Superoxide Dismutase in the Anterior Chamber and the Vitreous of Diabetic Patients

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Total superoxide dismutase (SOD) activity was examined in the anterior humor of 32 diabetic patients and 34 nondiabetic controls during cataract extraction. Median age (95% confidence interval) was 77.5 yr (73.3–81.0) and 79.3 yr (76.0–83.2), respectively. The SOD activity also was examined in posterior vitreous sampled peroperatively in 10 diabetics with proliferative retinopathy and post-mortem in seven diabetic patients and 35 nondiabetic controls. Ages were 57.2 yr (35.0–73.9), 74.4 yr (40.7–83.6), and 73.8 yr (65.0–80.2), respectively. In nondiabetic patients, the total SOD activity was much lower in the anterior chamber, 9.9 U/ml (8.1–12.6), than in the posterior vitreous, 106.3 U/ml (range 65.6–119.0), P < 0.001. We found no difference between the SOD levels in the anterior chamber of nondiabetic controls and diabetic patients, who had 9.6 U/ml (7.6–13.7). The SOD activity in posterior vitreous in diabetic patients sampled peroperatively, 23.9 U/ml (8.9–39.2), P < 0.0001, and post-mortem, 39.5 U/ml (6.5–214.2), P < 0.04, was significantly lower than in the controls sampled post-mortem, 106.3 U/ml (65.6–119.0). Low levels of SOD in the anterior chamber may be involved in cataract development, in diabetic patients and nondiabetic controls. That diabetics had decreased SOD activity in the posterior vitreous points to a possible role of SOD in the complex process of diabetic retinopathy development. Invest Ophthalmol Vis Sci 33:3131–3135, 1992

Oxygen is essential for all living animals, but it also exerts toxic effects. This toxicity is mediated by activated forms of oxygen, such as the superoxide anion radical (•O₂) and other toxic reduction products. Such compounds may react with and damage a host of different biologic substances.1–3 Superoxide dismutases (SOD) protect against the superoxide radical by catalyzing its dismutation:

\[
\text{SOD} \quad \quad \downarrow \\
\text{O}_2^+ + \text{O}_2^+ + 2\text{H}^+ \rightarrow \text{O}_2 + \text{H}_2\text{O}_2 \quad (1)
\]

High activity levels of superoxide dismutases are found in virtually all types of cells in the body,4 whereas the activity is much lower in extracellular fluids.5

In the advanced stages of diabetic retinopathy, large hemorrhages from fragile retinal vessels leak into the vitreous body.6–8 Recently, it was reported that SOD is decreased in the retinas of rats9 and rabbits10 that have long-standing experimentally induced diabetes. These observations may indicate that low levels could play a role in the development of retinopathy in diabetic humans.

The prevalence of senile cataract is greater in a diabetic population than in a nondiabetic population.11 Low SOD levels may play a role in the development of senile cataract in humans, because defective SOD molecules accumulate with age in the human lens.12

In the present study, our aim was to analyze the levels of SOD in the lens and retinal environments of diabetic patients, ie, the anterior chamber and posterior vitreous space. Therefore, we determined the SOD activity in the anterior chamber humor of diabetic patients undergoing lens extraction, in the posterior vitreous body of diabetic patients undergoing vitrectomy, and in post-mortem cases.

Materials and Methods

In 66 patients referred from other clinics, anterior chamber humor was collected during planned extracapsular cataract extraction. Thirty two of these had established diabetes controlled by internists or general practitioners, including 14 insulin-dependent patients. Median age of the diabetic subjects was 77.5 yr (95% confidence interval 73.3–81.0), and median age of the 34 nondiabetic controls was 79.3 (76.0–83.2).
Table 1. Patient age, diabetes duration, and total SOD activity in aqueous humour (median; 95% confidence interval)

<table>
<thead>
<tr>
<th>Nondiabetics (n = 34)</th>
<th>Diabetics (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>79.3 (76.0–83.2)</td>
</tr>
<tr>
<td>Duration (yr)</td>
<td>13 (4–15)</td>
</tr>
<tr>
<td>SOD (U/ml)</td>
<td>9.9 (8.1–12.6)</td>
</tr>
</tbody>
</table>

Wilcoxon’s test for unpaired variables applied. NS, not significant.

