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Curcumin ameliorates testicular damage in diabetic rats by suppressing cellular stress-mediated mitochondria and endoplasmic reticulum-dependent apoptotic death

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ABSTRACT

In the present study, we sought to explore whether curcumin plays any beneficial role against STZ induced testicular abnormalities in diabetic rats, and if so, what possible mechanism it utilizes to provide protection. Exposure to STZ (50 mg/kg body weight, i.p., once) reduced testis-to-body weight ratio, enhanced blood glucose level and intracellular ROS, altered testicular markers, diminished serum testosterone and impaired cellular redox balance. Administration of curcumin at a dose of 100 mg/kg body weight for 8 weeks effectively normalized all the alterations. Curcumin also showed inhibitory effect on the elevation of pro-inflammatory cytokines and translocation of NFκB into the nucleus and promoted the activation of the transcription factor Nrf-2 to provide protection against oxidants. To protect cells from STZ-induced stress-mediated damage, curcumin acted on the key mediators of the apoptotic cell death such as JNK and p38. In addition, this active molecule upregulated Bcl-2 expression, blocked the expression of pro-apoptotic proteins (Bax, Bad and Bid), decreased intracellular Ca^{2+} level, inhibited active caspase cascade and attenuated PARP cleavage. These results suggest that curcumin provides protection against cellular stress-mediated mitochondrial and endoplasmic reticulum-dependent apoptotic death of the testicular cells under diabetic condition and suggests the possibility of using this molecule as a potential therapeutic in the treatment of stress-mediated diabetic testicular dysfunction.

1. Introduction

Diabetes mellitus (DM), a chronic endocrine metabolic disorder, has become a major concern due to its associated complications and rising global incidence [1–3]. It results from either lack of insulin secretion (type-1 diabetes) and/or reduced sensitivity of tissues to insulin (type-2 diabetes) [4]. These events lead to hyperglycemia, and persistent hyperglycemia induces excessive production of reactive oxygen species (ROS) in diabetic subject [5]. Evidence suggests that DM-mediated oxidative stress and altered antioxidant defense of male reproductive system results in infertility/subfertility in the diabetic patient [6–10]. Mammalian sperm cells contain a high amount of polyunsaturated fatty acid that undergoes oxidation to affect sperm motility and fertility [11]. Multiple events, such as irregular hormonal regulation in the process of spermatogenesis, abnormal spermatogenesis, anomalous behavior of sperm, penile erection, ejaculation, germ cell death and varying degree of the testicular lesions are involved in this pathogenesis [12–14]. Along with oxidative stress, recent investigations also depicted the adverse effect of ER stress on male sex organ and fertility in diabetic condition [15]. ER is a eukaryotic cellular organelle present within the cytoplasm and formed by the interconnected network of a series of folded membranes (continue its expansion with the outer membrane of the nucleus) and flattened sacs. SR (sarcoplasmic reticulum) is a type of ER which acts mainly as a store of Ca^{2+} and helps pumping it out on stimulus response. ER plays an important role in protein folding, export and processing. However, sustained hyperglycemia, oxidative stress, excessive Ca^{2+} release and protein load impairs ER homeostasis and results in ER stress, and severe ER stress ultimately causes testicular cell death by activating apoptotic pathways [16]. Since cellular apoptosis is responsible for hyperglycemia-induced oxidative stress-mediated testicular damage in the diabetic pathophysiology, an in vivo model of diabetes has been made to conduct a study employing antihyperglycemic, antioxidative and antiapoptotic remedies to manage this detrimental complication. In the present study, streptozotocin (STZ), a nitrosourea derivative, has been used as a diabetogenic agent that induces

Abbreviations: CAT, catalase; CUR, curcumin; ER, endoplasmic reticulum; GPx, glutathione peroxidase; G6PD, glutathione-6-phosphate dehydrogenase; GR, glutathione reductase; GSH, glutathione; GSSG, glutathione disulfide; GST, glutathione S-transferase; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; MDA, malondialdehyde; MMP, mitochondrial membrane potential; NFκB, nuclear factor kappa B; Nrf-2, nuclear factor erythroid 2-related factor; PARP, poly(ADP-ribose) polymerase; PI3K, phosphatidylinositide 3-kinase; ROS, reactive oxygen species; SDH, sorbitol dehydrogenase; SOD, superoxide dismutase; STZ, streptozotocin

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hyperglycemia by the production of excessive free radicals and thus provides a useful model to investigate the efficiency of antioxidant substances [17,18].

For this purpose curcumin, an active yellow colored phenolic pigment of turmeric, isolated from the rhizome of *Curcuma longa*, draws our attention, as it possesses various beneficial biological activities such as antidiabetic, anti-inflammatory, anti-tumor, antioxidant, etc. [19–22]. Besides, this molecule is present in the commonly consumed foodstuff and considered safe compared to the available expensive commercial drugs. Although a number of reports suggest the protective action of curcumin against oxidative stress-mediated cardiomyopathy, neuropathy, nephropathy and hepatic injury; testicular dysfunction [23–29]; etc., no mechanistic approach has yet been taken to describe its detailed beneficial role in oxidative and ER stress-induced testicular damage in diabetes. The present study has been designed to investigate the molecular mechanism of curcumin against hyperglycemia-induced cellular stress-mediated testicular dysfunction. For this purpose, we have determined the effect of curcumin on the sperm count, motility and structural abnormalities in diabetic animals. In addition, the role of this molecule on serum testosterone level and SDH activity; the protein expression of Nrf-2 (stress-induced defense protein), NFκB and upstream signaling components (PI3K, Akt, etc.); ER and oxidative stress-induced apoptotic signaling molecules and stress-induced proteins (JNK and p38) have also been explored. This study might shed some light on the use of curcumin as an effective therapeutic in the treatment of stress-mediated diabetic testicular dysfunction.

