

Modulation of curcumin on PERK- CHOP signaling pathway in pancreas of type 2 diabetic rats

Objective: To investigate whether curcumin can decrease blood glucose by affecting the PERK- CHOP signaling pathway in pancreas.

Methods: A total of 45 male SD rats were randomly divided into normal control group, diabetes model group and curcumin treatment group, 15 rats in each group. The rat was given high fat as well as high sugar diet and intraperitoneal injection of streptozotocin (35 mg/kg, twice, once /d) to establish type 2 diabetes rat model with the model success level as "fasting glucose ≥ 11.1 mmol /L. After the model was successfully established, the rats in curcumin treatment group were treated with curcumin through gastric tube for 30 days at 200 mg/kg per day. ELISA method was adopted to detect serum adiponectin level, glucose oxidase method was applied to measure blood glucose and Western blotting as well as Real-time PCR method were respectively used to detect the key molecules of PERK- CHOP expression of PERK, CHOP protein, signaling pathway, and mRNA.

Results: Compared with normal control group, the blood glucose level was significantly higher and serum adiponectin level significantly lower in diabetes model group ($P < 0.05$); compared with diabetes model group, the blood glucose level of curcumin treatment group decreased significantly and serum adiponectin level significantly increased ($P < 0.05$). The content of PERK and CHOP mRNA in pancreatic tissue of diabetes group significant increased compared with normal control group ($P < 0.05$) and compared with diabetes group, the expression of PERK and CHOP mRNA in curcumin treatment group was lower ($P < 0.05$).

Conclusion: Curcumin can down regulate expression of PERK and CHOP in the pancreas of diabetic rats, correct the abnormality of PERK-CHOP signal transduction pathway during diabetes and it can also play a role in lowering blood glucose by enhancing the level of adiponectin, the sensitivity of target tissues to insulin as well as the level of adiponectin.

Keywords: curcumin • rat • type 2 diabetes mellitus • pancreas • PERK-CHOP signaling pathway

Introduction

Type 2 diabetes is the most common form of diabetes and it refers to a lack of insulin with the ability to produce insulin in patients not completely lost but the body no more sensitive to insulin, which finally leads to reduced hypoglycemic effect. If blood glucose remains high for a long time, the endothelial cells in vessels would be easily damaged with the incidence of atherosclerosis, which is much likely to induce cardiovascular disease and stroke [1]. Diabetes is mainly treated by interventions on diet and exercise, which, however, can be easier realized by additionally taking

curcumin [2]. A related study has shown that [3] curcumin can prevent and alleviate diabetes with the improvement in insulin sensitivity. Endoplasmic Reticulum Stress (ERS) has been a hot spot in recent researches into the pathogenesis of type 1 and type 2 diabetes [4]. It can promote insulin resistance and β cell apoptosis. (PERK) -C /EBP- CHOP, as a classical signaling pathway in ERS, plays a key role in pancreatic islet β cell apoptosis [5,6]. Further research on the signaling pathway of PERK- CHOP helps to elucidate the pathogenesis of diabetes and provide new insights into the treatment. In this study, we studied the hypoglycemic effect of

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curcumin in the early stage and found that it can significantly reduce blood sugar. But the mechanism by which curcumin lowers blood sugar remains unknown. Therefore, we explored the effect of curcumin on the regulation of pancreatic signaling pathway of PERK-CHOP in the rats, expecting to reveal the hypoglycemic mechanism of curcumin and lay a foundation for further treatment of type 2 diabetes by curcumin.

Materials and methods

▪ Experimental animals

A total of 45 male clean grade SD rats with the body weight of (210 ± 15) g provided by an animal experimental center.

▪ Experimental drug and reagents

Curcumin was purchased from Hebei Tian Xu Biological Science and Technology Co., Ltd.. Streptozotocin (STZ) was purchased from American Sigma company, which was made into 2% solution with the addition of sodium citrate-citric acid citrate buffer (pH 4.4) while being used; blood glucose assay kit was purchased from Shanghai Rongchuang Biotechnology Co., Ltd.; rat adiponectin ELISA kit was purchased from Shanghai Lanji Bio-Technology Co., Ltd; polyclonal antibody of Rabbit anti CHOP and PERK was purchased from Wuhan, Jia Hao Biological Technology Co. Ltd; IgG second antibody of Goat anti Rabbit was purchased from Nanjing Shengxing Biotechnology Co., Ltd.; BCA protein assay kit was purchased from Hangzhou Lianke Biological Technology Co. Ltd and Super ECL Plus ultra sensitive luminescent was purchased from Beijing pulilai Gene Technology Co Ltd.

