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Taurine ameliorate alloxan induced oxidative stress and intrinsic apoptotic pathway in the hepatic tissue of diabetic rats

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ABSTRACT

Oxidative stress is associated with various diabetic complications and taurine plays an important role in ameliorating those difficulties. In the present study we, therefore, investigated whether taurine plays any beneficial role against diabetes induced liver dysfunction and if it does, what cellular mechanism it follows during protective action. Induction of diabetes by alloxan (ALX) (at a dose of 120 mg/kg body weight, i.p., once) reduced body weight and plasma insulin level, enhanced blood glucose and serum markers related to hepatic injury, accelerated ROS production, disturbed the intra-cellular antioxidant machineries and disintegrated hepatic cells near central vein. This pathophysiology leads to apoptotic cell death as evidenced from DNA fragmentation and TUNEL aasay. Studies on the mechanism of apoptosis showed that ALX accelerated the markers of mitochondrial dependent apoptotic pathway (enhanced cytochrome C release in cytosol from mitochondria, altered the expression of Bax, Bcl-2, Apaf-1, caspase-9, caspase-3). Treatment with taurine (1% w/v for three weeks) post-hyperglycemia, however, could restore all the alteration caused by ALX. Moreover, taurine activates hepatic PI3Kinase, Akt, hexokinase and augments the translocation of GLUT 2 to hepatic membrane in diabetic rats. Combining all, as a potential therapeutic, taurine may normalize the complications of diabetic liver injury.

1. Introduction

Worldwide diabetes mellitus (DM), a chronic metabolic disorder is the most serious and common cause of morbidity and mortality in modern civilization (Chinenye et al., 2012). This disorder is the result of long time persistence of high glucose level in blood and metabolic defects of important biomolecules such as carbohydrates, proteins and lipids (Baynes, 1991). DM is of two types IDDM (insulin dependent diabetes mellitus or type1 diabetes) and NIDDM (non-insulin dependent diabetes mellitus or type2 diabetes). IDDM is associated with impairment in the secretion of insulin from the β-cells of pancreas and NIDDM is the consequence of insulin resistance (Chuhwak and Pam, 2007). Earlier research suggests that in diabetes, oxidative stress plays an important role due to increase in production of reactive oxygen species (ROS). Glucose-autooxidation and non-enzymatic protein glycosylation are the main sources of this free radical generation which leads to tissue damage in diabetic complications (Brownlee, 2001; Wolff and Dean 1987). Apart from hyperglycemia, hyperlipidemia, cardiovascular complications, nephropathy and retina damage which are the major disorder of diabetes (Baynes, 1991; Ceriello, 2000; Yim et al., 2007), hepatic injury has also been identified as major secondary complication in diabetic patients (Harrison, 2006). Mainly IDDM is responsible for chronic liver injury (Kim et al., 2009a,b) as liver plays a unique role in glucose homeostasis (König et al., 2012). Anatomical position of liver makes it able to control the systemic supply of absorbed nutrients. It is one of the two organs which participates in both glucose consumption and production, also maintains blood glucose by complex regulation of metabolic substrates, insulin and other hormones (Michael et al., 2000). Hepatic glucose and lipid metabolism also get impaired due to hyperglycemia and hypoinsulinaemia.

Evidences from a number of research groups suggest that chronic hyperglycemia and increased oxidative stress are the main reason of liver dysfunction in diabetes. Scientists are, therefore, looking for suitable agents which possess both antioxidant as well as hypoglycemic effects (Manna et al., 2010a,b) for the treatment of oxidative stress mediated liver dysfunction. At the same time, these agents should have minimal side effects. For this purpose, special focus has been given lately on the sulfur containing non-protein β amino acid, taurine (2-aminoethanesulfonic acid), as this molecule is synthesized in the liver from cysteine and methionine.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; ALX, alloxan monohydrate; ATP, adenosine tri phosphate; CAT, catalase; DAB, 3,3′-diaminobenzidine tetrahydrochloride; GLUT 2, glucose transporter type 2; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, glutathione disulfide; GST, glutathione-S-transferase; MDA, malonaldehyde; ROS, reactive oxygen species; SOD, superoxide dismutase; TAU, taurine; TUNEL, terminal transferase mediated dUTP nick end-labeling.

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Besides, it is also present in our dietary supplements. This compound is well known as it possesses antioxidant and antiapoptotic properties and also participates in membrane stabilization, regulation of intracellular Ca^{2+} concentration, reduction of the levels of pro-inflammatory cytokines in various organs and blood pressure control (Aerts and Van Assche, 2002; Das et al., 2008, 2009a,b, 2010a–c, 2011a,b). Earlier studies from our laboratory reports that taurine exhibits hypoglycemic effect and suppress mitochondrial dependent apoptosis in renal and cardiac tissues of alloxan induced diabetic rats (Das and Sil, 2012; Das et al., 2012). As a continuation, we investigated the role of taurine in diabetes induced hepatotoxicity in the present study as liver is the main organ for detoxification of different xenobiotics. On this account we investigated the status of the physiological parameters related to hyperglycaemic liver disease, intracellular antioxidant enzymes, GSH/GSSG ratio, and the involvement of mitochondria dependent apoptotic pathway under diabetic condition. Output of this study may serve as important information for the treatment of oxidative stress mediated diabetic liver injury.

