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The sesame lignan sesamin attenuates vascular dysfunction in streptozotocin diabetic rats: Involvement of nitric oxide and oxidative stress

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ABSTRACT

The effect of chronic administration of sesamin was studied on aortic reactivity of streptozotocin diabetic rats. Male diabetic rats received sesamin for 7 weeks after diabetes induction. Contractile responses to KCl and phenylephrine and relaxation response to acetylcholine were obtained from aortic rings. Maximum contractile response of endothelium-intact rings to phenylephrine was significantly lower in sesamin-treated diabetic rats relative to untreated diabetics and endothelium removal abolished this difference. Meanwhile, endothelium-dependent relaxation to acetylcholine was significantly higher in sesamin-treated diabetic rats as compared to diabetic ones and pretreatment of rings with nitric oxide synthase inhibitor N(G)-nitro-L-arginine methyl ester significantly attenuated the observed response. Two-month diabetes also resulted in an elevation of malondialdehyde and decreased superoxide dismutase activity and sesamin treatment significantly improved these changes. Therefore, chronic treatment of diabetic rats with sesamin could prevent some abnormal changes in vascular reactivity in diabetic rats through nitric oxide and via attenuation of oxidative stress and tissue integrity of endothelium is necessary for its beneficial effect.

1. Introduction

Cardiovascular disorders continue to constitute major causes of morbidity and mortality in diabetic patients in spite of significant achievements in their diagnosis and treatment (Coccheri, 2007). Changes in vascular responsiveness to vasoconstrictors and vasodilators are mainly responsible for development of some vascular complications of diabetics (Nasri et al., 2011). Most of these complications are due to increased serum glucose and augmented generation of reactive oxygen species which finally lead to endothelium dysfunction (Naito et al., 2011).

Sesamin, one of the major lignans in sesame seed and oil, and its isomers have beneficial physiological effects, acting as an antioxidant (Ikeda et al., 2003), anti-carcinogen (Hirose et al., 1992), anti-hypertensive (Kita et al., 1995; Nakano et al., 2003) and are capable of reducing serum lipids (Rogi et al., 2011). There are also indications that sesamin isomers could enhance plasma levels of α- and γ-tocopherol in rats (Yamashita et al., 1995). Recent work demonstrated that sesamin metabolites induce nitric oxide-dependent vasorelaxation in vitro (Nakano et al., 2006) and sesamin feeding enhances endothelium-dependent relaxation in deoxycorticosterone acetate-salt hypertensive rats (Nakano et al., 2003). It has also been reported that the aqueous leaf extract of sesame induces dose-dependent vasorelaxation in guinea-pig aortas (Konan et al., 2008). Nevertheless, the exact underlying mechanisms of in vivo protective effects of sesamin on vascular system are not completely understood. Therefore, this study was undertaken to assess the beneficial effect of chronic sesamin treatment on aortic reactivity of streptozotocin-diabetic rats and to investigate the involvement of nitric oxide, prostanoids, and oxidative stress.

2. Materials and methods

2.1. Animals

Male albino Wistar rats ($n=48$) (Pasteur's institute, Tehran, Iran) weighing 240–300 g were housed in an air-conditioned colony room at 21 ± 2 °C and supplied with standard pellet diet and tap water ad libitum. Procedures involving animals and their care were

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conducted in conformity with NIH guidelines for the care and use of laboratory animals.

2.2. Experimental protocol

The rats were rendered diabetic by a single intraperitoneal dose of streptozotocin (60 mg/kg) freshly dissolved in ice-cold 0.1 M citrate buffer (pH 4.5). Age-matched normal animals that received an injection of an equivalent volume of buffer comprised a non-diabetic control group. One week after streptozotocin injection, overnight fasting blood samples were collected and serum glucose concentrations were measured using glucose oxidation method (Zistchimie, Tehran). Only those animals with a serum glucose level higher than 250 mg/dl were selected as diabetic. During the next weeks, diabetes was reconfirmed by the presence of polyphagia, polydipsia, polyuria, and weight loss. Normal and hyperglycemic rats were randomly allocated and similarly grouped into six groups (eight in each): normal vehicle-treated control, sesamin-treated controls in two subgroups, diabetic, and sesamin-treated diabetics in two subgroups. Sesamin was daily administered p.o. (using gavage needle) at doses of 10 and 20 mg/kg dissolved in 0.5% carboxymethylcellulose throughout the experimental period for 7 weeks. Changes in body weight were regularly recorded during the study.

