Antioxidant enzyme status in alloxan-diabetic rat lenses

Mehmet BALCI[1], Ömer AKYOL[2], Nazmi ZENGİN[3], Gülcan KURAL[4]

[1]Eye Clinic, Soma State Hospital, Manisa, [2]Dept. of Biochemistry, Medical School of Ankara University, Ankara
[3]Dept. of Ophthalmology, Medical School of Selçuk University, Konya, and [4]First Eye Clinic, Numune Hospital, Ankara, TURKEY

We investigated the possible antioxidant enzyme activity alterations in alloxan-diabetic rat lenses. Six weeks after injecting 150 mg/kg of alloxan intraperitoneal^, lenses were extracted and activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH Px) of the diabetic lenses were compared to the controls. In the diabetic group (n=10) SOD and GSH Px activities were found to be 37.14±4.23 U/mg protein and 0.638±0.125 U/mg protein, respectively. In the control group (n=6) SOD activity was found to be 49.46±14.28 U/mg protein, and the GSH Px activity was found to be 0.356±0.165 U/mg protein. Student's t test revealed a statistically significant decrease in the SOD activity, and a statistically significant increase in the GSH Px activity in the diabetic group. These results indicate that oxidative stress might play a role in the pathogenesis of diabetic group. These results indicate that oxidative stress might play a role in the pathogenesis of diabetic cataracs. [Turk J Med Res 1994; 12(1): 1-4]

Key Words: Diabetes, Cataract, Superoxide dismutase, Glutathione peroxidase

Amongst the possible mechanisms that lead to cataract formation oxidative stress is the most investigated one. The reactive nature of oxygen and the intermediate products of oxygen metabolism, eg, singlet oxygen (O_2), superoxide anion (O_2), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH') are thought to play an important role in the genesis of tissue injury (1). The two major protective mechanisms are the antioxidant enzyme systems, eg, superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH Px), glutathione reductase (GSS Rd) system, and the nonenzymatic free radical scavengers, eg, glutathione, ascorbate, vitamin A, vitamin E, and metal-binding proteins (2-5).

In some metabolic events, an increase in xanthine oxidase levels or a decrease in SOD, and GSH Px levels result in free radical production. If allowed to remain unscavenged, these radicals can initiate deleterious reactions such as oxidation of protein and non-protein -SH, peroxidation of cytosolic and membrane lipids, and depolymerization of macromolecules such as proteins and mucopolysaccarides. In the eye, these reactions may trigger abnormalities in the water-ion balance of the lens that eventually lead to cataract formation (6,7). The participation of free radicals has been well established in postischemic cell damage in liver, heart, brain, kidney, intestine and skeletal muscle, etc (8-13). Free radicals have also been implicated in conditions such as aging, carcinogenesis, inflammation, phagocytosis, and atherosclerosis (14-16).

Although the osmotic effect of sorbitol has been proposed as a major factor, some recent findings suggest that free .radicals might play a role in diabetic cataractogenesis (17-20). Studies on SOD levels of diabetic lenses supported this view but some contradictory results were also reported (21). It is, therefore, reasonable to investigate other enzymes of the antioxidant system, such as GSH Px.

We undertook this study to determine the antioxidant status in diabetic rat lenses by concomitant SOD and GSH Px assays.

MATERIALS AND METHODS

Sixteen female Wistar albino rats of three months old, weighing 150-200 gr, were used throughout this study. Ten rats were assigned to a diabetic group and six to a control group.

Received: March. 29, 1993 Accepted: Dec. 21, 1993

Correspondence: Ömer AKYOL
Fakülteler Mah. Keskin Sok. 18/4
Cebeci-ANKARA
TURKEY

Table 1. SOD activity in lenses (U/mg protein)

Diabetic group		Controls	
Rat No.	SOD activity	Rat No.	SOD activity
1	38.36	1	31.23
2	38.46	2	40.71
3	33.48	3	43.75
4	45.49	4	54.41
5	37.51	5	72.55
6	32.51	6	54.10
7	34.01		
8	36.82		
9	37.51		
10	36.7?		
ti«10	37.14±4.23*	n=S	49.46+14.28*

' Meanistandard deviation
t-2.60, p<n.02

Diabetic rats were produced by an injection of 150 mg/kg alloxan intraperitoneal^. Intracardiac blood was obtained weekly, and glucose concentration was measured by the glucose oxidase method (22). At the end ox sixth week, rats were sacrificed, and the eyes wore enucleated immediately. Lenses were then extracted and wet weights were determined by sensitive mechanic scale (Gebr, Bosch S200, Germany).

