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Effect of streptozocin-induced diabetes mellitus on expression of methionine sulfoxide reductases and accumulation of their substrates in mouse lenses

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ABSTRACT

To determine the influences of early-stage diabetes mellitus on methionine sulfoxide reductases (Msrs) expression in lenses, streptozocin (STZ)-induced diabetic mice as animal models were used in this study. The results showed that the contents of methionine sulfoxide (MetO), protein carbonyl (PC) and Malondialdehyde (MDA) in the lenses of STZ-induced diabetic mice after 14 days were significantly higher than that in the normal control, level of total sulfhydryl groups (TSH) was 60% of normal control, and mRNA expressing levels of the MsrA and MsrBs were significantly decreased compared with normal group, as was the expression of MsrB1 protein. These results suggest that STZ not only causes increased oxidative stress, but also suppresses Msr mRNA and MsrB1 protein expression during early-stage diabetes in mice. However, the mechanism remains to research.

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1. Introduction

Cataract, which is characterized by cloudiness or opacity of the eye lens, is the leading cause of blindness worldwide (McCarthy and Taylor, 1996) and one of the major complications of both type 1 and 2 diabetes mellitus. Oxidative stress is believed to play a major role in cataract formation. Methionine sulfoxide (MetO) is an oxidative stress product produced by the oxidation of methionine (Met) (Brennan and Kantorow, 2009; Truscott and Augusteyn, 1977) that reaches levels as high as 60% in cataract while being essentially absent from clear lenses (Kantorow et al., 2004). The results from Garner and Spector (1980) showed that Met oxidation occurred in all selected fractions of lens tissue homogenates, and higher sulfur oxidation states were observed in the membrane-related fractions in cataract lenses. Furthermore, the oxidation of Met affects a multitude of biological functions through the direct inactivation of proteins (Marchetti et al., 2005).

Mammals have two methionine sulfoxide reductases (Msrs), MsrA and MsrB, which catalyze the thioredoxin-dependent reduction of S-MetO and R-MetO derivatives to Met (Kim and Gladyshev, 2004, 2005, 2007; Hansel et al., 2005), respectively. A combination of MsrA and MsrB can fully repair oxidized Met residues (Grimaud et al., 2001; Kim and Gladyshev, 2007). Marchetti et al. (2005) showed that 40% of the Msr enzyme activity present in the lens was due to MsrB, whereas the remaining enzyme activity was due to MsrA. Both enzymes are important for lens cell function, resistance to oxidative stress, and, potentially, cataractogenesis (Hawse et al., 2004; Marchetti et al., 2005). In mammals, there is only one gene encoding MsrA, but there are at least three genes encoding MsrBs (MsrB1, MsrB2 and MsrB3) (Kim and Gladyshev, 2004). All three MsrB genes are required for lens cell viability, and their silencing in lens cells results in increased oxidative stress-induced cell death (Marchetti et al., 2005). The selenoprotein family, which includes mammalian MsrB1 (also known as SelR or SelX), consists of 25 proteins containing the amino acid selenocysteine that have been found to be important mediators of the beneficial role of selenium in human health and which are catalytically active in redox processes; thus, they represent a major enzymatic antioxidant system (Kim and Gladyshev, 2004; Arbogast and Ferreiro, 2010). The presence

Abbreviations: Methionine, Met; Methionine sulfoxide, MetO; Streptozocin, STZ; methionine sulfoxide reductases, Msrs; protein carbonyl, PC; Malondialdehyde, MDA; total sulfhydryl groups, TSH; methionine sulfoxide reductases A, MsrA; methionine sulfoxide reductases B, MsrB; complementary DNA, cDNA; reverse transcription polymerase chain reaction, RT-PCR; polyvinylidene fluoride, PVDF; Tris-buffered saline containing Tween-20, TBST.

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of selenium in MsrB1 is relevant to the previously proposed role of selenium as an antioxidant.