▪ Model making and drug intervention

A total of 45 SD rats were randomly divided into normal control group, diabetes model group and curcumin treatment group, 15 rats in each group. The rat was given high fat as well as high sugar diet and intraperitoneal injection of streptozotocin (35 mg/kg, twice, once/d) to establish type 2 diabetes rat model with the model success level as "fasting glucose ≥ 11.1 mmol/L. After the model was successfully established, the rats in curcumin treatment group were treated with curcumin through gastric tube for 30 days (200 mg/kg per day); normal control group and diabetes model group were given normal saline of the same quantity with the same duration as curcumin treatment group.

▪ Experimental materials

The rats in each group were fasted for over 12 h after treatment, then under the anesthesia by intraperitoneal injection of 10% chloral hydrate, they were given thoracotomy and killed after exaction of blood from heart followed by quick collection, the pancreas was taken out with a part of them directly preserved in liquid nitrogen and another part ground preserved in liquid nitrogen after being ground by adding into Trizol after.

▪ Histological examination of pancreas

The pancreas in rats of each group were given paraffin section and HE staining followed by the observation on pancreatic isle structure in all rats under light microscope.

▪ Detection of serum adiponectin and blood glucose level

The collected blood was centrifuged at the rate of 3000 R/min at 4°C for 20 min followed by collection of serum. Blood glucose level was measured by glucose oxidase method and adiponectin level ELISA method.

▪ Detection of PERK and CHOP expression by Western blotting assay

The total protein was extracted from 100 mg pancreas tissue, and the concentration of total protein was quantified by bicinchoninic acid (BCA) protein kit. The protein sample was 100 g and given 12%, 8%, 8% and 12% SDS-PAGE gel electrophoresis followed by transmembrane, closed with 5% skim milk powder for the overnight; the first antibody (PERK 1:500, CHOP 1:500, β -actin 1:1000) was incubated at room temperature for 2 h; second antibody (1:2000 diluted) was incubated at room temperature for 1 h; Super ECL Plus ultra sensitive luminescent liquid was used to develop the photo with the scan conducted on film. Quantity One-v 4. 6.2 software was used to analyze the developing band with the ration of "gray scale of target band to gray scale of β -actin band" as the relative expression level of each target protein.

▪ Detection of the expression of PERK, CHOP and mRNA by Real-time PCR

Total RNA was extracted by Trizol and reversely transcribed into cDNA, specific primers as well as relative standard curves were designed with glyceraldehyde phosphate dehydrogenase (GAPDH) as internal control, according to the difference in Cycle threshold (Ct), the difference in expression of the target

gene was assessed by delta method($\Delta\Delta$). PCR primer sequence: GAPDH Forward 5' -CCA TGG AGA AGG CTG GG -3', Reverse 5' - CCA TGG AGA AGG CTG GG-3'; PERK Forward 5' -GATGACTGCAATTACGCTATCAAGA-3', Reverse 5' -CCTTCTCCCGTGCCAACTC-3';CHOP Forward 5' - CCA GCA GAG GTC ACA AGC AC- 3' Reverse 5' - CGC ACT GACCAC TCT GTT TC - 3'. The PCR primer was designed and synthesized by Shanghai Engineering Biology Co., Ltd..

▪ **Statistical analysis**

Statistical analysis was performed on SPSS 21 software and the result was described as "mean \pm standard deviation", Comparison of mean in multiple groups was conducted with analysis of variance one-way (ANOVA) and comparison between the two groups was performed with LSD test, P < 0.05 was considered as statistical significance.

Results

▪ **Observation of HE staining in each group**

The HE staining results showed that the pancreatic islets of rats in the normal group were oval-shaped with equable size, regular sharp as well as clear boundary, pump in shape and closely arranged (**FIGURE 1A**); islets of rats in the model group were seen obvious atrophy of irregularity with structure disorder, apart of exocrine pancreas went deep into the inside of islets and the kytoplasm of most islet cells were significantly reduced with vascular degeneration (**FIGURE**

1B); In curcumin treatment group, the islet was approximately circular or elliptic with clear boundary as well as no obvious atrophy and the cells were closed arranged with good shape and obviously reduced vacuolar degeneration (**FIGURE 1C**).

