Turkish Journal of Endocrinology and Metabolism, (2000) 1 : 1-4 **ORIGINAL ARTICLE**

Lens Superoxide Dismutase and Catalase Activities in Diabetic Cataract

Dilek Özmen* **Bilgin Özmen**** **Esin Erkin***** **İnci Güner*** **Sara Habif*** **Oya Bayındır***

* *Ege University, Faculty of Medicine, Department of Clinical Biochemistry, Bornova, İzmir*

** *Celal Bayar University, Faculty fo Medicine Department of Internal Medicine, Manisa*

*** *Celal Bayar University, Faculty of Medicine Department Ophthalmology, Manisa*

Biochemical evidence suggests that oxidative damage of the lens proteins is involved in the genesis of senile cataract and the degenerative manifestations of diabetes such as diabetic cataract. This damage either decreases the antioxidant capacity or decreased antioxidant capacity results in oxidative damage. To test this hypothesis the antioxidant status in senile (n = 26) and diabetic (n=18) cataractous lenses was investigated by determining Cu, Zn-superoxide dismutase (Cu, Zn-SOD) and catalase activities by enzymatic and colorimetric methods respectively. Both Cu, Zn-SOD and catalase levels were significantly lower in the diabetic cataractous lenses. The following results were obtained: (mean ± SEM, diabetic and senile cataractous lenses respectively) Cu, Zn-SOD 8.052 ± 0.428 (µ/g prot. and 18.216 ± 0.827 (µ/g prot. ($p < 0.05$); catalase 0.326 ± 0.031 kU /g prot. and 0.665 ± 0.063 kU /g prot. $p<0.001$).

The decreased activities pointed to a decrement of the antioxidant capacity in the diabetic cataractous lenses suggesting the implication of antioxidant enzymes in the genesis of diabetic cataract.

Key words : Diabetic cataract, Cu, Zn superoxide dismutase, catalase

Introduction

The oxidative stress which is associated with diabetes mellitus, might play an important role in the initiation and progression of diabetic complications (1). It has been suggested that free oxygen radicals trigger cataract, one of the degenerative manifestations of diabetes (2, 3). The toxic effects of the reactive oxygen species are neutralized in the lens by antioxidants such as ascorbic acid, vitamin E, the glutathione system (GSH peroxidase, GSH reductase), superoxide dismutase and catalase (4). The enzymatic (superoxide dismutase, glutathione peroxidase, catalase) and non-enzymatic (ascorbate, glutathione, cysteine) antioxidant system activities are decreased in the lens and aqueous humor during aging and in the development of senile cataract (5. 6).

While enzymatic antioxidant activities such as superoxide dismutase, catalase and glutathione peroxidase decrease in liver, kidney and heart tissues of patients with diabetes mellitus, the levels of the reactive oxygen species such as superoxide anion radicals increase (7, 8). These alterations suggest that free oxygen radicals and antioxidant mechanisms might play an important role in the pathogenesis of diabetic cataract. In diabetic

Correspondence address:

Dilek Özmen
Ege Üniversitesi Tıp Fakültesi
Klinik Biyokimya Bilim Dalı
(35100) Bornova - İzmir, Türkiye

patients high lens copper ion concentrations and low Cu, Zn-SOD activities were observed (9).

In view of these observations, the purpose of this study was to determine the activities of Cu, Zn-superoxide dismutase and catalase in diabetic and non-diabetic senile cataractous lenses and to designate the role of these enzymes in the development of diabetic cataract.

Materials and methods

Eighteen Type II diabetic, cataractous patients (10 female, 8 male, mean age 62) and 26 senile cataractous patients (11 female, 15 male, mean age 68) were included in this study. The mean duration of diabetes was 19 years.

Participants in the study had no clinical evidence of any disease state which might affect the antioxidant capacity. All diabetics were treated with insulin and none of them had any clinically diagnosed macrovascular, microvascular or neurological complications of diabetes mellitus.

Extracorpuscular cataract extraction and posterior chamber intraocular lens implantation was performed on all of the cataractous patients at the Ophthalmology Department of the Medical School of Celal Bayar University.

After extraction, the weighed lenses were immediately homogenized in 0.1 molar phosphate buffer pH 7.0 at 0 °C. The homogenized material was stored at - 20 °C until analysis.

Protein : Protein concentrations were measured by the method of Lowry (10).

Cu, Zn- SOD : Lens Cu,Zn-SOD levels were determined by the spectrophotometric indirect inhibition technique of Sun et al., based on the inhibition of nitroblue tetrazolium (11). In the assay, the xanthine - xanthine oxidase system was used as a superoxide generator. The absorbance of the reduction product (formazane) was measured at 560 nm.

