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Curcumin protects islet beta cells from streptozotocin-induced type 2 diabetes mellitus injury via its antioxidative effects

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Abstract

Introduction: Streptozotocin (STZ)-induced diabetes rodent models are widely used to study the pathogenesis and metabolic function in diabetes (DM). The aim of this study was to assess the antioxidant effect of curcumin in STZ-induced type 2 diabetes mellitus (T2DM).
Material and methods: In this research, rats were randomly divided into 3 groups (8 in each group): a nondiabetic group (Control), a diabetic group (DM), and a curcumin treatment group (DM + Cur 200 mg/kg group).
Results: after intraperitoneal injection (*i.p.*), associated-oxidative stress parameters were observed, malondialdehyde (MDA) was decreased, and glutathione peroxidase (GPX) and super oxide dismutase (SOD) were restored in pancreatic tissues of curcumin-treated DM rats. In addition, curcumin improved the survival and function of islet cells with decreased cell apoptosis in Langerhans islet and increased insulin secretion in the STZ-induced T2DM rat model.
Conclusion: Our findings suggest that curcumin is a potent candidate for the prevention and therapy of DM. **(Endokrynol Pol 2022; 73 (6): 942–946)**

Key words: *antidiabetic activity; antioxidant activity; curcumin; diabetes mellitus; pancreatic beta cell toxicity*

Introduction

Diabetes mellitus (DM), which is characterized by hyperglycaemia and insufficiency of secretion or action of endogenous insulin, is a chronic metabolic disorder with a rapidly increasing prevalence [1, 2]. Diabetes mellitus was classified as type 1 (T1DM), type 2 (T2DM), and other specific types of DM, and gestational diabetes. One subtype of T2DM occurs due to insulin resistance coupled with insufficient production of insulin [3]. However, obesity, physical inactivity, viral infection, autoimmune disease,s and environmental factors are known to be major risk factors for DM [4–8]. It is widely accepted that increased oxidative stress participates in the process of DM development and its complications [9]. Hence, antioxidants are proposed to have a beneficial effect on reducing the oxidative injury derived from the above risk factors. In addition, chemical induction of DM by streptozotocin (STZ) administration is one of the most frequently used animal models for experimental type 1 [10] and type 2 [11] diabetes mellitus. DNA alkylating activity and beta cell selective toxicity of STZ contributes to the pathogenesis of diabetes mellitus. In this research, a simple model of STZ-induced T2DM was constructed to study curcumin's anti-diabetic activity. Curcumin, a kind of natural phenol extracted from *Curcuma longa* plants, possesses a wide range of pharmacological properties, including antioxidant, anti-inflammatory, and anticancer [12, 13]. It was reported to counter the tissue injury of alkylating agents with decreased oxidative stress [11]. The ability of curcumin to suppress oxidative stress may contribute to its anti-diabetic activity.

Both GPX and SOD are major antioxidant enzymes in cells. SOD enzymes are indispensable and ubiquitous antioxidant defences maintaining the steady-state levels of $O_2^{\cdot-}$ [14], while GPXs are important enzymes in the glutathione-ascorbate cycle for catalysing the reduction of H_2O_2 or organic hydroperoxides to water [15]. However, MDA is one of the final products of lipid peroxidation. An increase in free radicals causes overproduction of MDA. The malondialdehyde level is commonly used as a marker of oxidative stress and the antioxidant status in cancerous patients [16].

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In the present study, a rat model of T2DM, which was established by STZ induction, was used to investigate the protective effects of curcumin on the pathogenesis of DM. Taken together, curcumin showed potential antidiabetic effects (which may be partially due to its antioxidant functions) in STZ induced T2DM rats. The study showed that curcumin decreased oxidative injury and retarded the development of DM.

Material and methods

Ethics statement

Four-week-old male Wistar rats, weighing 60–80 g, were obtained from the Laboratory Animal Centre of Guilin Medical University. Experimental design and handling procedures were performed in accordance with the National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee of Guilin Medical University. All efforts were made to reduce the animals' suffering and the number of animals used for this experiment.