Both lenses of the same animal were homogenized with distilled water by using a homogenizer (B. Braun Melsungen AG, Type 853 202, W. Germany). The suspension was added same amount of ethanol: chloroform (5:3, v/v) and centrifugated at 15000x g for 15 minutes. The resulting supernatant was assayed for SOD and GSH Px activities. Soluble protein was determined as described by Lowry et al (23). The nitroblue tetrazolium (NOT) reduction method of Sun et al (24) was used to determine SOD activity. Amount of protein required to inIlibit NBl reduction by 50% was defined as one unit (U) of GSH Px.

Results are expressed as meantSD. Student's t test was used for statistical analysis of significance.

RESULTS

Fourtyeighi hours attei injection of alloxan, bloud samples revealed that all rats became diabetic. Minimum blood glucose level was found to be 200 mg/100 ml. Weekly controls demonstrated that this level was maintained during the research period.

Superoxide dismutase

SOD levels of diabetic rat lenses were found to be lower as compared with control (Table 1). While mean SOD activity was 37.14±4.23 U/mg protein in diabolic lenses, it was 49.46± 14.28 U/mg protein in controls. The difference between the two groups was statistically significant (Student's test, p<0.02. t-2.60).

Table 2. GSH Px activity in lenses (U/mg protein)

Diabetic group		Controls	
Rat No.	GSI i-Hx activity	Rat No.	GSH-Px activity
1	0.723	1	0.313
2	0.794	2	0.654
3	0.796	3	0.208
4	0.772	4	0.361
5	0.600	5	0.382
6	0.515	6	0.20?
Ü	0.486		
8	0.511		
9	0.630		
10	0.646		
n-10	0.638+0.125	n=6	C.356±0.165*

Mean±standard deviation
t=3.91, p<0.05

Glutathione peroxides

GSH Px assays showed higher levels in the diabetic group than in the controls (Table 2). Moan GSH Px activity war; 0.638±1.25 U/mg protein in tho diabetic gronp and 0.356±0.165 in the controls. The difference between two groups was si ttistically significant (Student's t test, p<0.005, t-3.91).

DISCUSSION

Antioxidant system alterations noted in experimental diabetes can result primarily f. liabetic state, and secondarily from weight loss. Direct effects of a diabotoge.iic agent unrelated 1». i diabetes-inducing actions can also be a contributing factor but we feel it's unlikely for the following reasons. First, alloxan is a rapidly metabolized agent (26) and would, therefore, not be expected to exert direct effect over the 6 weeks period oi this study Second, previous Studies failed to detect any alterations in tissue antioxidant enzyme activities in ihe alloxan injected animals that failed to develop diabetes. Finally, insulin treatment completely reversed tne alterations observed in the diabetic tissues (27).

The classical explanation of tire pathogenesis of cataracts is the loss of lens tiunsparenoy by osmotic factors. There is, now, fairly convincing evidence that tree radical mechanisms are involved in cataraotogenesis (20-30). Osmotic stress has also long been held accountable tor diabetic cataracts, however, results of some recent researches support an oxidati -e mechanism (17-20). Statislici-.lly significant reduced SOD activity, and enhanced GSH Px activity found in the present study suggest that diabetes can alter the antioxidant enzyme status of lenses which, in turn, might be expected to impact upon lens transparency.

Reduced SOD activity noted in our study must bo related to an increase in the oxidant activity. This finding is consistent with those from cataractous human lenses (2829). in experimental diabetes, Scharf (21) found the SOD activity significantly increased In the lenses of moderately diabetic rats but in normal limits in the severely diabetic ones The increat.0 in moderately diabetic rats, as stated by the author can be compensatory, and can be related to the severity and duration of diabetic state. In the present study, we didn't attempt to correlate our findings with the severity of diabetes because SOD activity of diabetic lenses were not as high as the mean activity found in the control group. In addition, duration of diabetes in Scharf's study is shorter than ours (4 vs 6 weeks). Therefore, increased activity reported by Scharf seems more likely to be a compensatory feature of early and mild diabetes whereas our results can show the effects of longei duration and established disease.