Catalase : Lens catalase activities were determined by Goth's colorimetric method, in which serum was incubated in H_2O_2 substrate and the enzymatic reaction was stopped by the addition of ammonium molibdate (12). The intensity of the yellow complex formed by molibdate and H_2O_2 was measured at 405 nm.

Statistics. The data are presented as means ± SEM. Studentís t-test for unpaired data was used to determine differences between diabetic and senile cataractous lenses. The limit of statistical significance was set at $p < 0.05$.

Results

Lens Cu, Zn superoxide dismutase and catalase levels of the patients with diabetic and senile cataract are presented in Table 1.

Table 1. Lens Cu,Zn-Superoxide dismutase and calatase values (mean ± SEM) of diabetic and senile cataractous subjects.

Parameter	Diabetic cataract (n = 18)	Senile cataract (n = 26)
Cu,Zn-Superoxide dismutase (µg/g protein)	8.052 ± 0.428*	18.216 ± 0.827
Catalase (kU/g protein)	0.326 ± 0.030**	0.665 ± 0.063

* $p < 0.05$
** $p < 0.001$

Lens Cu,Zn-SOD levels were significantly lower in the diabetic patients compared with the senile cataractous patients 8.052 ± 0.428 vs 18.216 ± 0.827 µg / g protein ($p < 0.05$).

Lens catalase activities were also significantly lower in the diabetic patients 0.326 ± 0.030 vs 0.665 ± 0.063 kU/ g protein ($p< 0.001$).

Discussion

Oxidative stress is thought to play a major role in cataract formation and diabetic complications. The glycometabolic imbalance is also an important cataractogenic factor in diabetics. Several pathogenetic mechanisms have been proposed to explain the accelerated cataractogenesis in diabetes. These mechanisms include the increased glycation and browning of lens cristallins and increased sorbitol-pathway activity (13).

The aqueous humor normally contains hydrogen peroxide (H_2O_2), a compound capable of genera-

ting reactive oxygen species. The enzymatic systems such as Cu, Zn-SOD and catalase, which protect the lens from oxidative damage are localized beneath the lens capsule.The loss of or decrease in enzyme activities allows H_2O_2 and free radicals to induce irreversible deleterious effects on the lens including a decrement in Na- K ATPase activity (14, 15).

In various studies which investigated the role of oxidant stress in the development of cataract, lens lipid peroxides are reported to be increased. On the other hand, an insufficiency of enzymatic and nonenzymatic antioxidant systems with aging is also reported (4, 5, 16-19). Obara reported that lens lipid peroxides and oxidized lipoprotein levels are increased and Cu, Zn-SOD activities are decreased due to the accelerated generation of reactive oxygen species, especially hydroxyl radicals (OH) inside cataroctous lenses. This author stated that lens glucose, glycated protein and lipid peroxides were higher in diabetic cataractous patients when compared with senile cataract (6). In a previous study we also observed higher lens lipid peroxide levels in diabetic cataractous lenses than in senile cataractous lenses (20).

In rat lenses exposed to a hyperglycemic environment (50mM glucose) Cu, Zn-SOD and catalase mRNA levels decreased (21). Yan et al. investigated the inactivation of SOD and catalase by sugars and the loss of antigenicity that was monitored by the loss of activity. In this study, incubation by sugars resulted in a time dependent inactivation of the enzymes (22).

Besides such alterations, the increased level of copper ions is likely to be related with the increased peroxidative changes (6). Lin found that the copper ion concentrations are higher in cataractous lenses thani in clear lenses. He observed that copper ion concentrations were significantly higher in diabetic cataractous lenses than in senile ones. In that study protein-unconjugated copper ions were higher than the protein conjugated ions, and as a result the superoxide anion radical scavenging capacity was reduced (9). Ascorbic acid and metal ions such as Cu+ made a major contribution to hydrogen peroxide formation (6, 23). So, changes in the metal ion content of the aqueous humor can affect lens viability.

In conclusion, the decrease in Cu, Zn-SOD and catalase activities was more pronounced in diabetic cataractous lenses than in senile ones In the light of our findings and the evidence from previous reports, we propose that diabetes itself might be a condition which leads to the acceleration of the aging process in the lens tissue.

