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Taurine rescues vascular endothelial dysfunction in streptozocin-induced diabetic rats: Correlated with downregulation of LOX-1 and ICAM-1 expression on aortas

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ABSTRACT

Macroangiopathy is a major complication of diabetes mellitus in which dysfunction of vascular endothelium induced by excessive oxidative stress is an early and key determinant. As an endogenous antioxidant, taurine possesses endothelial protective effect *in vitro*. LOX-1 is an endothelial receptor for oxidized low-density lipoprotein (oxLDL) which might mediate endothelial dysfunction and subsequent atherogenesis in diabetes. We used streptozotocin-induced rats as models of type 1 diabetes to evaluate the protective effect of taurine against vascular endothelial dysfunction in type 1 diabetes and the possibly involved molecule mechanism. Eight male Wistar rats were used as normal control group. Sixteen diabetic rats induced by one single injection of streptozocin (60 mg/kg, i.p.) were randomly divided into two groups after the diabetes onset: diabetes mellitus group and taurine-treated diabetes group. 6 weeks afterward, endothelium-dependent vasodilation of isolated thoracic aorta, serum oxLDL and soluble intercellular adhesion molecule (sICAM-l) levels, LOX-1 and intercellular adhesion molecule (ICAM-1) expression on aortas were determined respectively. Streptozocin-induced diabetic rats were complicated with excessive oxidative stress and endothelial dysfunction: increased serum oxLDL and sICAM-1, inhibited endothelium-dependent vasodilator responses to acetylcholine (1 nM–0.1 μM). Simultaneously, LOX-1 and ICAM-1 expression were enhanced in aortas of diabetic rats; whereas blunted endothelium-dependent vasodilator responses to acetylcholine, increased serum oxLDL and sICAM-1 level as well as overexpression of LOX-1 and ICAM-1 were all attenuated significantly by taurine treatment. In conclusion, taurine improves vascular endothelial dysfunction induced by experimental type 1 diabetes and this effect might be associated with downregulation of LOX-1 and ICAM-1 expression on aortic vascular endothelium via its antioxidative property.

1. Introduction

Under proatherosclerostic conditions such as hypercholesterolemia, hypertension, diabetes or aging, endothelial dysfunction precedes the obviously structural vascular changes and development of atherosclerosis. Oxidized low-density lipoprotein (oxLDL) is recognized as a major cause of endothelial dysfunction in the diseases related to atherogenesis, which has been suggested to affect endothelium-dependent vasodilation through decreasing production of endothelium-derived nitric oxide (NO) and enhancing expression of adhesion molecules on the endothelium via lectin-like oxLDL receptor-1 (LOX-1) (Xu et al., 2007; Stewart and Nagarajan, 2006). Recent studies show that LOX-1 expression is upregulated in vessel tissues from animals and humans suffering from atheroscelosis and the related diseases (Chen et al., 2000; Hamakawa et al., 2004). Expression of LOX-1 in endothelial cells may provide a molecular link for incorporation of oxLDL into the cells, inducing the generation of superoxide anions that could activate oxidative stress-sensitive factors such as NF-KappaB, p38MAPK (mitogen-activated protein kinase) (Mehta et al., 2003; Li et al., 2002) and subsequently induce overexpression of endothelin-1 and adhesion molecules such as P-selectin, VCAM-1 (vascular cell adhesion molecule-1) and ICAM-1 (intercellular adhesion molecule-1) or impair endothelial nitric oxide synthase (eNOS) activation (Chavakis et al., 2001), which were all involved in the pathomechanism of endothelial dysfunction. LOX-1 was increasingly viewed as a mediator and biomarker of endothelial or vascular dysfunction (Vohra et al., 2006). As a marker of oxidative stress, oxLDL and other molecules related to oxidative stress could induce LOX-1 expression (Xu et al., 2007; Halvorsen et al., 2001), whereas antioxidant treatment could suppress its expression, which suggested a redox sensitive regulation of LOX-1 expression (Nagase et al., 2001).

Macroangiopathy is a major complication of both type 1 and type 2 diabetes, and its occurrence essentially is the process from endothelial dysfunction to atherosclerosis plaque, in which oxidative stress induced by chronic hyperglycemia plays a key role (Jay et al., 2006). As an endogenous semi-essential amino acid, taurine has been reported to act as a thermoregulator and an anticonvulsant without

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any found side effect hitherto (Huxtable, 2000). It also has been shown that when administered *in vivo*, taurine could improve endothelial function and inhibits apoptosis of endothelial cell as well as decreases plasma level of LDL, which prevents initiation and progression of atherosclerosis (Fennessy et al., 2003; Sethupathy et al., 2002). There is evidence that the level of taurine is reduced in plasma and tissues of type 1 diabetic animals (Colivicchi et al., 2004; Militante et al., 2000; Pop-Busui et al., 2001) and taurine supplement may be a promising agent in the treatment of diabetes related to its hypoglycemic, hypolipidemic or anti-inflammatory activity (Tenner et al., 2003; Alvarado-vasquez et al., 2003; Casey et al., 2007). Recent studies showed that *in vitro* taurine could protect against endothelial dysfunction induced by oxLDL and by high glucose (Tan et al., 2007; Ulrich-Merzenich et al., 2007). However, whether taurine administration *in vivo* could educe this endothelial protective effect in diabetes and the possibly involved molecular mechanism were still unknown.

Therefore, the present study aimed to investigate the protective effect of taurine on endothelial dysfunction induced by experimental diabetes as well as the possibly involved molecular it's mechanism indeed via studying endothelium-dependent vasodilator of isolated aortas, serum oxLDL and sICAM-1 levels as well as LOX-1 and ICAM-1 expression on aortas. As a generally accepted animal model more closely resembled to type 1 diabetes, streptozocin-induced diabetic rats were used in our current investigation.

