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Curcumin protects rat liver from streptozotocin-induced diabetic pathophysiology by counteracting reactive oxygen species and inhibiting the activation of p53 and MAPKs mediated stress response pathways

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ABSTRACT

Curcumin (CUR) is a highly pleiotropic molecule and possesses anti-inflammatory, hypoglycemic, antioxidative, wound-healing and antimicrobial activities. The present study was carried out to investigate whether CUR plays any beneficial role in streptozotocin (STZ) induced hepatic pathophysiology in diabetic rats. STZ exposure increased hepatic damage associated serum markers (ALT, ALP and LDH) as well as NO production in the liver tissue. Moreover, the same exposure enhanced ROS generation and lipid peroxidation; reduced GSH levels and antioxidant enzyme activities. Hyperglycemia induced hepatic pathophysiology also activated stress response pathways (involving phosphorylation of p38, ERK1/2 MAPKs and p53) and reduced mitochondrial membrane potential which in turn led to cellular apoptosis as evidenced from increased hepatic DNA fragmentation as well as FACS analysis. However, treatment with CUR effectively counteracts diabetes-induced, oxidative stress mediated hepatic damage and could act as a therapeutic in lessening liver dysfunction in diabetic subjects.

1. Introduction

Diabetes is the most common endocrine disorder now-a-days. It is basically a group of metabolic diseases characterized by hyperglycemia and resulting from the defects in insulin secretion, insulin action or both [1]. Hyperglycemia contributes to the progression and maintenance of overall oxidative environment. Increasing evidence from both the experimental and clinical studies indicates that oxidative stress plays a major role in the diabetic pathophysiology [2]. Reactive oxygen species (ROS) are generated disproportionately in diabetes by various pathways [3]. Among these various pathways, high glucose (and also other sugars) activates the polyol pathway, increased formation of AGEs (advanced glycation end products) along with the expression of the receptor for AGEs, activation of protein kinase C (PKC) isoforms

Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; CAT, catalase; ERK1/2, extracellular signal regulated kinases 1/2; FRAP, ferric reducing antioxidant power; GSH, glutathione; GSSG, glutathione disulphide; GST, glutathione S-transferase; GPx, glutathione peroxidase; GR, glutathione reductase; LDH, lactate dehydrogenase; MDA, malondialdehyde; NAPQI, N-acetyl-p-benzoquinone imine; MAPK, mitogen-activated protein kinases; PSA, prostate-specific antigen; ROS, reactive oxygen species; SOD, superoxide dismutase; STZ, streptozotocin; TPTZ, 2,4,6-tripyridyl-s-triazine.

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and increased activity of the hexosamine pathway were reported to be important [4]. ROS usually damage different organs of the body by peroxidation of membrane lipids, oxidation of proteins, DNA and other intracellular macromolecules. Changes in oxidative stress biomarkers, including glutathione, superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, some vitamins and associated genes may serve as a quantitative measurement of oxidative damage in diabetes [5]. Our aim was to find out easily available and inexpensive antioxidant molecules which can effectively reduce hepatic oxidative overload under diabetic conditions. For this purpose we have chosen curcumin, a commonly used foodstuff and an important component of Indian herbal medicine. Curcumin is a diaryl heptanoid and is the principal curcuminoid of the popular South Asian spice, turmeric. It is a well-known antioxidant [6] and highly pleiotropic molecule that has been reported to exert a wide range of pharmacological activities like antibacterial [7] anti-inflammatory, anti-cancer, anti-oxidant [8], hypoglycaemic [9,10], anti-atherosclerotic, anti-microbial [10], wound healing [11], etc. Moreover, curcumin has been found to interact directly with various intracellular signalling molecules [12]. Its ameliorative effects have been indicated to be mediated through the modulation of multiple cell signalling molecules like apoptotic proteins, cyclooxygenase (COX)-2, NF-κB [13], STAT3, IKKβ, interleukin [IL]-1β, IL-6 [14], endothelin-1, C-reactive protein (CRP) [15], GST [16,17], PSA, pro-inflammatory cytokines (tumour necrosis factor [TNF]-α [18], VCAM, prostaglandin E2, malondialdehyde (MDA), glutathione (GSH) [19], pepsinogen, phosphorylase kinase (PhK) [20], creatinine, transferrin receptor, total cholesterol, transforming growth factor (TGF)-β, triglyceride, HO-1 [21], etc. Most importantly, curcumin has been reported to have the capacity to directly scavenge ROS [22].

