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Xanthine oxidase and lens oxidative stress markers in diabetic and senile cataract patients ☆

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ABSTRACT

Xanthine oxidase (XOD) is a prooxidant enzyme possibly implicated in diabetic lens injury and genesis of senile cataract (SC). We evaluated the impact of diabetes on XOD activity and its relationships with lens oxidative stress markers in patients operated on for SC. Serum and lens XOD activities, lens malondialdehyde (MDA), conjugated dienes, superoxide dismutase (SOD), glutathione peroxidase (GPx) and reduced glutathione (GSH) levels were measured in 62 non-diabetic and 29 diabetic patients operated on for SC. Lens XOD, SOD, GPx and GSH levels were gradually declining, while MDA and serum XOD were increasing with patient's age. Lens XOD activity was positively correlated with conjugated dienes concentration (rho = 0.316; $p = 0.003$) while being inversely correlated with age (rho = −0.371; $p < 0.001$), indicating that low ocular expression of XOD could be related to lower intensity of oxidative stress and delayed occurrence of SC. When samples were adjusted for confounding factors, serum XOD ($p < 0.001$), lens XOD ($p = 0.003$) and conjugated dienes ($p = 0.002$) were significantly higher in diabetic than in non-diabetic group. Lens SOD and GPx were moderately increased while MDA and GSH were unchanged in diabetic, compared with non-diabetic SC group. Blood HbA_{1C} concentration was positively correlated with lens XOD (rho = 0.346; $p < 0.001$) as well as serum XOD activity (rho = 0.485; $p < 0.001$). These results suggest that poor glycemic control may upregulate systemic and ocular XOD activities contributing to lens oxidative stress and possibly to earlier onset of cataract.

1. Introduction

Senile cataract (SC) is an age-related, progressive lens opacification and the leading cause of curable blindness worldwide. The prevalence of disease is greater in patients with type 2 diabetes mellitus, especially in women, representing the major ocular complication of chronic hyperglycemia (Swetha, Jeganathan, Wang, & Wong, 2008). Its morphological characteristics in diabetics are however similar to normoglycemic subjects, although some clinical types may be more frequent. Mechanisms underlying the genesis of SC are highly complex and most probably related to long-term ocular oxidative stress, stemming from overproduction of oxidants, their insufficient removal, or combination of both (Vinson, 2006).

The lens and the surrounding ocular tissues are chronically exposed to environmental and intrinsic oxidants, such as superoxide anion radicals and hydrogen peroxide, produced during mitochondrial respiration and photo-oxidation of endogenous UV filters, ascorbate and structural proteins, the crystallines. Normally, superoxide is spontaneously or via superoxide dismutase (SOD) converted to hydrogen peroxide, which is thereafter reduced to water by glutathione peroxidase (GPx) and/or catalase. Excessively high levels of oxidants can be toxic to the lens and impose irreparable damage, including cross-linking and aggregation of crystallines, loss of cellular redox balance, oxidation of DNA bases and lipid peroxidation (LPO) of polyunsaturated fatty acids. Lipid hydroperoxides with conjugated diene bonds are unstable, primary LPO products that typically absorb at 234 nm (Chajes, Sattler, Stultschnig, & Kostner, 1996). They subsequently rearrange and undergo oxidative carbon–carbon bond cleavage forming relatively stable lipid-derived aldehydes, such malondialdehyde (MDA).

Numerous studies have evidenced that chronic, uncontrolled influx of glucose in insulin-independent lens tissue induces mitochondrial dysfunction, activation of polyol pathway and protein kinase C, osmotic swelling, and post-translational modifications of

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enzymes and other long-lived proteins (Kyselova, Stefek, & Bauer, 2004). However, xanthine oxidoreductase was also suggested to mediate the lens injury in hyperglycemic conditions (Cekic, Bardak, Totan, Akyo, & Zilelioglu, 1999; Taysi et al., 2011). This enzyme exists in two interconvertible forms, constitutively expressed in many ocular tissues (Cejkova & Lojda, 1996; Fox & van Kujik, 1998; Cejkova, Ardan, Cejka, & Luyckx, 2011). It is the rate-limiting enzyme of purine metabolism, catalyzing the hydroxylation of hypoxanthine to xanthine and its oxidation to uric acid, with concomitant formation of superoxide and hydrogen peroxide. Either reversibly, following oxidation of critical cysteine residues, or irreversibly by limited proteolysis, the dominant NAD^+-dependent xanthine dehydrogenase form is converted to xanthine oxidase (XOD; EC 1.1.3.22). Because of lower affinity for NAD^+, a reduced FAD cofactor of XOD readily reacts with oxygen and in hypoxic conditions generates mainly hydrogen peroxide (Kelly et al., 2010), which is the principal lens oxidant.

Despite evidences that some well known risk factors implicated in cataractogenesis, like UVB radiation and hyperglycemia, can promote the conversion of xanthine reductase to XOD form (Cejkova et al., 2011; Taysi et al., 2011; Portugal-Cohen & Kohen, 2009), its role in genesis of SC has not been extensively examined. Given that XOD may be the source of oxidants, especially in hyperglycemic conditions, this study was undertaken to explore its relationship with ageing and with ocular oxidative stress in type 2 diabetes mellitus patients with SC and to compare them with non-diabetic SC subjects.

