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Taurine exerts hypoglycemic effect in alloxan-induced diabetic rats, improves insulin-mediated glucose transport signaling pathway in heart and ameliorates cardiac oxidative stress and apoptosis

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ABSTRACT

Hyperlipidemia, inflammation and altered antioxidant profiles are the usual complications in diabetes mellitus. In the present study, we investigated the therapeutic potential of taurine in diabetes associated cardiac complications using a rat model. Rats were made diabetic by alloxan (ALX) (single i.p. dose of 120 mg/kg body weight) and left untreated or treated with taurine (1% w/v, orally, in water) for three weeks either from the day of ALX exposure or after the onset of diabetes. Animals were euthanized after three weeks. ALX-induced diabetes decreased body weight, increased glucose level, decreased insulin content, enhanced the levels of cardiac damage markers and altered lipid profile in the plasma. Moreover, it increased oxidative stress (decreased antioxidant enzyme activities and GSH/GSSG ratio, increased xanthine oxidase enzyme activity, lipid peroxidation, protein carbonylation and ROS generation) and enhanced the proinflammatory cytokines levels, activity of myeloperoxidase and nuclear translocation of NFκB in the cardiac tissue of the experimental animals. Taurine treatment could, however, result to a decrease in the elevated blood glucose and proinflammatory cytokine levels, diabetes-evoked oxidative stress, lipid profiles and NFκB translocation. In addition, taurine increased GLUT 4 translocation to the cardiac membrane by enhanced phosphorylation of IR and IRS1 at tyrosine and Akt at serine residue in the heart. Results also suggest that taurine could protect cardiac tissue from ALX induced apoptosis via the regulation of Bcl2 family and caspase 9/3 proteins. Taken together, taurine supplementation in regular diet could play a beneficial role in regulating diabetes and its associated complications in the heart.

Introduction

Diabetes is the most common and serious metabolic disease. Diabetes is mainly found to be of two types. In type 1, which is also known as IDDM or insulin dependent diabetes mellitus, insulin is produced in lesser amount. In type 2, also known as NIDDM or noninsulin dependent diabetes mellitus, the pancreas is usually producing enough insulin, but for unknown reasons the body cannot use the insulin effectively, a condition called insulin resistance. The chronic disorders related to diabetes apart from hyperglycemia and hyperlipidemia are cardiovascular complications, nephropathy and retina damage (Baynes and Thorpe, 1999; Ceriello, 2000; Yim et al., 2007).

The major role of insulin in diabetes is to maintain whole body glucose homeostasis via glucose transporter 4 (GLUT4), expressed in adipose tissue, skeletal and cardiac muscles (Charron et al., 1999; Haruta et al., 1995). During insulin stimulation, intracellular vesicles that store GLUT4, translocate to the plasma membrane and facilitate glucose uptake (Bryant et al., 2002; Pessin et al., 1999). Under diabetic condition, reduced expression of GLUT4 causes impairment of insulin signaling and stimulates glucose production in the liver. These alterations lead to high glucose concentrations in blood (Nizamutdinova et al., 2009).

Hyperglycemia-induced generation of free radicals (oxidative stress) contributes to the development and progression of diabetes and other related complications. Therefore, an agent that possesses both hypoglycemic and antioxidant activities would be a therapeutic tool for diabetic patients (Manna et al., 2010a,b). Taurine, a sulfur containing beta amino acid, is present in most animal tissues and is

Abbreviations: ALX, alloxan; CAT, catalase; DAB, 3,3′-diaminobenzidine tetrahydrochloride; EtBr, ethidium bromide; FACS, fluorescence activated cell sorting; GLUT4, glucose transporter type 4; GSH, glutathione; GSSG, glutathione disulfide; GST, glutathione S-transferase; GR, glutathione reductase; HDL, high-density lipoprotein; IL-6, inter leukin6; IR, insulin receptor; IRS1, insulin receptor substrate 1; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; MDA, malondialdehyde; NF-κB, nuclear factor kappa B; ROS, reactive oxygen species; SOD, superoxide dismutase; STE buffer, sodium chloride-tris-EDTA buffer; TAU, taurine; TNF-α, tumor necrosis factor alpha; TUNEL, terminal transferase mediated dUTP nick end-labeling.