2. Materials and methods

2.1. Chemicals

Taurine (2-aminoethane sulfonic acid), alloxan, bovine serum albumin (BSA), Bradford reagent were purchased from Sigma–Aldrich Chemical Company, (St. Louis, MO) USA. 1-Chloro-2,4-dinitrobenzene (CDNB), 5,5′-dithiobis(2-nitrobenzoic acid) [DTNB, (Ellman's reagent)], reduced nicotinamide adenine dinucleotide (NADH), nitro blue tetrazolium (NBT), oxidized glutathione (GSSG), phenazine methosulphate (PMT), reduced glutathione (GSH), thiobarbituric acid (TBA) and other reagents were bought from Sisco research laboratory, Andheri, Mumbai, India. All the antibodies were purchased from Abcam (Cambridge, Cambridgeshire, UK). Kits for the measurement of blood glucose, ALT and ALP were purchased from Span Diagnostic Ltd., Surat, Gujarat, India. TUNEL assay kit was purchased from Invitrogen, Eugene, Oregon, USA.

2.2. Animals

For this experiment, adult Wister male rats, weighing approximately 160–180 g were purchased from M/S Ghosh Enterprises, Kolkata, India. The animals were acclimatized for two weeks prior to the experiments and were maintained under standard conditions of temperature (23 ± 2^0C) and humidity ($50 \pm 10\%$) 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. 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.

2.3. Experimental induction of diabetes

Diabetes was induced in the experimental rats after overnight fasting with a single dose of ALX (Kim et al., 2008) i.e. 120 mg/kg body weight in citrate buffer, pH 4.5, by intraperitoneal injection (Verma et al., 2010). After 3 days of ALX injection the fasting blood glucose level was determined in the experimental animals using an Advanced Accu-check glucometer (Boehringer Mannheim, Indianapolis, IN, USA). The rats with blood glucose above 300 mg/dL (Winiarska et al., 2009) were considered to be diabetic and then they were used for the experiments as necessary.

2.4. Experimental design for in vivo treatments

Experimental design for in vivo experiments in present study has been summarized in Fig. 1: The animals were randomly assigned to four groups each consisting of six rats.

Group 1 – Normal group: Rats received neither ALX nor taurine only water as vehicle.
Group 2 – TAU group: Rats received only 1% taurine (w/v in water, orally).
Group 3 – ALX group: Rats received single dose of ALX, considered as diabetic control group.
Group 4 – ALX + TAU post treatment group: Rats received taurine (1% w/v in water, orally) from the 4th day after ALX injection for 21 days.

2.5. Collection of blood and liver

After alloxan induction rats in each group were bled every 3 days from the lateral vein of the tail and 100 μL blood was taken for the measurement of plasma glucose. The experimental rats were euthanized under light ether anesthesia after 3 weeks of treatment with taurine. Liver was removed, either stored at −80 °C till biochemical analysis or fixed in 10% buffered formalin for TUNEL and histological assessments. The body weight and liver weight were measured and compared between groups. Blood samples were drawn from the caudal vena cava. Blood was collected in test tubes containing heparin solution and centrifuged at 1500*g* for 10 min to obtain plasma. The plasma was immediately stored at −80 °C until use.

2.6. Preparation of mitochondrial, cytosolic and membrane fractions

The liver tissue was minced, washed with saline buffer and homogenized in a Dounce glass homogenizer in homogenizing buffer (50 mM phosphate buffer/1 mM EDTA, pH 7.5, containing 1.5 mM $MgCl_2$, 10 mM KCl, and supplemented with protease and phosphatase inhibitors). The homogenates were spun down for 10 min at 500*g* at 4 °C. The supernatant was collected and recentrifuged at 2000*g* for 10 min. Again the supernatant was collected and recentrifuged at 12,000*g* for 10 min at 4 °C, and pellet was resuspended in 200 mM mannitol, 50 mM sucrose, 10 mmol/L Hepes-KOH (pH 7.4) and stored as mitochondrial fraction at −80 °C as described by the method of Jang et al. (2004) with some modifications. The final supernatant was taken and centrifuged for 1 h at 40,000*g*. The resultant supernatant was used as cytosolic fraction and stored at 4 °C. The pellet was resuspended in the above-mentioned homogenizing buffer, containing 1% (v/v) Triton X-100, by sonication for 7×1 s cycles and again centrifuged for 1 h at 40,000*g*. The supernatant now contained the extracted membrane fraction and stored at 4 °C. In the present study mitochondrial fraction has been used for the determination of membrane potential, cytosolic fraction has been used for all other western blot analyses,

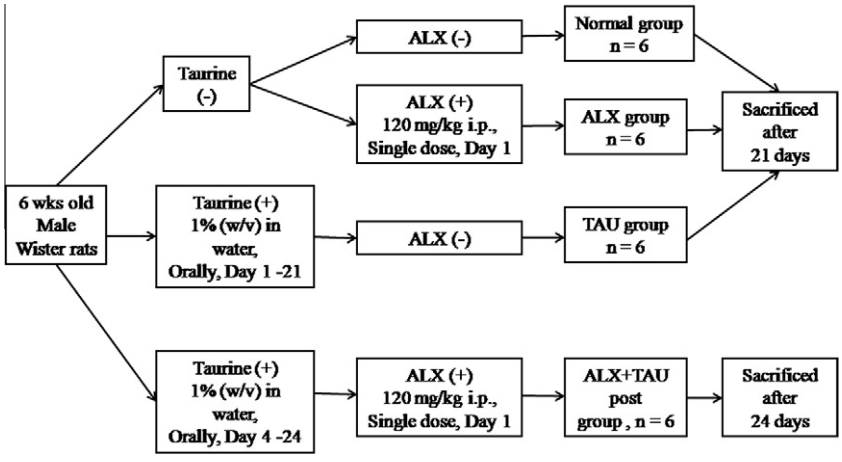

Fig. 1. Schematic diagram of in vivo experimental protocol.