Table 2. Patient age, diabetes duration, sampling time, and total SOD activity in vitreous body (median; 95% confidence interval)

<table>
<thead>
<tr>
<th>Examined post-mortem</th>
<th>Diabetic duration (yr)</th>
<th>Time elapsed (days)</th>
<th>SOD death to sampling (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiabetics (n = 35)</td>
<td>73.8 (65.0–80.2)</td>
<td>2 (2–2)</td>
<td>106.3 (65.6–119.0)</td>
</tr>
<tr>
<td>Diabetics (n = 10)</td>
<td>74.4 (40.7–83.6)</td>
<td>12 (8–26)</td>
<td>39.5 (6.5–214.2)</td>
</tr>
</tbody>
</table>

a compared to b, not significant. a to (b and c), P < 0.03. d to e, not significant. f to g, P < 0.009. h to i, P < 0.04. k to (l and j), P < 0.0001. k to j.

Wilcoxon’s test for unpaired variables applied.
younger than subjects examined post-mortem \( (P < 0.03; \text{Table 2}) \). However, we found no correlation between SOD activity and age, either among the diabetics (\( \tau = 0.29 \), not significant; Fig. 1) or among the nondiabetic controls (\( \tau = -0.10 \), NS). Diabetes duration \( (n = 17) \) and SOD levels did not correlate \( (\tau = -0.31 \), NS). However, for all 42 subjects, elapsed time between death and examination correlated with SOD activity \( (\tau = 0.321 \), \( P = 0.003 \)).

Diabetic patients were examined on post-mortem day 1 (1–2) and nondiabetic controls were examined on day 2 (2–2), which is a significant difference \( (P < 0.009) \). The presence of diabetes and posterior vitreous SOD activity correlated significantly \( (\tau = 0.49 \), \( P < 0.0001 \)). When elapsed time was held constant, diabetes and SOD still correlated \( (\tau = 0.41 \), \( P < 0.001 \)). However, when diabetes was held constant, elapsed time and SOD did not correlate \( (\tau = 0.16 \), NS). There was a large variability in the vitreous SOD activity among individuals (Table 2). To test for the possibility that the location of the needle in the vitreous and other elements in the sampling procedure were critical to the results, samples were drawn from the right and left eyes of 13 of the controls. As shown in Figure 2, there was a good correlation between the eyes \( (\rho = 0.98 \), \( P < 0.001) \), suggesting that our findings reflect true inter-individual differences.

**Discussion**

The SOD activity in anterior chamber and posterior vitreous in diabetic patients was much lower than the activity in human tissues, most of which contain 10,000–40,000 U/g wet weight. The activity in human plasma is about 25 U/ml and thus lies between that in the anterior chamber and that in the posterior vitreous.

The SOD levels in posterior vitreous body were significantly lower in diabetic patients with proliferative retinopathy than in nondiabetic controls examined post-mortem. For obvious reasons, normal vitreous was unavailable. The findings in Figure 2 (right and left eyes) indicate that the sampling procedure was not responsible for differences found between individuals. The diabetic patients were younger than the control subjects. There was no correlation between the SOD activity and age, in diabetic patients or nondiabetic controls. Thus, an age difference cannot explain the lower SOD activity in the diabetic patients.

A post-mortem leakage of SOD from adjacent ocular tissues may explain the higher SOD activity of posterior vitreous in the controls compared to the diabetics. This explanation seems to be less likely, because when time after death was kept constant, SOD levels still significantly correlated to diabetes. Keeping diabetes constant did not result in significant correlation between SOD levels and time after death. Thus, diabetes better explains the low vitreous SOD levels than time after death.