▪ **Comparison of blood glucose and serum adiponectin level among groups**

Compared with normal control group, the blood glucose level was significantly higher and serum adiponectin level significantly lower in diabetes model group(P<0.05); compared with diabetes model group, the blood glucose level of curcumin treatment group decreased significantly and serum adiponectin level significantly increased (P<0.05) as shown in **TABLE 1**.

▪ **Comparison of PERK and CHOP proteins among groups**

The content of PERK and CHOP in diabetes model group significant increased compared with normal control group (P<0.05) and the expression of PERK and CHOP in curcumin treatment group was lower than that of diabetes model group (P<0.05), as shown in **TABLE 2** and **FIGURE 2**.

▪ **Comparison of PERK and CHOP mRNA among groups**

The content of PERK and CHOP mRNA in diabetes model group significant increased compared with normal control group (P<0.05) and the expression of PERK and CHOP mRNA in curcumin treatment group was lower than

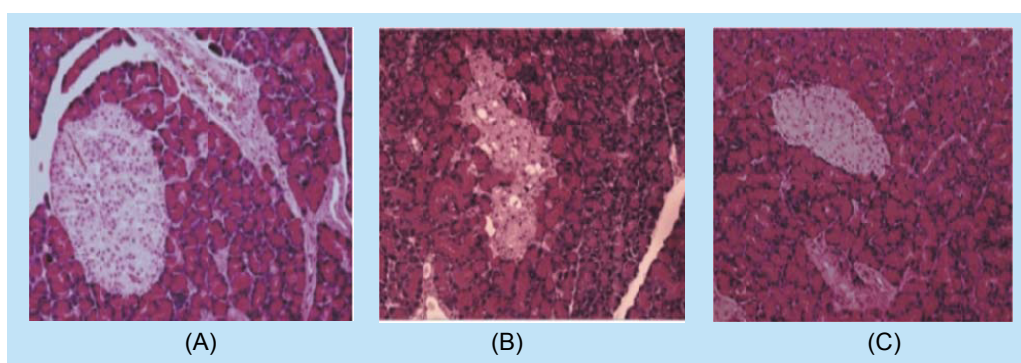


Figure 1. Pathological changes of pancreas islets in rats of each group (HE, *200). (A) normal control group (B) diabetes model group (C) curcumin treatment group.

| Table 1. Comparison of blood glucose and serum adiponectin level among groups. | | | |
|---|----------|-------------------------------|--------------------------|
| Group | n | Blood glucose (mmol/L) | Serum adiponectin |
| Normal control group | 15 | 10.73 \pm 1.54 | 68.32 \pm 12.61 |
| Diabetes model group | 15 | 30.04 \pm 3.25 | 53.91 \pm 9.08 |
| Curcumin treatment group | 15 | 14.86 \pm 2.77 | 94.12 \pm 16.34 |
| F | | 48.187 | 8.052 |
| P | | 0.000 | 0.020 |

that of diabetes group ($P < 0.05$), as shown in **TABLE 3**.

Discussion

Diabetes mellitus (DM) is one of the most common chronic noncommunicable diseases in the world with increasingly high incidence and serious burden of disease in patients. According to the “global diabetes report” by WHO in 2016, there are 422 million people (or 8.5% of the population) suffering from diabetes in 2014. Diabetes and its complications have seriously affected the quality of human life and become an important issue of public health [7]. Therefore, it is of great value and significance to actively explore new Chinese medicines which can effectively prevent or cure diabetes mellitus and improve its complications.

Curcumin is a natural polyphenolic compound extracted from the rhizome of plants of curcuma genus (such as curcuma, curcuma aromatica and curcuma zedoary) [8]. In Asia countries like China and India, curcuma is used as medicine and food and now it has been widely used as a natural pigment food additive both at home and abroad [9].

However, it is found by many studies in nearly more than half a century that curcumin also has a very wide pharmacological effects, including anti-inflammatory, antioxidant, lowering blood sugar, immune regulation and antiproliferative activity [10,11]. It is a natural Chinese medicine with great potential and many of its biological functions are beneficial to improve the progression of diabetes. A related study [12] has shown that curcumin not only protects pancreatic β cells and increases insulin sensitivity, but also helps with the treatment for a variety of complications. In addition, some scholars [13,14] also have studied the effect of curcumin on cell signal transduction pathway and made it clear that curcumin can inhibit nuclear transcription factor kappa NF B or AP - 1 through inhibition of signaling pathways of MAPK, SphK1 - S1P, or PKC- α and PKC - β 1, activate the signaling pathway of AMPK, upregulate the expression of Smad7 and activate nuclear transcription factor Nrf2 to play the effects of antiinflammation, antioxidation, anti-proliferation, blood sugar reduction and immunity regulation. Excessive and persistent ERS leads to activation of PERK,

