

Activity of superoxide dismutase obtained from senile cataract lens — effect of diabetes mellitus[★]

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The activity of Cu,Zn superoxide dismutase in the fluid obtained from eye lens capsules after cataract surgery was investigated in samples obtained from patients with senile cataract and with senile cataract combined with diabetes mellitus. Two parameters were measured and compared: the frequency of occurrence of detected superoxide dismutase activity and the relative activity of the enzyme in samples derived from senile cataract patients *versus* those from the patients affected additionally by diabetes mellitus. It was confirmed that the decrease of superoxide dismutase activity during cataract was additionally promoted by diabetes mellitus.

Keywords: Cu,Zn superoxide dismutase activity, senile cataract, diabetes mellitus

INTRODUCTION

A number of hypotheses concerning the etiology of cataract have been put forward (Francis *et al.*, 1999; Harding 2002). Most of them assume an involvement of reactive oxygen species in the primary stages of the pathology (Taylor, 1995). Therefore, studies of the defense mechanisms against reactive oxygen species and the status of defense systems during cataract might shed light on specific pathways of the cataract-associated degeneration. One of the key anti-oxidative enzymes present in the eye lens is Cu,Zn superoxide dismutase (Cu,Zn-SOD) (Behndig *et al.*, 1998) localized mainly in the cytosol of differentiated eye lens cells, forming inside the lens extremely elongated fibers without nuclei and mitochondria. Due to the lack of these organelles, the only source of the enzyme present in the eye lens fluid is the cytosol. The Cu,Zn superoxide dis-

mutase is relatively resistant to various stress conditions. Due to this feature it can be studied more readily than other enzymes as cataract progresses, because in most cases the enzyme retains partial activity in primary stages of cataract formation.

MATERIALS AND METHODS

The material was obtained from the Ophthalmology Clinic; it was the fluid remaining after flushing of the interior of the lens capsule after surgical removal of cataract nucleus by nucleus expulsion. There were 73 samples of senile cataract collected over a period of three months. For each sample collected, additional medical information about the patient was provided. After collection the fluid was frozen and kept at -20°C until the measurements. The salts used for buffer preparation

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Abbreviations: Cu,Zn-SOD, SOD, superoxide dismutase.

were purchased at POCh (Gliwice, Poland). Adrenaline was purchased from Sigma-Aldrich (Milwaukee, USA). The Folin reagent used for protein determination was from Merck (Germany). Before measurements the fluid was thawed, thoroughly mixed and divided into small aliquots suitable for measurements of protein content and enzyme activity. The protein content was measured by the Lowry method (Lowry *et al.*, 1951). The superoxide dismutase activity was measured by the adrenalin method (Misra, 1985). Briefly, 100 μ l of sample was diluted in 2.8 ml of carbonate buffer, pH 10.2, incubated at 30°C and placed into 1 cm quartz spectrophotometer cuvette. The reaction was started by adding 100 μ l of 10 mM adrenalin solution in 10 mM HCl. In the basic pH the adrenalin was spontaneously oxidized, with the kinetics recorded by measuring the increase of absorbance at 480 nm over time. The kinetics of adrenalin oxidation in the presence of the sample was compared with the oxidation rate of adrenalin alone. The activity of Cu,Zn-SOD was calculated as the inhibition of the rate of adrenalin oxidation. The relative activity of SOD was calculated by normalization to the protein content in the sample. The statistical significance of the differences in enzyme activity determination was checked by Student's unpaired *t*-test.

RESULTS AND DISCUSSION

The samples were assigned to the following independent pairs of categories: primary stages of the cataract (average age of patients 70.5 ± 9.5 years, $n=25$) *versus* mature stages of cataract (average age of patients 69.4 ± 11.3 years, $n=48$), and samples from patients without diabetes mellitus *versus* those obtained from diabetes mellitus patients.

Figure 1 shows the occurrence of measurable Cu,Zn-SOD activity in primary stages of cataract *versus* frequency of this enzyme activity at mature

stages of cataract. It can be seen, that samples with non-detectable activity of Cu,Zn-SOD are substantially more frequent in mature cataract.

There is a tendency of earlier impairment of Cu,Zn-SOD when cataract occurs together with diabetes mellitus, as shown in Fig. 2A. The average age of patients with primary stages of cataract without diabetes mellitus was 68.4 ± 9.8 years ($n=12$), while the average age of patients with primary stages of cataract and with diabetes mellitus was 73.4 ± 8.7 years ($n=7$).

