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Professional paper

Alloxan Induced Cataract in a Rat

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A B S T R A C T

We have measured lipid peroxidation and the activity of antioxidant enzymes in lenses of alloxan injected rats. After 12 weeks alloxan treated rats developed lens cataract. Diabetes rats had both lower lens weight and lower level of proteins in soluble fraction of lens homogenate. Alloxan treatment is associated with a significant increase of thiobarbituric acid reactive substances and the activity of antioxidant enzymes superoxide dismutase and catalase. However, diabetes decreased the activity of glutathione peroxidase in rat lenses. These results show that alloxan, which changes antioxidant status in rat lenses, may cause complications associated with diabetes.

Introduction

Insulin dependent diabetes mellitus is a multifactorial autoimmune disease with a series of complications affecting many organs[1]. There is the evidence of oxygen free radicals participating in the pathogenesis of diabetes mellitus[1,2]. Alloxan is a rapid and potent inducer of diabetes in experimental animals due to its pronounced damaging effect on pancreatic islet beta cells[1–3]. It is known that alloxan induced toxicity is mediated by the formation of oxygen free radicals such as superoxide anion and hydroxyl radical[4]. Oxygen free radicals exert their cytotoxic effects on the function of cell macromolecules and lipid cell membranes[1,2,4]. An increase in free radicals production or a reduction in antioxidant defenses may enhance the level of oxidative stress in different organs such as lung, kidney or ocular tissue[2,4].

Antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase protect cells and tissues against the oxidative injury[5]. Different factors, which induce the development of lens cataract, may lead to modification in activities of these enzymes leading to an increase in oxygen free radicals production[4–6]. In this study we measured the level of thiobarbituric acid substances and the activity of antioxidant enzymes in diabetic rat lenses.

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Material and Methods

Diabetes was induced in male Wister rats weighing 170–200 g using alloxan (alloxan monohydrate, Sigma USA). Alloxan was administered in 10 rats by a single intraperitoneal injection of 60 mg/kg. Control and alloxan treated animals had free access to food and water. Hyperglycemia was determined two days after treatment with glucose oxidase method[7]. Lens cataracts were determined by slit lamp biomicroscopy. At the end of the 12th week rats were anaesthetized with ether. The eyes were enucleated and lenses immediately dissected. Both lenses of each rat were weighted and homogenized with 2,0 mL of 50 mM phosphate buffer (pH 7.5). Obtained homogenate was centrifuged at 6000 × G to separate soluble and insoluble fraction of rat lenses[8].

Soluble fraction was aspirated and immediately used to determine the activity of antioxidant enzymes and lipid peroxides. Lipid peroxidation was estimated by measuring malondialdehyde formation of thiobarbituric acid by the method of Ohkawa et al[9]. Results were expressed in micromoles of thiobarbituric acid reactive substances per mg protein. Superoxide radical was analyzed and determined by the reduction of cytochrome c[10]. Superoxide dismutase activity was determined by the method of Marklund and Marklund[11], and catalase by the method of Johansson and Hakanborg. The metabolism of glutathione enzymic system, glutathione peroxidase and glutathione reductase were determined by the method of Paglia and Valentine[13] and Rathbum et al[14]. Protein concentration in rat lenses was measured by the folin fenol reagent[15]. Data were analyzed using Student t-test, with statistical significance of $p<0.05$.

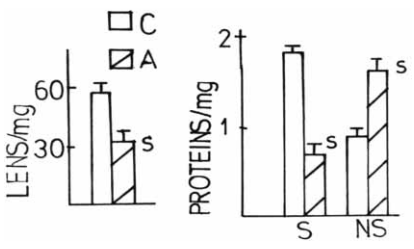

Fig. 1. Lens weight and protein level in soluble (S) and non-soluble (NS) fraction of rat lenses.