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essential for the normal functioning of different organs (Brosnan and Brosnan, 2006). Taurine exhibits antioxidative properties, membrane stabilizing effect, regulates intracellular Ca^{2+} concentration, inhibits apoptosis, reduces the levels of pro-inflammatory cytokines in various organs and controls blood pressure (Aerts and Van Assche, 2002; Das et al., 2008, 2009a,b, 2010a–c, 2011a,b; Kontny et al., 2000; Manna et al., 2008a,b, 2009; Racasan et al., 2004; Sinha et al., 2007, 2008a,b). Taurine is found at high concentrations inside glucagon and somatostatin-containing cells in the pancreatic islets and increases insulin secretion, sensitivity and glucose uptake (Cherif et al., 1998; De la Puerta et al., 2010; Kaplan et al., 2004) in different experimental conditions. Literature suggests a considerable variation about the opinion on the mode of action of taurine in diabetes. Kulakowski and Maturos (1984) reported that the hypoglycemic effect of taurine was not mediated via increased insulin release; on the other hand, Pandya et al. (2010) and Chang and Kwon (2000) reported that taurine increased insulin secretion in streptozotocin-induced diabetic animals. Besides, Brons et al. (2004) reported that taurine supplementation had no effect on insulin secretion and glucose level in prediabetic human. The authors, however, also expressed the opinion that they could not rule out the possibility of hypoglycemic effect of taurine if diabetic subjects had been taken (as reported by Elizarova and Nedosugova (1996)).

The beneficial effects of taurine in diabetes mellitus and its cardioprotective role in other pathophysiological conditions have been known (Das et al., 2011a; Gavrovskaya et al., 2008; Ghosh et al., 2009; Winiarska et al., 2009), although the exact mechanism of hypoglycemic action of taurine in cardiac associated complications is not properly defined. The aim of this present study has, therefore, been set to investigate the mechanisms of possible beneficial action of taurine against ALX (2,4,5,6-tetraoxypyrimidine; 2,4,5,6-pyrimidinetetrone)-induced diabetes and its associated complications in cardiac tissue of rats. In the present study, attempts have, therefore, been made to investigate the mechanisms of hypoglycemic, antioxidant, antiinflammatory and antiapoptotic action of taurine in ALX-induced diabetic heart. The outcome, we believe, might provide important information about its usefulness as a therapeutic agent for the treatment of diabetes and related cardiac dysfunctions.

Materials and methods

Chemicals

Taurine (2-aminoethane sulfonic acid), alloxan, bovine serum albumin (BSA), Bradford reagent, anti Bcl 2, anti Bcl XL, anti caspase 3, anti Akt, and anti NFkB antibodies were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Other antibodies, like, anti IR, anti IRS1, and anti GLUT4 were purchased from Abcam (Cambridge, Cambridgeshire, UK). Kits for measurement of blood glucose, LDH, uric acid and total cholesterol were purchased from Span Diagnostic Ltd., Surat, Gujarat, India. All other chemicals were bought from Sisco Research Laboratory, Andheri, Mumbai, India. TUNEL assay kit was purchased from Invitrogen, Eugene, Oregon, USA.

Animals

Adult male Wistar rats weighing approximately 160–180 g were purchased from M/S Gosh Enterprises, Kolkota, India. Animals were acclimatized under laboratory conditions for two weeks prior to experiments. All the experiments with animals were carried out according to the guidelines of the institutional animal ethical committee (IAEC), Bose Institute, Kolkata 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.

Experimental design for in vivo treatments

Experimental design needed for the present in vivo study has been summarized (in Fig. 1) as follows: Forty-five rats were randomly assigned to five groups.

Group 1—Normal group: Six rats received neither ALX nor taurine and received only water as vehicle. No mortality was found.
Group 2—TAU group: Six rats received only 1% taurine (w/v in water, orally). No mortality was found.
Group 3—ALX group: Thirteen rats received single dose of ALX (Verma et al., 2010) at a dose of 120 mg/kg body wt in citrate buffer, pH 4.5, i.p. After 3 days of ALX injection, rats having blood glucose level in excess of 300 mg/dL were considered as diabetic. Six rats died during the experimental period.
Group 4—ALX&TAU simultaneous treatment group: Ten rats received taurine (1% w/v in water, orally, 1 h before ALX injection) from the day on which ALX was injected for 21 days. Two rats died during the experimental period.
Group 5—ALX + TAU post treatment group: Ten rats received taurine (1% w/v in water, orally) from the 4th day after ALX injection for 21 days. Three rats died during the experimental period.

Collection of blood, pancreas and heart

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. Pancreases and hearts were removed. Hearts were either stored at −80 °C till biochemical analysis later or fixed in 10% buffered formalin for TUNEL and histological assessments. Pancreases were fixed in 10% buffered formalin for only histological assessments. The body weight and heart 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.

Preparation of nuclear, mitochondrial, cytosolic and membrane fractions

The hearts were 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. The pellet was resuspended in the same buffer and taken as nuclear fraction and stored at −80 °C as described by the method of Lizotte et al. (2009). The supernatant was 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 (Huisamen et al., 2001).

In the present study, the nuclear fraction has been used for the western blot analysis of NFκB; membrane fraction has been used for