Table 2. Comparison of PERK and CHOP contents among groups.

| Group | n | PERK | CHOP |
|--------------------------|----|-------------|-------------|
| Normal control group | 15 | 1.36 ± 0.25 | 0.41 ± 0.16 |
| Diabetes model group | 15 | 2.24 ± 0.43 | 0.92 ± 0.11 |
| Curcumin treatment group | 15 | 1.12 ± 0.31 | 0.65 ± 0.07 |
| F | | 9.512 | 13.754 |
| P | | 0.014 | 0.006 |

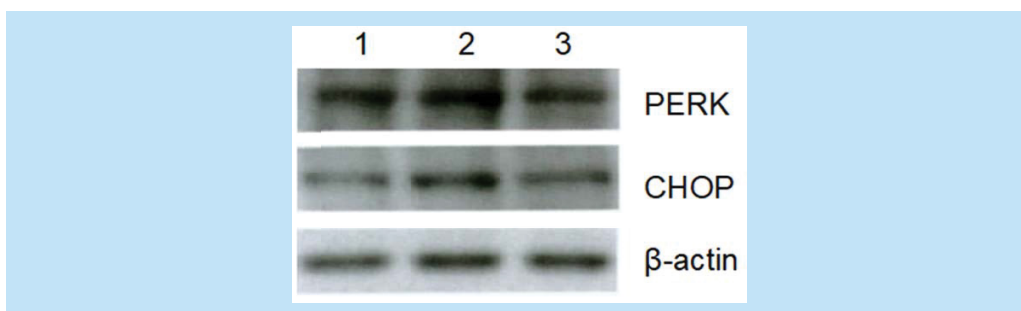


Figure 2. The expression of PERK and CHOP proteins of pancreatic tissues in each group. (1) normal control group (2) diabetes model group (3) curcumin treatment group.

Table 3. Comparison of PERK and CHOP mRNA among groups.

| Group | n | PERK/GAPDH | CHOP/GAPDH |
|--------------------------|----|-------------|-------------|
| Normal control group | 15 | 1.05 ± 0.31 | 1.02 ± 0.27 |
| Diabetes model group | 15 | 1.64 ± 0.28 | 1.18 ± 0.33 |
| Curcumin treatment group | 15 | 0.85 ± 0.07 | 0.53 ± 0.04 |
| F | | 9.128 | 7.247 |
| P | | 0.015 | 0.025 |

thereby activating ATF4 to separate the complex of ATF4/CHOP followed by activation of CHOP, which can induce ER associated cell death through signaling pathways like Bcl -2/Bax. At present, there are few reports about the effect of curcumin on lowering blood glucose and on PERK-CHOP signaling pathway. In this research we mainly studied whether curcumin plays a role in lowering blood sugar by affecting pancreatic PERK- CHOP signaling pathway and found that the content of PERK and CHOP mRNA in pancreatic tissue of diabetes group significant increased compared with normal control group ($P<0.05$) and compared with diabetes group, the expression of PERK and CHOP mRNA in curcumin treatment group was lower ($P<0.05$), all of which that curcumin can improve the abnormality of PERK- CHOP signal transduction pathway in islet beta cell so as to protect the the cell with decreased apoptosis and increased insulin secretion, thereby lowering blood sugar. The results of HE staining also confirms that curcumin could improve the structure of pancreatic islet and protect the beta cells in rats.

Adiponectin is a fatty factor secreted by adipocytes and has the effects of regulating glucose movement as well as influencing insulin sensitivity [15]. The results of this study showed that compared with normal control group, the blood glucose level was significantly higher and serum adiponectin level significantly lower in diabetes model group ($P<0.05$); compared with diabetes model group, the blood glucose level of curcumin treatment group decreased significantly and serum adiponectin level significantly increased ($P<0.05$), all of which suggested that curcumin can raise the sensitivity of target tissue to insulin by increasing the level of adiponectin and thus play the effect of reducing blood sugar.

In conclusion, curcumin can enhance the sensitivity of target tissue to insulin by increasing the level of adiponectin; more importantly, it can, through up regulation of expression of factors related to PERK- CHOP signaling pathway in islet beta cell, improve the abnormal changes of the PERK- CHOP signaling pathway, thereby maintaining normal cell cycle as well as proliferation, delaying function decline in pancreatic beta cells and eventually achieving the purpose of reducing the blood glucose.

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