Several previous studies (28) have reported a decrease in the enzymatic activity of GSH Px during cataractogenesis, but that effect was not obseived in the present study. The reasons for this discrepancy are not clear but it may be a spesific feature of diabetic state in the ions. Wohaieb and Godin (27) have studied ihe alterations in froo radical scavenging enzymes in various tissues of strepiozocin-induced diabetic rat model. They found decreased .SOD but increased GSH Px activities in some tissues, as was the case in our study. They have proposed that the in creases were compensatory (usually involving enzymes whose activity in control tissue is low), and the decreases wore due to a direct inhibitory effect resulting from an increased tissue antioxidant activity. In our opinion, the compensatory incroaso in the activity r.t GSH Px might be due to an inorea.se in the substrate (H_2O_2) lovols, which can result from **non**-enzyntatfo oxidation of ascorbate (31 -32) or from an increase in p oxidation in the absence of insulin (33) ol by reactive products generated by autooxidation of glucose (34). Howevei, further research is needed to elucidate the exact mechanism of these compensatoiy changes.

In conclusion, result of the present study confirm ihe view that oxidative stress can be a contributing faciei, may bo the major one, in diaboiic . .. togenesis. However, more detailed studies which correlate the severity and duration of diabetes with all elements of the antioxidant system are indicated.

enzimlerin durumu

Alloksanla diabet oluşturulan ratlann lenslerİndeki oî'tsı antiuksidan enzim değişikliklerini ¿1 rjtndık intraperitoneal olarak 150 mg/kg alloxan enjek siyonundan 6 hafta sonra lensier çıkarılarak Jiabe tik lenslerin süpeı oksit dismutaz (SOD) t/c gluta tyon poroksidaz (GSH Px) düzoyleri konttoi leıinkiyle karşılaştırıldı. Diabetik grupta (n=10) SOD ve GSH Px aktiviteleri sırasıyla 37.14±4.23 U/mg protein ve 0.638±0.12 U/mg protein bulun du. Kontıol grubunda (0-6) SOD aktivitesi 46.46±14 28 U/mg protein, GSH Px akiıvitesi 0.356±0.165 U/mg protein bulundu. Student i testiyle diabaıik grupta istatistiksel olarak SOD aktivı tesinde anlamlı bir azalma, GShl Px aktivltesinde ise anlamlı bir artına olduğu belirlendi. Bu sonuçlar oksidatif stresin diabetik kataraktın patogenezindc rol oynayabileceğini düşündürmektedir. [Tuı J Mod Res 12(1): 1-4]

REFERENCES

1. Srivastava SK, Ansari Nil, Liu S, et al. The effects ot oxidants on biomembranes and cellular metabolism. Mol Cell Biochem 1989; 91:149-57.
2. Simmons KJ. Defense against free radicals has therapeutic implications. Jama 1984; 251: 2189-92.
3. Halliwell B, Gutteridge JML Lipid peroxidaiton, oxygen radicals, cell damage and antioxidant therapy, lancet 1984; 1: 1396-7.

4. Freeman BA, Crapo JD. Biology of disease. Free radicals and tissue injury. Lab invest 1982; 47: 412-26.

5. Hallliwell B, Borish E, Pryon NA, et al. Oxygen radicals and human disease. Ann Intern Med 1987; 107: 526-45.

6. McCord JM,. Oxygen-derived free radicals in post-ischemic tissue injury. N EnglJ Med 1985; 312:159-63.

7. Bhuyan KC, Bhuyan DK, Podos SM. Evidence of increased lipid peroxidation in cataract. IRCS 1981; 9:126-9.

8. Jennische E. Possible influence of glutathione on post-ischemic liver injury. Acta Pathol Microbiol Scand 1984; 92: 55-64.

9. Vasko KA, DeWall RA, Riley AM. Effect of allopurinol on renal ischemia. Surgery 1971; 71: 787-97.

10. Hess ML, Manson NH, Okabe E. Involvement of free radicals in the pathophysiology of ischemic heart disease. Can J Physiol Pharmacol 1982; 60: 1382-9.

