Antioxidant enzyme status in alloxan-diabetic rat lenses

Mehmet BALCI, Ömer AKYOL, Nazmi ZENGİN, Gülcan KURAL

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 cataracts. [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 (O2), superoxide anion (O2), hydrogen peroxide (H2O2), 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 macro-
molecules 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.
Table 1. SOD activity in lenses (U/mg protein)

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>SOD activity</th>
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<tr>
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<tr>
<td>1</td>
<td>38.36</td>
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<tr>
<td>2</td>
<td>38.46</td>
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<tr>
<td>3</td>
<td>33.48</td>
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<td>4</td>
<td>45.49</td>
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<td>5</td>
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<td>6</td>
<td>32.51</td>
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<tr>
<td>10</td>
<td>36.77</td>
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<tr>
<td></td>
<td>Mean±standard deviation</td>
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<tr>
<td></td>
<td>n=10 37.14±4.23*</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>GSH-Px activity</th>
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</tr>
<tr>
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<tr>
<td>2</td>
<td>0.794</td>
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<tr>
<td>3</td>
<td>0.796</td>
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<td>4</td>
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<tr>
<td>5</td>
<td>0.600</td>
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<tr>
<td>6</td>
<td>0.515</td>
</tr>
<tr>
<td></td>
<td>Mean±standard deviation</td>
</tr>
<tr>
<td></td>
<td>n=10 0.638±0.125</td>
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</tbody>
</table>

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 of sixth week, rats were sacrificed, and the eyes were 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 15000xg 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 inhibit NBI reduction by 50% was defined as one unit (U) of GSH Px.

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

RESULTS

Fourtyeight hours after injection of alloxan, blood 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 diabetic 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).
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Glutathione peroxidases

GSH Px assays showed higher levels in the diabetic group than in the controls (Table 2). Mean GSH Px activity was 0.638±0.12 U/mg protein in the diabetic group and 0.356±0.165 in the controls. The difference between two groups was statistically significant (Student's t test, p<0.005, t=3.91).

DISCUSSION

Antioxidant system alterations noted in experimental diabetes can result primarily from diabetic state, and secondarily from weight loss. Direct effects of a diabetogenic agent unrelated to 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 of this study. Second, previous studies failed to detect any alterations in tissue antioxidant enzyme activities in the alloxan injected animals that failed to develop diabetes. Finally, insulin treatment completely reversed the alterations observed in the diabetic tissues (27).

The classical explanation of the pathogenesis of cataracts is the loss of lens transparency by osmotic factors. There is, now, fairly convincing evidence that tree radical mechanisms are involved in cataractogenesis (17-20). Osmotic stress has also long been held accountable for diabetic cataracts, however, results of some recent researches support an oxidati -e mechanism (17-20). Statistically 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 be related to an increase in the oxidant activity. This finding is consistent with those from cataractous human lenses (28,29). 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 increa.t.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 longer 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 observed in the present study. The reasons for this discrepancy are not clear but it may be a specific feature of diabetic state in the ions. Wohabe and Godin (27) have studied the alterations in free radical scavenging enzymes in various tissues of streptozocin-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 increases were compensatory (usually involving enzymes whose activity in control tissue is low), and the decreases were due to a direct inhibitory effect resulting from an increased tissue antioxidant activity. In our opinion, the compensatory increase in the activity of GSH Px might be due to an increase in the substrate (h2O2) levels, which can result from non-enzymatic oxidation of ascorbate (31-32) or from an increase in p oxidation in the absence of insulin (33) ol by reactive products generated by autoxidation of glucose (34). However, further research is needed to elucidate the exact mechanism of these compensatory changes.

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

enzzmlerin durumumu

Alloksanla diabet oluşturan ratlann lenslerindeki otsu antioksidan enzim değişikliklerini @1 rımdık intraperitoneal olarak 150 mg/kg alloxan enjek sionundan 6 hafta sonra lenser çıkarırken Jabe tik lenserin süper oksit dismutaz (SOD) lcv glut ton poroksidaz (GSH Px) düzeyleri kontroi leiniyle 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 bulundu. Kontrol grubunda (0-6) SOD aktivitesi 46.46±14.28 U/mg protein, GSH Px aktivitesi 0.356±0.165 U/mg protein bulundu. Student i tes­tiyle diabaik grupla istatistiksel olarak SOD aktiviti inesinde anlamlı bir azalma, GSH Px aktivitesinde ise anlamlı bir artına olduğu belirlendi. Bu sonuçlar oksidatif stresin diabetik kataraktan patogenezindir rol oynayabileceğini düşündürmektedir. [Tur J Mod Res 12(1): 1-4]

REFERENCES