve the insulin resistance in type II DM rat models. Meanwhile, the results of this study also revealed that curcumin medication can significantly decrease the levels of FFA and TNF-α in serum of type II DM rat models. Thus, we inferred that the mechanism of curcumin in ameliorating the insulin-resistance might be correlated with the decrease in levels of FFA and TNF-α in serum. The results of this study are expected to enhance the understanding the pathogenesis of DM, and to provide theoretical evidence for discovering the molecular biological mechanism of curcumin in treatment of type II DM.

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