Establishment of rat T2DM model

Rats were randomly divided into 3 groups (8 each group): a nondiabetic group (Control), a diabetic group (DM), and a curcumin treatment group (DM + Cur 200 mg/kg group). Curcumin and streptozotocin (STZ) were all obtained from Sigma-Aldrich China (Shanghai, China). All rats were fed with normal chow for 8 weeks and then received single intraperitoneal injection of vehicle (0.1 mol/L citrate buffer) or 100 mg/kg STZ (dissolved in 0.1 mol/L citrate buffer, pH 4.5). Curcumin (200 mg/kg/day) was given intragastrically the day after STZ injection for one week. The DM and control groups were intragastrically given vehicle (CMC-Na) only. At the end of the experimental period, after 12 h fasting, the rats were anesthetized with 5 mg/kg urethane and sacrificed. Blood samples were collected via cardiac puncture and then centrifuged at 4000 rpm, 4°C for 15 min to obtain the serum. Pancreas were immediately removed and divided into 2 parts for histopathological detection and stored at −80°C for subsequent assays. Effective doses for curcumin were selected similarly to an earlier work reported in a previous study [17].

Histopathological examination

Pancreases were fixed in 4% paraformaldehyde overnight at 4°C, embedded in paraffin, and sectioned into 4-μm thick slides. The slides were dewaxed, hydrated, and then stained with haematoxylin and eosin (HE). The morphology of the pancreas was evaluated under a light microscope.

Immunohistochemistry (IHC) assays for insulin expression in Langerhans islet

Slides were dewaxed and hydrated and then underwent antigen retrieval by high-temperature induced epitope retrieval (HIER) in citrate buffer (0.1 M, pH 6.0) in a high-pressure cooker for 3 minutes. Then the endogenous peroxidases were blocked by 3% H_2O_2 for 10 minutes. The mouse monoclonal primary antibody of insulin (SC-8033, Santa Cruz Biotechnology) with a dilution of 1:100 were incubated with the slides for one hour. The secondary antibody, MaxVision™ HRP-Polymer anti-Mouse antibody (KIT-5001, Maxim Biotechnology), was incubated for 20 minutes. Then DAB was incubated for 5 minutes. Between each of the above steps, the slides were washed with PBS (0.1M, PH 7.2) 3 times for 3 minutes. After DAB coloration, the slides were counterstained with haematoxylin, dehydrated, and mounted with resinene.

Detection of blood glucose and serum insulin levels

Blood glucose levels were detected by a blood sugar instrument (ACCU-CHEK® Aviva, Roche). Serum insulin levels were measured using a mouse/rat insulin enzyme-linked immunosorbent assay (ELISA) kit (Cat. Number EZRMI-13K, Merck Millipore, Germany) following the protocols provided by the company.

Detection of oxidative associated factors

Pancreatic tissue was harvested and homogenized in 50 mmol/L phosphate buffer and then centrifuged at 3000 rpm at 4°C for 15 min. The levels of superoxide dismutase (SOD), malondialdehyde (MDA, a product of lipid peroxidation), and glutathione peroxidase (GPX) in the resultant supernatant were measured according to the protocol provided by corresponding detection kits (Nanjing Jiancheng Bioengineering Institute, Nanjing China).

Terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) assays

TUNEL assays were performed following the manufacturer's instructions (Roche Applied Science, USA). Briefly, tissue slides were dewaxed, rehydrated, and then incubated with 1% Triton X-100 at room temperature (RT) for 15 min. The endogenous peroxidase was blocked with 3% H_2O_2-methanol solution at RT for 10 min. Subsequently, the sections were digested by 20 mg·mL^{-1} proteinase K at 37°C for 15 min and then incubated with TdT-enzyme at 37°C for 1 h. After washing with phosphate-buffered saline (PBS) 3 times, the sections were incubated with 100 μL of digoxigenin (conjugated to horseradish peroxidase, POD). 3,3'-diaminobenzidine (DAB) was used as a chromogen. The nuclei of apoptotic cells were stained brown and randomly counted under a microscope at 400× magnification (Leica DM2500, Germany).