Results

Forty-eight hours after the alloxan injection, 80% of rats developed diabetes. Plasma glucose in diabetic rats was found to be about 420 mg/dL = 23 mmol/I. Several measurements demonstrated that this level of glucose was maintained during the study. Twelve weeks after treatment with alloxan rats had two-fold lower lens weight than control rats. Protein levels in soluble fraction of diabetic lenses significantly decreased compared to the control group (Figure 1).

Injected alloxan induced the release of superoxide radical in diabetic lenses. Thiobarbituric acid, hich is a damage index of cell membrane, increased approximately three-fold in diabetic lenses. The activity of superoxide dismutase, catalase and glutathione reductase increased in diabetic lenses, while the activity of glutathione peroxidase was lower in lenses of treated rats (Table 1).

Discussion

The results indicate the possibility that the loss of water in lens structure presents an important factor in development of cataract. Injected alloxan induces release of superoxide in rat lens, which corresponds with the results of several researches that show that alloxan produces oxygen free radicals[1–4]. Hyperglycemic condition such as alloxan induced diabetes and increased production of free radicals may arise from glucose auto-

TABLE 1
SUPEROXIDE (O'), LIPID PEROXIDATION (LP), SUPEROXIDE DISMUTASE (SOD), CATALASE (CAT), GLUTATHIONE PEROXIDASE (GSH-P) AND GLUTATHIONE REDUCTASE (GSH-R) ACTIVITIES IN RAT LENSES IN 12-WEEK ALLOXAN TREATMENT

	Control group	Diabetic group (8)
LP in moles/mg protein	0.34 ± 0.03	0.95 ± 0.08*
O' in moles/lens	0.0	2.12 ± 0.02
SOD U/mg protein	0.97 ± 0.09	2.77 ± 0.20*
CAT U/mg protein	1.93 ± 0.18	3.07 ± 0.03*
GSH-P U/mg protein	200.42 ± 23.4	109.31 ± 11.23*
GSH-R U/mg protein	8.06 ± 0.83	± 1.02

* $p<0.05$

oxidation and also from a non-enzymatic protein glycation[16]. Oxidative damage in many tissues is a consequence of imbalance between the pro-oxidant and antioxidant activity.

Injected alloxan increased the level of thiobarbituric acid reactive substances in rat lenses. This increase suggests that there is a high level of oxygen free radicals in lenses and their production may destroy the lens and other parts of ocular tissue. A lens contains antioxidant enzymes, which destroy free oxygen metabolites. This study shows that alloxan increases the activity of all antioxidant enzymes except glutathione peroxidase. Superoxide dismutase removes superoxide radical by converting it into hydrogen peroxide, which is then decomposed in water by catalase and glutathione peroxidase. The increased activity of superoxide dismutase and catalase probably decreases the harmful effect of oxygen free radicals in rat lenses.

We can conclude that there is an increase of thiobarbituric acid reactive substances and superoxid radicals in diabetic lenses. In such conditions antioxidant enzymes decrease the damaging effect of oxygen free radicals whose presence is associated with the development of diabetic cataract.

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ALOKSAN INDUCIRANA KATARAKTA U ŠTAKORA

S A Ž E T A K

Analizirali smo lipidnu peroksidaciju i aktivnost antioksidativnih enzima u lećama štakora kojima smo injicirali alloxan. Nakon 12 tjedana u pokusnih štakora tretiranih alloxanom razvila se katarakta. Pri tome je kod dijabetičkih štakora bila manja težina leće i razina proteina u topivoj frakciji lećnog homogenata. Terapija alloxanom povezana je sa znatnim porastom tiobarbiturne kiseline te aktivnošću antioksidativnih enzima superoksid dizmutaze i katalaze. Kao zaključak možemo reći da dijabetes doprinosi smanjenju aktivnosti glutation peroksidaze u lećama štakora. Ovi rezultati ukazuju da alloxan koji mijenja antioksidativni status u lećama štakora može znatno doprinijeti komplikacijama povezanim sa šećernom bolešću.