References

1. Vucic M, Gavella M, Bozikov V, Ashcroft SJH, Rocic B. Superoxide dismutase activity in lymphocytes and polymorphonuclear cells of diabetic patients. *Eur J Clin Chem Clin Biochem* **35** (7): 517 -521, 1997.
2. Bhuyan DK, Bhuyan KC. Oxy radicals in the eye tissues of rabbits after diquat in vivo. *Free Radic Res Commun* **2:** 621-627, 1991.
3. Altomare E, Vendemiale G, Grattagliano I, Angelini P, Micelli-Ferrari T, Cardia L. Human diabetic cataract: Role of lipid peroxidation. *Diabetes & Metabolism* **21:** 173 -179, 1995.
4. Simonelli F, Nesti A, Pensa M, Romano L, Savastano S, Rinaldi E, et al. Lipid peroxidation and human cataractogenesis in diabetes and severe myopia. *Exp Eye Re.* **49:** 181 -187, 1989.
5. Babizhayev MA, Deyev AI, Linberg LF. Lipid peroxidation as a possible cause of cataract. *Mech Age De* **44:** 68 -89, 1988.
6. Obara Y. The oxidative stress in the cataract formation. Nippon-Ganka-Gakkai-Zasshi **99** (12): 1303-1341, 1995.
7. Asayama K, Hayashibe H, Dobashi K, Niitsu T, Miyao A, Kato K. Antioxidant enzyme status and lipid peroxidation in various tissues of diabetic and starved rats. *Diabetes Res* **12** (2): 85-91, 1989.
8. Giugliano G, Ceriello A, Paolisso G. Diabetes Mellitus, Hypertension and cardiovascular disease: Which role for oxidative stress? *Metabolism* **44** (3): 363 -368, 1995.
9. Lin J. Pathophysiology of cataracts : Copper ion and peroxidation in diabetics. *Jpn J Ophthalmol* **41**(3): 130 -137. 1997.
10. Lowry CH, Rosebrough NJ, Farr L, Randall RJ. Protein measurement with Folin Phenol reagent. *J Biol Chem* **193:** 265, 1951.
11. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. *Clin Chem* **34** (3): 497-500, 1988.
12. Goth L. A simple method for determination of serum catalase activity and revision of reference range. *Clin Chim Acta* **196:** 143-152, 1991.
13. Lyons TJ, Silvestri G, Dunn JA, Dyer DG, Baynes JW. Role of glycation in modification of lens crytallins in diabetic and nondiabetic senile cataract. *Diabetes* **40:** 1010-1015, 1991.

14. Green K. Free radicals and aging of anterior segment tissues of the eye: a hypothesis. Ophtalmic Res 27 Suppl **1:** 143-149, 1995.

15. Reddan JR., Steiger CA., Dziedzic DC., Gordon SR. Regional differences in the distribution of catalase in the epitelium of the ocular lens. *Cell Mol Biol* **42** (2): 209-219, 1996.

16. Fecondo JV, Augusteyn RC. Superoxide dismutase, catalase and glutathione peroxidase in the human cataractous lens. *Exp Eye Res* **36** (1): 15-23, 1983.

17. Ohrloff C, Hockwin O, Olson R, Dickman S. Glutathione peroxidase, glutathione reductase and superoxide dismutase in the aging lens. *Curr Eye Res* **3** (1): 109-115, 1984.

18. Babizhayev MA, Arkhipenko IUV, Kagan VE. Antioxidative enzyme activity and metabolism of peroxide compounds in the crytalline lens during cataractogenesis. *Biull Eksp Biol Med* **103** (2): 143-146, 1987.

19. Lian H, Li S, Cao X, Pan S, Liang S. Malonaldehyde, superoxide dismutase and human cataract. *Yen Ko Hsueh Pao* **9** (4): 186-189, 1993.

20. Özmen D, Mutaf I, Özmen B, Menteş J, Bayındır O. Lens lipid peroxides and glutathione concentrations in diabetic cataract. *Ann Clin Biochem* **34:** 190-192, 1997.

21. Khanna P, Wang L, Ansari NH. Semi-quantitation of mRNA by polymerase chain reaction. Levels of oxidative defence enzymes and aldose reductase in rat lenses cultured in hyperglycemic or oxidative medium. *Res Commun Mol Pathol Pharmacol* **92** (1): 3-18, 1996.

22. Yan H., Harding JJ. Glycation induced inactivation and loss of antigenicity of catalase and superoxide dismutase. *Biochem J* **328** (Pt 2): 599-605, 1997.

23. Spector A., Ma W., Wang RR. The aqueous humor is capable of generating and degrading H_2O_2. *Invest Ophtalmol Vis Sci* **39** (7): 1188-1197, 1998.