Statistical analysis

Data are presented as the mean ± SD. Statistical analysis was performed using one-way analysis of variance (ANOVA). Differences were considered statistically significant at a P value <0.05.

Results

Curcumin increased insulin secretion and decreased blood glucose in DM Rats

To investigate the protective effects of curcumin on the function of islet in DM rats, serum insulin was detected by ELISA, and insulin expressed by islet cells was detected by IHC. As shown in Figure 1A, serum insulin significantly decreased in the DM group, and curcumin partially restored the serum insulin levels. Meanwhile, blood glucose was decreased by curcumin treatment in DM rats (Fig. 1B). Insulin expression data in situ on tissue slides were consistent with the data in sera (Fig. 1C). The data imply that curcumin partially restored the secretion function of beta cells that had been injured by STZ treatment.

Curcumin inhibited the oxidative status of the pancreas induced by STZ treatment

To investigate the oxidative status of the pancreas, the antioxidant defence system parameters GPX and SOD, and the oxidative stress parameters MDA in pancreatic tissue homogenates were detected. As shown in Figure 2, curcumin partially restored the GPX

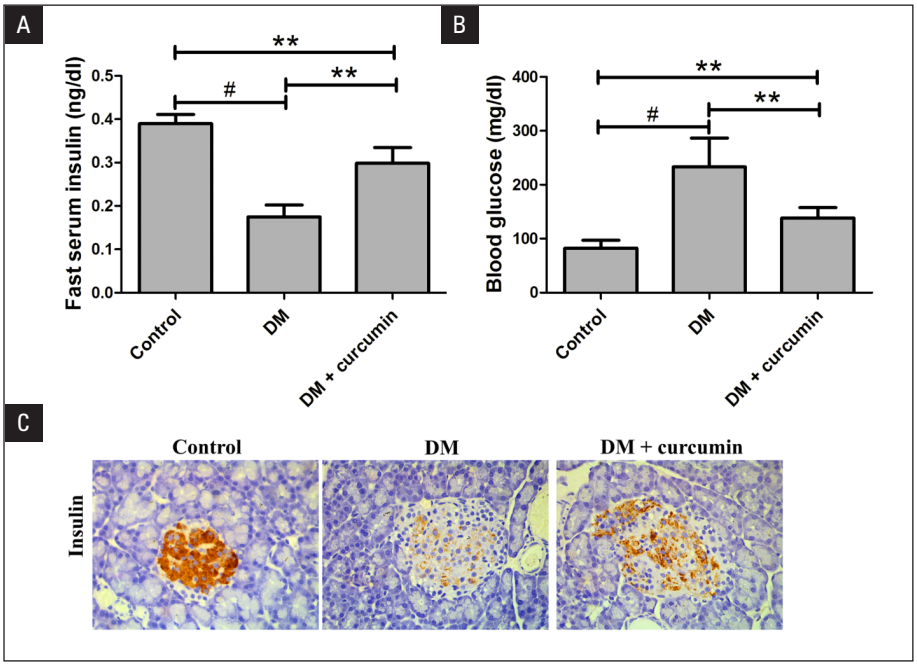

Figure 1. *Curcumin restored insulin expression and decreased blood glucose levels in streptozotocin (STZ)-induced diabetes mellitus (DM) rats.* **A.** *Curcumin restored serum insulin levels;* **B.** *Curcumin decreased blood glucose levels.* **C.** *Immunohistochemistry showed that curcumin partially restored the expression of insulin in pancreatic Langerhans islets.* $^{**}p < 0.01$; $^{\#}p < 0.001$