Finally, the rats were anesthetized with diethyl ether, decapitated, descending thoracic aorta was carefully removed and placed in a petri dish filled with cold Krebs solution containing (in mM): NaCl 118.5, KCl 4.7, $CaCl_2$ 1.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, and glucose 11. The aorta was cleaned of excess connective tissue and fat and cut into rings of approximately 4 mm in length. Aortic rings were suspended between the bases of two triangular-shaped wires. One wire was attached to a fixed tissue support in a 50 ml isolated tissue bath containing Krebs solution (pH 7.4) maintained at 37 °C and continuously aerated with a mixture of 5% CO_2 and 95% O_2. The other end of each wire attached by a cotton thread to a F60 isometric force transducer (Narco Biosystems, USA) connected to a computer. In all experiments, special care was taken to avoid damaging the luminal surface of endothelium. Aortic rings were equilibrated at a resting tension of 1.5 g for at least 45 min. In some experiments, the endothelium was mechanically removed by gently rubbing the internal surface with a filter paper. Isometric contractions were induced by the addition of phenylephrine (1 μM) and once the contraction stabilized, a single concentration of acetylcholine (1 μM) was added to the bath in order to assess the endothelial integrity of the preparations. Endothelium was considered to be intact when this drug elicited a vasorelaxation $\geq$50% of the maximal contraction obtained in vascular rings precontracted with phenylephrine. The absence of acetylcholine relaxant action in the vessels indicated the total removal of endothelial cells. After assessing the integrity of the endothelium, vascular tissues were allowed to recuperate for at least 30 min.

At the end of the equilibration period, dose–response curves with KCl (10–50 mM) and phenylephrine (10^{-10}–10^{-5} M) in the presence and absence of endothelium were obtained in aortic rings in a cumulative manner. To evaluate acetylcholine (10^{-9}–10^{-4} M)-induced vasodilatation in rings with endothelium, they were preconstricted with a submaximal concentration of phenylephrine (10^{-6} M) which produced 70–80 % of maximal response. The sensitivity to the agonists was evaluated as pD2, which is the negative logarithm of the agonist concentration required to produce 50% of the maximum response.

To determine the participation of nitric oxide, rings were incubated 30 min before the experiment with N(G)-nitro-L-arginine methyl ester (100 μM, a non-selective nitric oxide synthase inhibitor). To determine the participation of endothelial cyclooxygenase-derived prostanoids in response to acetylcholine, segments were preincubated with indomethacin (10 μM, an inhibitor of cyclooxygenase-derived prostanoid synthesis) 30 min before the experiment with acetylcholine.

After each vasoreactivity experiment, aortic rings were blotted, weighed, and the cross-sectional area (csa) was calculated using the following formula: Cross-sectional area (mm^2)=weight (mg)/[length (mm) × density (mg/mm^3)]. The density of the preparations was assumed to be 1.05 mg/mm^2 (Abebe et al., 1990).

2.3. Determination of malondialdehyde concentration in aortic rings

After removing aortic segments and cleansing them of extra tissues, they were blotted dry and weighed, then made into 5% tissue homogenate in ice-cold 0.9% saline solution. A supernatant was obtained from tissue homogenate by centrifugation (1000 × *g*, 4 °C, 5 min). The malondialdehyde concentration (thiobarbituric acid reactive substances) in the supernatant was measured as described before (Roghani and Baluchnejadmojarad, 2009). Briefly, trichloroacetic acid and thiobarbituric acid reactive substances reagent were added to supernatant, then mixed and incubated at 100 °C for 80 min. After cooling on ice, samples were centrifuged at 1000 × *g* for 20 min and the absorbance of the supernatant was read at 532 nm. Thiobarbituric acid reactive substances results were expressed as malondialdehyde equivalents using tetraethoxypropane as standard.

2.4. Measurement of superoxide dismutase activity in aortic rings

The superoxide dismutase activity of supernatant was measured as described earlier (Baluchnejadmojarad and Roghani, 2008). Briefly, supernatant was incubated with xanthine and xanthine oxidase in potassium phosphate buffer (pH 7.8, 37 °C) for 40 min and nitro blue tetrazolium was added. Blue formazan was then monitored spectrophotometrically at 550 nm. The amount of protein that inhibited nitro blue tetrazolium reduction to 50% maximum was defined as 1 nitrite unit of superoxide dismutase activity.

2.5. Drugs

Phenylephrine, sesamin, streptozotocin, acetylcholine, indomethacin, and N(G)-nitro-L-arginine methyl ester were purchased from Sigma Chemical (St. Louis, USA). All other chemicals were purchased from Merck (Germany) and Temad (Iran). Indomethacin solution was prepared in ethanol in such a way that the maximal ethanol concentration of the medium was less than 0.001% (v/v).

2.6. Data and statistical analysis

All values were given as means ± S.E.M. Contractile response to phenylephrine was expressed as grams of tension per cross-sectional area of tissue. Relaxation response for acetylcholine was expressed as a percentage decrease of the maximum contractile response induced by phenylephrine. Statistical analysis was carried out using repeated measure ANOVA (for body weight and serum glucose level) and one-way ANOVA (for data of vascular reactivity) followed by Tukey post-hoc test. A statistical *P* value less than 0.05 considered significant.

3. Results

After 8 weeks, the weight of the vehicle-treated diabetic rats was found to be significantly decreased as compared to controls