As the cataract proceeds, the difference between the Cu,Zn-SOD activities in samples derived from cataract patients without and with diabetes mellitus disappears (Fig. 2B). The average age of patients with advance stages of cataract without diabetes mellitus was 69.7 ± 10.6 years ($n=36$) and that of patients with advanced stages of cataract and with diabetes mellitus was 70.9 ± 12.5 years ($n=12$). In both Fig. 1. and Fig. 2. variation within the groups is quite high, indicating difficulties in data interpretation. Table 1 shows data about circulatory pathologies in cataract patients from basic the two categories defined above.

Table 1.

Percentage of circulatory system pathologies in cataract patients	(n=73)
Senile cataract without diabetes mellitus	72.5% (n=54)
Senile cataract with diabetes mellitus	84.2% (n=19)

It can be seen that the occurrence of circulatory system diseases among cataract patients is substantially higher in patients with diabetes mellitus.

Further assays of clinical material obtained from cataract patients with diverse medical record and treated by other methods of cataract surgery

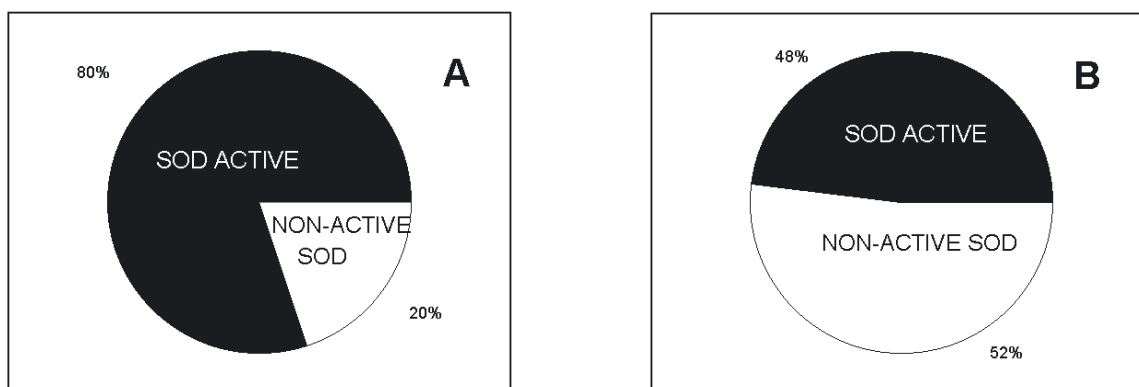


Figure 1. Occurrence of Cu,Zn superoxide dismutase activity in different stages of senile cataract.

A. Occurrence of Cu,Zn-SOD activity in primary stages of cataract ($n=19$); B. Occurrence of Cu,Zn-SOD activity in mature stages of cataract ($n=54$).

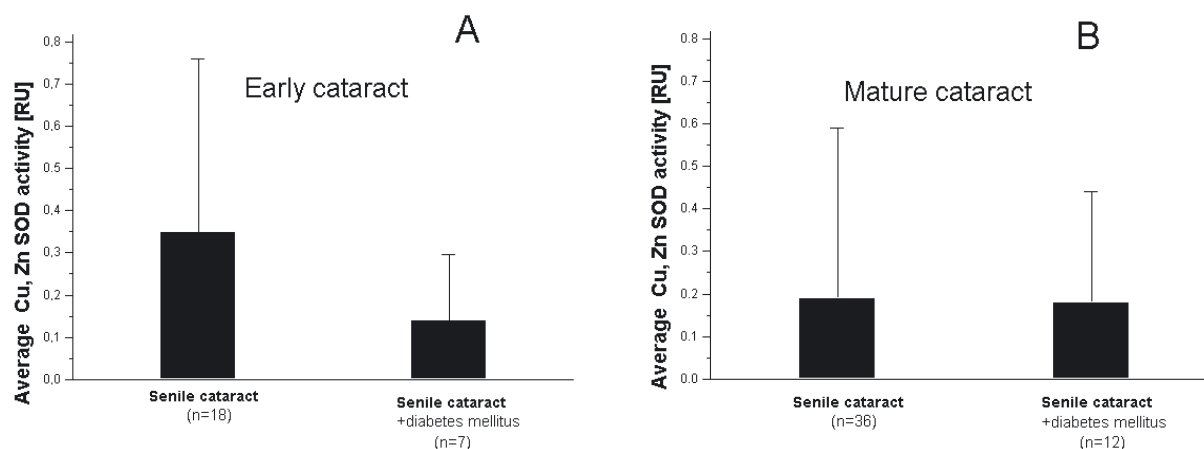


Figure 2. Average Cu,Zn-SOD activity in senile cataract from patients without and with diabetes mellitus. A. Activity of Cu,Zn-SOD in primary stages of cataract; B. Activity of Cu,Zn-SOD in mature stages of cataract.

are necessary for determination of weak points of antioxidant defense in cataract of diverse etiology.

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