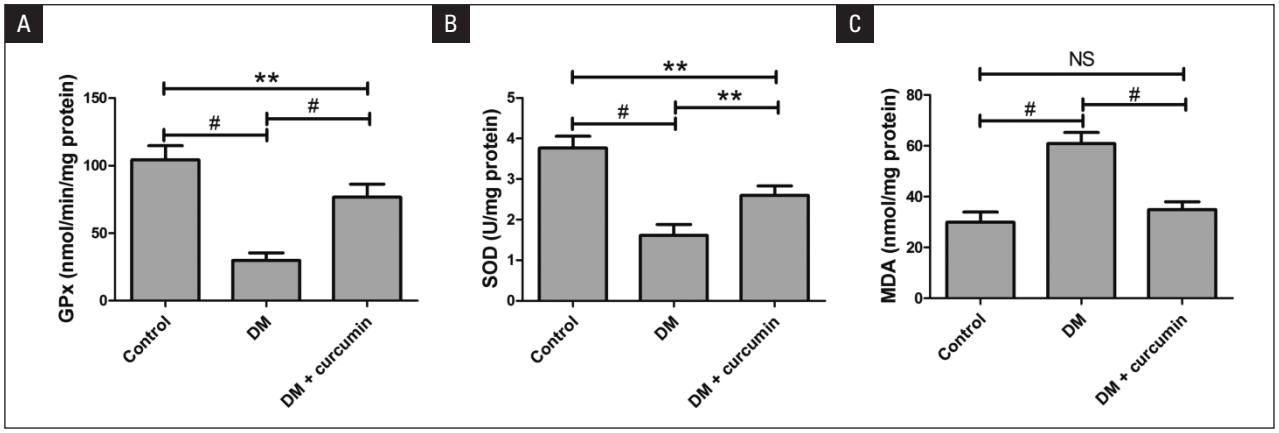

Figure 2. *Curcumin decreased oxidative stress in the pancreases of streptozotocin (STZ)-induced diabetes mellitus (DM) rats.* **A.** *Curcumin restored the glutathione peroxidase (GPx) levels in pancreatic tissue homogenates;* **B.** *Curcumin restored the superoxide dismutase (SOD) levels in pancreatic tissue homogenates;* **C.** *Curcumin decreased the malondialdehyde (MDA) levels in pancreatic tissue homogenates.* $^{**}p < 0.01$; $^{\#}p < 0.001$

and SOD (2 major antioxidant enzymes) levels in STZ-treated pancreatic tissues. Lipid peroxidative products, MDA, were significantly decreased by curcumin. These data imply that curcumin could decrease oxidative injury in pancreatic tissues.

Curcumin decreased cell apoptosis in the Langerhans islet of DM rats

To detect the protective effects of curcumin on islet cells, apoptotic cells were detected by TUNEL assays on pancreatic tissue slides. As shown in Figure 3, apoptotic cells were drastically decreased by curcumin treatment in DM rats. The data suggest that curcumin could protected islet cells from STZ-induced apoptosis.

Discussion

Injury factor-induced pancreatic β-cell apoptosis is a major contributor to the pathogenesis of diabetes [18]. In the present study, we investigated the protective effects of curcumin on pancreatic β-cells that were damaged in STZ-induced T2DM rats. Our data showed