Hemorrhages may occur in diabetic patients with proliferative retinopathy and conceivably could cause SOD level changes. Because erythrocytes contain about 20,000 U/ml CuZn SOD, an increase, rather than a decrease, in SOD activity would be the likely outcome of bleedings. Burke injected hemoglobin into nondiabetic rabbit vitreous and found increasing levels of vitreous SOD activity on the day after injection. Levels peaked on the 4th day and gradually returned to normal after 90 days. Thus, bleeding is a less likely explanation for the low SOD activity observed in our study. Burke also demonstrated increased superoxide production by monocytes and macrophages invading the vitreous after hemoglobin injection. Vitreous cavity hemorrhages thus may cause damage through free radical generation, while a decrease in...
SOD activity seems unlikely. On the other hand, in diabetics with low SOD levels, a hemorrhage may be expected to produce increased oxidative stress and damage.

In experimental diabetes induced by streptozocin or alloxan, decreased retinal SOD activity was reported.9-10 Nishida and coworkers9 did not find decreased SOD activity in cornea, lens, liver, kidney, or blood in rats after streptozocin induction of diabetes. This is in contrast to Loven et al,17 who reported decreased cytosolic SOD in liver, kidney, and erythrocytes in rats with streptozocin diabetes. Insulin treatment increased the SOD activity.

There are three SOD isoenzymes in the mammalian body. CuZn SOD is located in the cytosol and nucleus,18 Mn SOD is located in the mitochondrial matrix,18 and the secretary extracellular SOD is found in extracellular fluids5 and in the tissue interstitial space.19 Because of the scarcity of material, separation of isoenzymes was not possible in the present study. In three large vitreous specimens, separation with immobilized antibodies15 indicated that about 90% of the SOD activity was generated by CuZn SOD, and the rest was generated by extracellular SOD. No significant cyanide-resistant SOD activity (= Mn SOD) was found. Analysis of a pool of anterior chamber fluid indicated a similar relative composition. The enzymatic activity of CuZn SOD is sensitive to nonenzymatic glycation,20 and glycation may be a major cause of the decreased SOD activity we found in the vitreous in diabetes. This mechanism would be much more pronounced in the vitreous, with its slow rate of protein turnover, than in the anterior chamber humor. Unfortunately, hemoglobin A1 measurements were not available at the time most patients were examined.

In the human lens, the SOD activity decreases with age.12 It has been suggested that this may contribute to the development of senile cataract. The observation that animals fed with an SOD inhibitor develop cataract supports this notion.21 At least below age 65, cataract prevalence is higher among diabetic patients than nondiabetics.11,22 Above this age, results are less clear.23 In our lens extraction groups, median age was above 65 yr. Also, SOD activity in cataractous lenses has been reported to be higher in diabetic patients than in nondiabetics.22 The chamber humor partly controls the homeostasis of the lens. The anterior chamber fluid had a very low SOD activity, but there was no apparent difference between diabetic patients and controls. Note that the anterior chamber humor has a high ascorbate content, which contributes significantly to protection against superoxide.

There is a fluid flow from the vitreous to the choriocapillaris23 that keeps the retina attached, and SOD may contribute to protection against superoxide radicals in the retinal extracellular space. Increased amounts of oxygen radicals may be formed under many circumstances in the retina—from activated phagocytic cells in the inflammatory response,26-28 from activated phagocytosing retinal pigment epithelial cells,29 by photochemical reactions,30 and under conditions with hypoxia/ischemia followed by re-oxygenation.31 Retinal ischemia is considered to be a major cause of proliferative changes in diabetic retinopathy.31 The decreased SOD activity in the posterior vitreous body in diabetes conceivably may contribute to the development of diabetic complications.

Key words: anterior chamber humor, cataract, diabetes, oxygen radicals, proliferative retinopathy, superoxide dismutase (SOD), SOD activity, vitreous body

Acknowledgments

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References

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