11. Demopoulos HB, Flamm ES, Peitrongro DD, et al. The free radical pathology and the microcirculation in the major central nervous system disorders. Acta Physiol Scand 1980; 492:91-119.

12. Parks DA, Bulkley GB, Granger DN, et al. Ischemic injury in the cat small intestine. Role of superoxide radicals. Gastroenterology 1982; 82: 9-15.

13. Kagan V, Churakova TO, Karagodin VP, et al. Disturbances of the Ca^{++} transport enzyme system in membranes of the sarcoplasmic reticulum caused by hydroperoxides of phospholipids and of fatty acids. Bull Exp Biol Med 1979; 87: 124-8.

14. Change B, Sies H, Boveris A. Hydroxyperoxide metabolism in mammalian organs. Physiol Rev 1979; 59: 526-605.

15. Minnaker KL. Aging and diabetes mellitus as risk factors for vascular disease. Am J Med 1987; 92: (Suppl 13): 47-53.

16. IMorel DW, DiCorleto PA, Chisolm GM. Endothelial and smooth muscle cells after low density lipoprotein in vitro by free radical oxidation. Arteriosclerosis 1984; 4: 357-64.

17. Srivastava SK, Ansari NH, Hair GA, et al. Activation of human erytrocyte, brain, aorta, muscle and ocular tissue aldose reductase. Metabolism 1986; 35 (Suppl 1): 114-8.

18. Srivastava SK, Ansari NH, Hair GA, et al. Hyperglycemia induced activation of human erythrocyte aldose reductase and alteration of kinetic properties. Biochem Biophys Acta 1986; 870: 302-11.

19. Malone JI, Lowitt S, Cook WR. Nonosmotic diabetic cataract. Pediatr Res 1990; 27: 293-6.

20. Ross VM, Creighton MO, Trevithick JR. Modelling cortical cataractogenesis. VI. Induction by glucose in vitro or in diabetic rats: prevention and reversal by glutathione. Exp Eye Res 1983; 37: 559-73.

21. Scharf J, Mokady S, Zeidler A. Aldolase, glucose 6 phosphate dehydrogenase, superoxide dismutase and aldose reductase activity in the lenses of diabetic rat. Isr J Med Sci 1991;27:102-4.

22. Bergmeyer HU. Methods of enzymatic analysis. New York: Academic Press Inc, 1974; 457-8.

23. Lowry OH, Rosenbraug NJ, Farr AL, et al. Protein measurement with the folic phenol reagent. J Biol Chem 1951; 193: 265-75.

24. Sun Y, Oberty LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988; 34: 497-500.

25. Paglia DE, Valentine WN. Studies on the quantitavite and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; 70: 158-69.

26. Boquist L. Alloxan diabetogenicity: determinants of potentiation, protection and b-cell selectivity. DiabMetabolisme 1989; 15: 23-9.

27. Wohaieb SA, Godin DV. Alterations in free radical-tissue defense mechanisms in streptozocin-induced diabetes in rat. Effects of insulin treatment. Diabetes 1987; 36: 2014-18.

28. Fecondo JV, Augusteyn RC. Buperoxide dismutase, catalase and glutathione peroxidase in the human cataractous lens. Exp Eye Res 1983; 36: 15-23.

29. Tomba MC, Gandolfi SA, Maraini G. Search for an oxidative stress in human senile cataract. Hydrogen peroxide and ascorbic acid in the aqueous humour and and malondialdehyde in the lens. Lens Res 1985; 2: 263-76.

30. Simonelli F, Nesti A, Pensa M, et al. Lipid peroxidation and human cataractogenesis in diabetes and severe myoppia. Exp Eye Res 1989; 49: 181-7.

31. Spector A. Oxidation and cataract. In: Human cataract formation. Pitman, London (Ciba Foundation Symposium 106) 1984; 48: 64.

32. Nishikimi M. Oxidation of ascorbic acid with superoxide anion generated by the xanthine-xanthine oxidase system. Biochem Biophys Res Commun 1975; 63: 463-8.

33. Horle S, Ishii H, Suga T. Changes in peroximal tatty acid oxidation in diabetic rat liver. J Biochem 1981; 90: 1691-96.

34. Crabbe MJC. Diabetic and galactosaemic cataract. In: Human cataract formation Pitman, London (Ciba Foundation Symposium 106) 1984; 110-31.