Streptozocin (STZ) is widely used as an inducer of diabetes mellitus in experimental animals (Tsuji and Sakurai, 1998) through its toxic effects on pancreatic β-cells (Anwer et al., 2007; Yamagishi et al., 2001; Stefek et al., 2002; Kalendra et al., 2002; Kim et al., 2003). The cytotoxic action of STZ is associated with the generation of reactive oxygen species causing oxidative damage (Szkudelski, 2001; Anwer et al., 2007). Diabetes causes the increased oxidative stress, which is thought to play an important role in the pathogenesis of various diabetic complications, including cataract (Chung et al., 2003).

However, little information is available about how the lens changes during the early stages of STZ-induced diabetes in mice. In the present work, STZ-induced diabetic mice as animal models were used, the levels of oxidative stress markers, malondiadehyde (MDA), protein carbonyl (PC), and total sulfhydryl (TSH) were measured, and expressions levels of MsrA, MsrB1, MsrB2 and MsrB3 mRNA, MsrB1 protein and contents of MetO were assayed in the lenses of STZ-induced diabetic mice. Our work shows that STZ toxicity not only acts on pancreatic β-cells, but also induces oxidative stress in diabetic mouse lenses.

2. Materials and methods

2.1. Mice and tissue preparation

A total of 60 male Kunming mice (18–25 g each) were purchased from the Hubei Research Animal Center. The mice were kept in an air-conditioned animal house under a normal day/night cycle and fed mouse chow (purchased from Hubei Research Animal Center) and tap water. They were randomly divided into two groups: control and diabetic (30 mice each). The mice were given only water for 24 h before they were injected intraperitoneally with a single dose of STZ (Sigma, St. Louis, MO, USA) at 150 mg/kg body weight (dissolved in 0.1 M citrate buffer, pH 4.5). 4 h later, a 20% glucose solution was administered to the mice (0.5 ml/mouse) in the diabetic group, while the control mice were injected with citrate vehicle alone. At 96 h after STZ injection, those mice with blood glucose levels over 15 mM in whole blood samples obtained from the tail vein of overnight-fasted animals and measured using a glucose test strip (Roche Diagnostics, Indianapolis, IN, USA) were considered to be diabetic (Zeng et al., 2009; Zhou et al., 2009). The mice were fed 10 days after the initial blood glucose assay, 27 diabetic mice survived of the 30 that were treated in diabetic group. All the mice were sacrificed by cervical dislocation. Whole eyeballs were collected after washing in ice cold 0.9% NaCl solution three times and dried with absorbent paper. The lenses were microdissected from the eyeballs and used for the following detections or frozen and stored at −80 °C until analyzed. All experiments were performed within 20 days.

2.2. Native PAGE of total protein

A native PAGE was used for the detection of total protein constituent alternation of the lenses. The lenses were minced with stainless steel scissors and homogenized using a glass homogenizer in an ice bath with the buffer containing 10 mM Tris-base (pH 7.4), 0.1 mM EDTA, 0.01 M sucrose, and 0.8% (w/v) NaCl. Supernatants were collected by centrifugation (4 °C, 10,000 g) for 15 min. The protein concentration was determined using the Bradford assay. Total protein (40 μg) was analyzed by native PAGE according to the method of Laemmli (1970). Briefly, the protein was mixed with 5× sample buffer (0.30 M Tris–HCl, pH 6.8, 50% (v/v) glycerol, 1% (w/v) Bromphenol Blue) and the mixture was electrophoresed under native conditions at 4 °C, 80 V for 0.5 h in 5% stacking gel and 120 V for 3.5 h in 10% resolving gel. The protein marker (SM0671, Fermentas) was used for monitoring the protein separation. Additionally, the running buffer for the native PAGE contained 3.0 g/L Tris-base and 14.4 g/L Glycine. The gels were stained with Coomassie brilliant blue R250.