We have done a thorough search in the literature related to the beneficial effects of curcumin on diabetic rat liver. All the research articles published so far focuses mainly on curcumin's beneficial roles on the biochemical parameters [23,24]. Some recent reports also described other beneficial roles of curcumin against generalized diseased conditions; like its antioxidant effect [6], anti-diabetic effect [25], etc. Some of the structural analogues of curcumin were also investigated for the similar effects ([26]; [42]). However, detail mechanism was not carried out in any of those studies related to our experimental model. In our study, we have performed a detailed mechanistic investigation to assess not only the biochemical changes but also the molecular signalling pathways through which curcumin exert its beneficial effects. In the present study, we have investigated the protective action of curcumin, like in the enhancement of the activity of antioxidant enzymes (usually thought to be the first line of cellular defence against oxidative damage); elevation of the body weight; increase in the cellular antioxidant power (FRAP); elevation of cellular non-enzymatic antioxidant (GSH) content; increase in the mitochondrial membrane potential ($\Delta\psi_m$); amelioration of the tissue damage (histological assessment) and most importantly, inhibition of p53 and p38-ERK1/2 MAPKs mediated mitochondrial Bax translocation and subsequent intrinsic mitochondrial apoptotic pathway in diabetes-induced hepatic oxidative stress. The outcome of this study could help to determine the role of this bioactive molecule in reducing diabetes induced oxidative stress and might shed some light on the darkness of the gradual deterioration of this serious endocrine disorder worldwide.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

Curcumin, STZ, BSA, Bradford reagent, anti-Bcl-2, anti-Bcl-XL, anti-Bad and anti-Bax antibodies were purchased from Abcam (UK). Other antibodies like anti-ERK, anti-p53, etc. were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA). Kits for measurement of blood glucose and LDH were purchased from Span Diagnostic Ltd., India. All other chemicals were bought from Sisco Research Laboratory, India.

2.1.2. Animals

Adequate numbers of adult male Wistar rats weighing approximately 220–280 g were purchased from M/S Gosh Enterprises, Kolkata, India. All the animals were acclimatized under laboratory conditions for 2 weeks before any experiment. Animals were maintained under standard conditions of temperature ($23 \pm 2\,^{\circ}C$) and humidity ($50 \pm 10\%$) with alternating 12 h light/dark cycle. The animals were given free access to tap water and fed standard pellet diet (Agro Corporation Private Ltd., Bangalore, India). All the experiments involving 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 was approved by both IAEC and Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environment & Forests, New Delhi, India (the permit number is 95/99/CPCSEA).

2.2. Methods

2.2.1. Experimental design for in vivo treatments

Experimental design needed for the present in vivo study has been summarized as follows: Rats were randomly assigned to four groups and treated as follows:

Group 1: Normal group: rats received neither STZ nor curcumin, received vehicle only.
Group 2: CUR group: rats received only CUR (100 mg/kg body weight in olive oil) orally for 56 days (simultaneously with Group 4).
Group 3: STZ group: rats received single dose of STZ (STZ, 60 mg/kg body weight in citrate buffer, pH 4.5, i.p.) [27]. STZ-exposed rats with blood glucose level in excess of 300 mg/dL, 3 weeks after the exposure were considered as diabetic.
Group 4: STZ and CUR: post-treatment group: rats received CUR (orally, 100 mg/kg body weight in olive oil) after 3 weeks from the day on which STZ was injected until the 56th day.