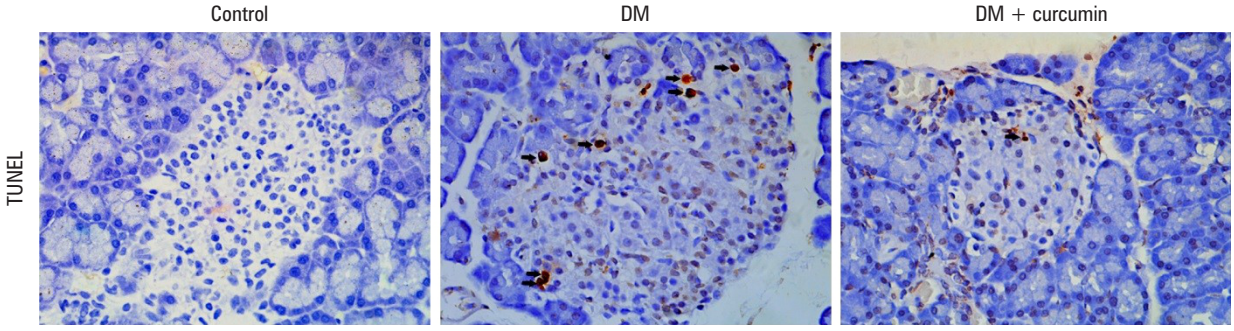

Figure 3. *Curcumin decreased apoptotic cells in Langerhans islets of streptozotocin (STZ)-induced diabetes mellitus (DM) rats. Curcumin decreased apoptosis of islet cells (arrow — apoptotic)*

that curcumin improved pancreatic beta cell function, and decreased oxidative stress and pancreatic β-cell death in STZ-induced DM rats. These data imply that curcumin had an antidiabetic property through decreasing oxidative stress-mediated injury of pancreatic beta cells and retarding the development of DM.

Rodent models are commonly used to study the pathogenesis and metabolic function in diabetes [19]. The STZ-induced DM rat model has long been used for the study of diabetes [20]. STZ, a glucosamine-nitrosourea compound, is used clinically as an alkylating antineoplastic agent that is particularly toxic to the beta cells of the pancreas in mammals [21]. STZ damages pancreatic cells as an alkylating agent and contributes to declining in quantity of cells [22]. After *i.p.* administration, STZ, as a pancreatic β-cell-specific cytotoxin, enters pancreatic beta cells via the glucose transporter type 2 (Glut-2) inducing DNA alkylation and fragmentation [23]. Despite its low bioavailability, curcumin proved to be safe and has good tolerability and effectiveness in various human diseases, including diabetes [24]. STZ preferentially accumulates in pancreatic beta cells via the low-affinity Glut-2 glucose transporter in the plasma membrane [25]. It is generally accepted that the toxicity of streptozotocin depends upon the DNA alkylating activity of its methyl-nitrosourea moiety [26]. Therefore, STZ also methylates proteins and DNA methylation is ultimately responsible for beta cell death, while protein methylation contributes to the functional defects of the beta cells [27]. Meanwhile, generation of ROS, including superoxide and hydroxyl radicals originating from hydrogen peroxide dismutation during hypoxanthine metabolism, may accelerate the process of beta cell destruction induced by STZ treatment [28]. In this study, curcumin partially restored the insulin levels and volume of beta cells in STZ-induced DM rats. Curcumin is reported to counter the cytotoxic effects of alkylating agents, which increase tissue oxidative stress and histological damage [29]. However, Adriana Bulboacă's [11] study groups suggested that curcumin treatment can reduce lipid peroxidation, demonstrated by the decrease of MDA in both curcumin pre-treatment groups [11]. The change of MDA in STZ-induced diabetes mellitus with curcumin pre-treatment was consistent with our data. This study showed that curcumin improved the survival and function of beta cells, which increased insulin secretion and decreased apoptosis of islet cells. Meanwhile, decreased MDA (a product of lipid peroxidation) and restored GPX and SOD (2 major antioxidant enzymes) were observed in pancreatic tissues of curcumin-treated DM rats. These data imply an improved oxidant status in STZ-treated rats.

Taken together, curcumin showed potential antidiabetic effects (which may be partially through its antioxidant functions) in STZ-induced DM rats. Our findings suggested that curcumin is a potent candidate for the prevention and therapy of T2DM.

Competing interests

The authors declare that they have no competing interests.

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Disclosure

The authors report no conflicts of interest in this work.

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Data availability

All data generated or analysed during this study are included in this published article.

Author contributions

X.L.Z. and S.J.X. were responsible for the study concept and design. J.L.D. and M.Q.Y. performed the analysis, acquired the data, and wrote the paper. Y.L. interpretated and analysed the data. All authors read, revised, and approved the final paper.

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