2. Materials and methods

2.1. Animals and experimental models

8-week-old male Wistar rats (180–200 g) were obtained from Experimental Animal Center of Shandong University. They received humane care in compliance with the 'Guide for the Care and Use of Laboratory Animals' published by the National Institutes of Health (NIH publication 86-23, revised 1986). Diabetes were induced by a single intraperitoneal injection of streptozocin (60 mg/kg body weight, Sigma, USA) freshly prepared in 0.1 M citrate buffer (pH 4.2). The normal control rats (NC group, $n=8$) were injected with equal volume of vehicle. After 72 h following streptozocin injection, animals showing blood glucose higher than 16.7 mM were considered as diabetic rats. Then randomly divided diabetic rats into two groups: diabetes mellitus group (DM group, $n=8$) and taurine-treated diabetes group (DM+TAU group, $n=8$; drinking water containing 1% taurine, taurine from Alphar Aesar, USA). All rats were maintained on a 12 h light/dark cycle and kept for 6 weeks with free access to food and water. All the protocols were in accordance with the institutional guidelines for animal research.

2.2. Blood collection and tissue preparation

At the end of experiment, after fasting overnight all rats were anaesthetized with 3% butaylone (50 mg/kg body weight, i.p.) and opened the thoracic cavity. After blood samples were collected from arteries, thoracic aortic tissues from the aortic arch to the diaphragm were rapidly isolated, upper half were for determination of endothelium-dependent vasodilator responses, Western blot and reverse transcription-PCR analysis. Subtus half were snapfrozen with OCT compound (Tissue-TeK, Tokyo, Japan) to make frozen sections and fixed in neutral buffered formalin and paraffin-embedded to make paraffin slices.

2.3. Determination of blood lipid and blood glucose

Blood lipid including serum levels of total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglyceride were determined by 7060 Hitachi biochemical instrument. Blood glucose was determined by blood glucose meter OneTouch Ultra (LifeScan, Inc., USA).

2.4. Determination of serum oxLDL and sICAM-1 levels

Serum oxLDL and sICAM-1 levels were measured by enzyme-labeled immunosorbent assay (ELISA) Kit (BPB Biomedicals, Inc., USA) according to the manufacturers instructions. The values were measured at 450 nm by microplate reader (Biotek). The standard curves measured by the ELISA Kits were linear from 0 to 50 ng/dl for serum oxLDL and linear from 0 to 1000 ng/ml for sICAM-1 respectively.

2.5. Functional assessment of isolated thoracic aortas

Segments of thoracic aortas were rapidly cut into rings of 3–5 mm length. The rings were suspended horizontally between two stainless steel wires and mounted in a 5 ml organ chamber filled with warmed (37 °C) and oxygenated (95% O_2 and 5% CO_2) Krebs' solution. The Krebs' solution had the following composition (mM): NaCl, 119.0; $NaHCO_3$, 25.0; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_47H_2O$, 1.2; $CaCl_2$, 2.5; and Glucose, 11.0. One of the ring ends was connected to a force transducer. The aortic ring was stretched with 2 g resting force and equilibrated for 60 min, and then precontracted with KCl (60 mM). After a maximal response to KCl was obtained, the rings were washed repeatedly with Krebs' solution and equilibrated again for 30 min. In order to measure vasodilator responses, rings were contracted with noradrenaline (NA, Sigma) to 50% of their maximal contraction. After the action stabilized, an accumulative concentration response curve to acetylcholine (1 nM–0.1 μM) was observed.

2.6. Immunohistochemistry

2.6.1. Determination of LOX-1 expression on aortic tissues by immunofluorescent staining

Serial 6um frozen sections were placed on 10% polylysine precoated slides and fixed in cold acetone for 30 min. After washing three times with PBS, the sections were incubated with 5% BSA (sigma, USA) for 30 min and then with anti LOX-1 IgG (diluted 1:200; Santa Cruz, USA., sc-11653) for 2 h at 37 °C, washed three times with PBS, then incubated with anti-goat FITC-IgG (diluted 1:200; Santa Cruz, USA., sc-2348) at 37 °C for 1 h. Finally, the sections were mounted in glycerol-PBS solution (glycerol/PBS, 9:1) and examined with an Olympus microscope equipped with fluorescence optics and appropriate filters. Pictures were taken when typical staining were showed among several sections. PBS replace anti-LOX-1 IgG to set negative control every experiments.

2.6.2. Determination of ICAM-1 expression on aortic tissues by SP immunostaining kit

Goat SP immunostaining kit was from Zhongshan Biotechnology Co, Ltd., China and the procedure was performed according to the manufacture's instructions. Briefly, 5–6 μm thick paraffin slices were roasted, deparaffinized and antigen repairing, then incubated in 0.3% H_2O_2 to removal endogenous peroxidase activity and 5%BSA in PBS to blocked non-specific staining. Subsequently incubated the slices with anti-rat ICAM-1/CD54 Antibody (diluted 1:200; R&D Systems, Inc. AF583) overnight at 4 °C and anti-goat biotinylation IgG for 1 h at 37 °C, then incubated with peroxidase-conjugated streptavidin for 30 min at 37 °C. After DAB coloration, hematoxylin courterstaining, dehydration, mounting in neutral gummi, Antibody binding was visualized and photographed under light microscope. PBS replace anti-ICAM-1 IgG to set negative control every experiment.

2.7. Western blot assay

50 mg freshly frozen samples of partial aortic tissues were subsequently homogenized and lysed for 10 min on ice in a 1 ml solution containing 10 mM HEPES (PH 7.6), 10 mM KCl, 1.5 mM $MgCl_2$, 0.5% NP-40, 1 mM DTT, and 0.5 mM PMSF. 50ug of protein were