2. Materials and methods

2.1. Chemicals

Curcumin, bovine serum albumin (BSA) and Bradford reagent were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Antibodies were purchased from Abcam (Cambridge, UK), Sigma (Missouri, USA) and Cell Signaling (Cell Signaling Technology Inc.,Danvers, MA). Kits for the measurement of blood glucose were purchased from Span Diagnostic Ltd., Surat, Gujarat, India. Streptozotocin (STZ) and all other chemicals were obtained from Sisco Research Laboratory, Andheri, Mumbai, India.

2.2. Animals and care

Eight-weeks-old adult male Wistar rats, weighing approximate 280-300 g, were purchased from M/S Ghosh Enterprises, Kolkota, India. Animals were acclimatized under laboratory conditions for 2 weeks prior to experiments and were maintained under standard conditions of temperature (23 ± 2 °C) and humidity (50 ± 10%) with an alternating 12 h light/dark cycles. They were fed standard pellet diet (Agro Corporation Private Ltd., Bangalore, India) and water ad libitum. All the experiments with animals were carried out according to the guidelines of the institutional animal ethical committee (IAEC), Bose Institute, Kolkata (the permit number is IAEC/BI/3(I) cert. /2010), and full details of the study were approved by both IAEC and CPCSEA (committee for the purpose of control and supervision on experiments on animals), Ministry of Environment and Forests, New Delhi, India (the permit number is 95/99/CPCSEA).

2.3. Induction of diabetes in the experimental animals

After overnight fasting, diabetes was induced in the experimental rats with a single intraperitoneal injection of streptozotocin (STZ) dissolved in 0.1 M sodium citrate buffer, pH 4.5, at a dose of 50 mg/kg body weight [16]. After 3 days of STZ injection, the fasting blood glucose level was determined using an Advanced Accu-check glucometer (Boehringer Mannheim, Indianapolis, IN, USA). The rats having blood glucose above 300 mg/dL were considered to be diabetic and were used for the experiments as necessary.

2.4. Solvent and route of administration

STZ was administered intraperitoneally in sodium citrate buffer. On the other hand, curcumin was given orally by oral gavages in olive oil.

2.5. Determination of dose- and time-dependent effect of curcumin

Dose- and time-dependent study has been done to determine the effective antidiabetic dose of curcumin. For this purpose, rats were randomly divided into 6 groups. Each group had six rats. First two groups were served as normal control (received only vehicle) and diabetic control (received STZ at a dose of 50 mg/kg body weight). Based on the earlier report [16], the remaining four STZ-exposed diabetic groups were further treated with four different doses of curcumin (40, 70, 100 and 130 mg/kg body weight) daily for 8 weeks or more (data are not shown). The effective dose of curcumin was selected by studying its effect on fasting blood glucose level, serum SDH and testosterone level. Eight weeks treatment with a dose, 100 mg/kg body weight, of curcumin has been taken in consideration as it effectively ameliorate all the above-mentioned alterations. Beyond the effective dose and treatment period, no significant effect was observed compared to the used regimen. Histological study was also done to confirm the effectual protective dose of this active molecule.

2.6. In vivo experimental design

After the determination of effective dose of curcumin, experimental design for the present in vivo study has been summarized as follows (Fig. 1):

Animals were randomly divided into four groups consisting of six animals in each group.

Group 1 (normal control) consists of six animals received only vehicle (olive oil).
Group 2 (curcumin group) consists of six animals received only curcumin at a dose of 100 mg/kg body weight orally, daily for 8 weeks to check whether it has any toxic effect or not.
Group 3 (diabetic control) consists of six animals received STZ at a dose of 50 mg/kg body weight once, intraperitoneally.
Group 4 (STZ + CUR) consists of six animals. After diabetic induction they were treated with curcumin at a dose of 100 mg/kg body weight daily for 8 weeks.

After 8 weeks of curcumin treatment animals were sacrificed.

2.7. Collection of blood, serum and testis

Rats in each group were bled after every 7 days from the lateral vein of the tail and 100 μL of blood was taken for the measurement of blood glucose level. Serum was prepared for the dose- and time-dependent assay of SDH and testosterone. After scarification, blood samples were also drawn from caudal vena cava and incubated at 37 °C for 30 min. To get serum, blood samples were centrifuged at 3000×*g* for 30 min; clear serum was obtained and stored at −80 °C until use. Testes were aseptically taken out from the rats and weighed. These tissues were either stored at −80 °C till further analysis or fixed in 10% buffered formalin for histological assessments.

2.8. Preparation of testicular tissue homogenate

Collected testes were minced, washed and homogenized in a Dounce glass homogenizer in 10 mM HEPES–KOH/1 mM EGTA buffer