2. Materials and methods

2.1. Patients and samples

This study included 91 patients (48 males and 43 females, aged 46−80 years) operated on for SC at Medical Centre of Kosovska Mitrovica. According to medical files, 29 patients were previously diagnosed with type 2 diabetes mellitus, considered as the diabetic SC group, while others (n=62) had no history of diabetes and comprised the non-diabetic SC group. Diabetic patients were on regular anti-diabetic medication: 24 were receiving sulfonylurea alone or in combination with biguanides and 5 were on combined insulin and oral hypoglycemic therapy. All patients underwent routine ophthalmologic, internistic and laboratory examination prior to surgery. Ethical clearance for the study was obtained from local Ethics Committee after written informed consent from participants was provided. This study excluded patients with mature cataract, proliferative retinopathy, or evidences of myocardial infarction, stroke, as well as malignant, autoimmune, hepatic or renal diseases.

The lens (nucleus plus cortex) was obtained during extracapsular cataract extraction followed by intraocular lens implantation. Aliquots of venous blood were taken preoperatively after an overnight fasting into Vacutainer tubes with and without EDTA anticoagulant, and spun at 3000×g for 15 min to obtain plasma or serum samples.

2.2. Preparation of lens extract

The lens was briefly homogenized on ice in a ten-fold volume (w/v) of a cold buffer (0.2 M potassium phosphate; 137 mM potassium chloride; 60 mM sodium dodecyl sulfate; pH 7.2). Ethanolic butylated hydroxytoluene solution (2%, v/v) was used to prevent lipid auto-oxidation. Insoluble material was removed by centrifugation (12,000×g; 40 °C; 20 min). If otherwise not stated, the hydrosoluble lens extract was used for biochemical measurements.

Biochemical measurements were carried out on an UV/VIS spectrophotometer equipped with a constant temperature cuvette compartment (SAFAS 2, Monaco).

2.3. Assessment of lens and serum XOD activity

To determine serum and lens XOD activity we employed the method of Roussos (Roussos, 1967) using xanthine as a substrate (xanthine, 3 mM; in 0.1 M NaOH/glycine buffer). The formation of uric acid was continuously monitored at $\lambda=293$ nm for 5 min. One unit of XOD activity was defined as 1 μmol/min uric acid formed at 37 °C. XOD activity was calculated using molar absorbance of $\varepsilon = 1.26\times10^4 L\times M^{-1}\times cm^{-1}$ after correction for pre-existing uric acid.

2.4. Measurement of lens antioxidant enzymes activities

SOD activity was determined by the rate of inhibition of adrenaline autooxidation to adrenochrome, continuously monitored at 25 °C for 3 min at $\lambda=480$ nm (Misra & Fridovich, 1972). SOD activity was computed against uninhibited control, using the molar absorbance of adrenochrome of $\varepsilon=4.02\times10^3\times L\times cm\times M^{-1}$. One unit of SOD activity was defined as the quantity of enzyme that inhibits autooxidation of 5 mmol adrenaline by 50%.

GPx activity was measured by colorimetric method with cumene hydroperoxide as a substrate (3 mM, in concentrated methanol) and freshly prepared glutathione as a co-substrate (6 mg in 10 mL of 50 mmol/L TRIS-HCl buffer, pH 8.9) (Rotruck et al., 1973). One unit of GPx activity was defined as the amount of enzyme that catalyses oxidation of 1 μM of glutathione per minute, at 37 °C.

2.5. Determination of glutathione

Tripeptide glutathione (GSH) is the major intracellular non-enzymatic antioxidant and reducing agent. Concentration of total GSH was determined using Ellman's reagent after deproteinization of hydrosoluble lens extract with 10% metaphosphoric acid (Beutler, Duron, & Kelly, 1963).

2.6. Measurement of lens lipid peroxidation markers

Baseline conjugated dienes concentration was assessed in liposoluble lens extract (Chajes et al., 1996). In brief, 50 μL of water-insoluble lens material was resuspended by vortex-mixing in 1.5 mL chloroform–methanol mixture (2:1; v/v), and spun for 5 min at 2000×g (4 °C). The lower chloroform layer was removed and evaporated to dryness under a stream of nitrogen. The lipid residue was redissolved in 1 mL concentrated cyclohexane and absorbance was measured in a quartz-glass cuvette at $\lambda=234$ nm. Concentration of conjugated dienes was calculated using molar absorbance of $\varepsilon = 2.95\times10^4\times L\times M^{-1}\times cm^{-1}$.

Concentration of MDA, which is a relatively stable LPO marker, was determined in hydrosoluble lens extract (Buege & Aust, 1978). Absorbance of pinkish trimethin complex formed in reaction of MDA with 2-thiobarbituric acid (2-thiobarbituric acid, 70 mM; NaOH, 50 mM) was measured at $\lambda=532$ nm. Concentration of MDA was calculated using the molar extinction coefficient of $\varepsilon=1.56\times10^5 L\times M^{-1}\times cm^{-1}$.

2.7. Determination of ferric reducing ability of the lens

The ferric reducing ability of the lens (FRAL) was assessed in order to evaluate concentration of total lens reducing agents, as previously described (Kisic, Miric, Zoric, Ilic, & Dragojevic, 2012). The absorbance was measured at $\lambda=593$ nm against reagent blank and expressed as micromoles of ferric ions reduced per lens.