2.3. Real-time PCR and agarose gel analysis

Total RNA was isolated from the lenses using Trizol reagent according to the supplier's instruction. Complementary DNA (cDNA) was prepared from the RNA using M-MLV reverse transcriptase, dNTPs, and oligo(dT) in Tris (hydroxymethyl) aminomethane hydrochloride (Tris–HCl) buffer (50 mM, pH 8.3). The reaction mixture was incubated at 37 °C for 60 min and stopped by heating at 95 °C for 10 min (Zeng et al., 2009).

Real-time PCR was performed using DNA Engine Opticon 2 (MJ Research, Watertown, MA, USA) with an SYBR Green PCR Master Mix kit (TOYOBO, Osaka, Japan) according to the vendor's protocol. The sequences of the primers used for MsrA, MsrB1, MsrB2, MsrB3, and GAPDH (internal control housekeeping gene) are listed in Table 1. The primers for MsrA, MsrB1, MsrB2, and MsrB3 were designed using Primer Premier 5.0 (http://www.PremierBiosoft.com/faq.html); the primers for GAPDH were described by Overbergh et al. (1999). All primers were synthesized by Sun-Biotechnology Co. (Beijing, China). The thermal cycle conditions were as follows: 94 °C for 5 min followed by 37 cycles of 94 °C for 30 s, 52 °C for 1 min, and 72 °C for 1 min for MsrA; 94 °C for 5 min followed by 37 cycles of 94 °C for 30 s, 49 °C for 1 min, and 72 °C for 1 min for MsrB1; 94 °C for 5 min followed by 37 cycles of 94 °C for 30 s, 51 °C for 1 min, and 72 °C for 1 min for MsrB2 and MsrB3; and 94 °C for 5 min followed by 25 cycles of 94 °C for 30 s, 60 °C for 1 min, and 72 °C for 1 min for GAPDH. All programs ended with a final extension at 72 °C for 10 min. The expression levels of the target genes were related to the expression level of GAPDH; relative expression levels were calculated using $2^{-\Delta\Delta Ct}$ rules.

Agarose gel analysis was performed according to Choquer et al. (2003). Aliquots (10 μl) of the RT-PCRs were size-separated on 1.2% agarose gel in 0.5× TAE (0.04 M Tris–acetate and 0.001 M EDTA, pH 8.0) prestained with 0.17 μg of ethidium bromide/ml. Electrophoresis was performed in a 10 × 10 cm agarose gel for 2.5 h at 50 V to ensure good separation of the products. The products were quantified directly by densitometric scanning of the fluorescence intensity under a UV light using the Chemi Doc System (Bio-Rad, Hercules, CA, USA). The density of each amplified band was measured using Bio-Rad Quantity One software.

Table 1
Primer sequences for real-time PCR.

Name		Sequence (5′–3′)
MsrA	MsrA-RV	GGC GGA TGT GGG ATA GAC
	MsrA-FW	TCA GTG GCA ACA GAA CGG
MsrB1	MsrB1-RV	TTG CCT TTA GGG ACG AAC
	MsrB1-FW	GAA ACC ATC CAC CCA GAC
MsrB2	MsrB2-RV	TGC TCG GCT ACT TTG TCC
	MsrB2-FW	GGG TCC CTA GGG ACG AAC
MsrB3	MsrB3-RV	GAA GAC TCG GCA GGA TGG
	MsrB3-FW	CTT GGC TGG TTG GTA TGG
GAPDH	GAPDH-RV	GGC ATG GAC TGT GGT CAT GA
	GAPDH-FW	TTC ACC ACC ATG GAG AAG GC

All gene sequences were obtained from NCBI (Genbank accession numbers are NM_026322 for MsrA, NM_013759 for MsrB1, NM_029619 for MsrB2 and NM_177092 for MsrB3); primer sequences for GAPDH is from Overbergh et al.'s work (1999). After denaturation at 94 °C for 5 min, polymerase chain reaction was carried out for the corresponding cycles. All the last cycle was followed by a final extension at 72 °C for 10 min.

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