CuZn superoxide dismutase, Mn superoxide dismutase, catalase and glutathione peroxidase in lymphocytes and erythrocytes in insulin-dependent diabetic children

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Abstract. CuZn superoxide dismutase, Mn superoxide dismutase, catalase and glutathione peroxidase activities in lymphocytes and erythrocytes were studied in 9 children with insulin-dependent diabetes mellitus (IDDM) as well as in 21 healthy children. The mean erythrocyte CuZn superoxide dismutase and glutathione peroxidase were statistically significantly lower in the IDDM group compared with the controls although almost all IDDM results fell within the mean ± 2 sd limits of the controls. The small differences found can hardly be assigned biological significance. Erythrocyte catalase as well as lymphocyte CuZn superoxide dismutase and Mn superoxide dismutase did not differ from the controls.

The two most commonly employed diabetogenic agents, alloxan and streptozotocin, both appear to damage islet B-cells by the means of toxic oxygen reduction products. Superoxide dismutase, catalase and hydroxyl radical scavengers all protect B-cells in vitro against alloxan (Grankvist et al. 1979a,b). In vivo protection against the diabetogenic activity of alloxan by means of parenterally administered superoxide dismutase has recently been demonstrated in mice (Grankvist et al. 1981a). Likewise, there are reports that parenteral superoxide dismutase partially protects against the diabetogenic activity of streptozotocin (Robbins et al. 1980; Gandy et al. 1982). However, failure of CuZn superoxide dismutase to protect against streptozotocin has also been reported (Gold et al. 1981). The reason for the high susceptibility of B-cells is unknown, so far. It may be that the metabolic speciali-
infections (Melin & Ursing 1958; Yoon et al. 1979) or by the effect of chemical agents on B-cells (Falkmer 1970).

The observations described above provide a rational basis for an investigation of the activities of CuZn superoxide dismutase, Mn superoxide dismutase, catalase and glutathione peroxidase in cases of insulin-dependent diabetes mellitus (IDDM) as compared to healthy controls. Defects in the superoxide dismutases, catalase and glutathione peroxidase may be fatal especially to B-cells which even normally are low in these enzymes. The Mn superoxide dismutase is also of interest in that information for its synthesis is located on chromosome No. 6 (McKusick 1982). There are indications that the gene or genes controlling IDDM are also located on chromosome No. 6 since a rather close association is seen between IDDM and the HLA-groups (Nerup et al. 1974), the complement C4 (Lamm et al. 1980) and the F1-phenotype of pro¬perdin (Raum et al. 1979) located on this chromosome.

Patients

Diabetic children
Blood samples were collected by venipuncture in hepa¬rinized tubes from 9 diabetic children 8–16 years of age each with a duration of diabetes of more than 2 years (mean 5.1 ± 3.0 st). Blood samples were collected in the morning after overnight fasting. The patients were in different degrees of metabolic control. HbA1 was an¬alyzed in each patient at sampling time and showed variations between 8.3 and 19.9% (mean 12.8% ± 3.0 so). Upper normal limit for the method in controls was 8%. No patient had signs of any infectious disease.

Control children
Blood samples were collected by venipuncture in hepa¬rinized tubes from 21 healthy children, 7–16 years of age after overnight fasting. No child had signs of any infectious disease at time of sampling.

Methods

Lymphoprep® was obtained from Nyegaard and Co., Oslo, Norway. KO2 was a product of Alfa Europe Pro¬ducts. Water was double distilled in quartz vessels.

Lymphocyte preparation
Lymphocytes from 10 ml blood were prepared essentially according to Böyum (1976). Heparinized blood was sepa¬rated on Lymphoprep®. Erythrocytes were lysed by ex¬posure to 0.75% NH4Cl. The preparations contained only a few per cent erythrocytes and polymorphonuclear granulocytes. The lymphocytes were disintegrated by sonication in 0.5 ml of 10 mm K phosphate, pH 7.4 + 30 mm KCl. The homogenates were allowed to stand for about 1 h at 4°C and were then centrifuged. Enzyme and protein analyses were performed on the supernatants.

Erythrocyte preparation
Whole blood was haemolysed in 10 volumes of 10 mm Na phosphate, pH 7.2. The haemolysate was then centri¬fuged and enzyme analysis was performed on the super¬natant. The enzymic activity was related to the haemo¬globin content of the haemolysate. Washed erythrocytes and whole blood gave the same results.

Superoxide dismutase
Superoxide dismutase was determined in terms of its ability to catalyze the disproportionation of O2− in alkaline aqueous solution. The disproportionation was directly studied in a spectrophotometer, essentially as described previously (Marklund 1976); the only difference being that both CuZn superoxide dismutase and the Mn superoxide dismutase were assayed at pH 9.5. One unit in the assay is defined as the activity that brings about a decay in O2− at a rate of 0.1 s−1 in 3 ml buffer. This corresponds to 8.3 ng human CuZn superoxide dismutase or 65 ng bovine Mn superoxide dismutase. The human Mn enzyme has not been investigated with this assay but its specific activity is probably similar to that of the bovine enzyme. The xanthine oxidase-cytochrome C assay for superoxide dismutase works at physiological conditions: neutral pH and low O2− concentration (McCord & Fridovich 1969). When bovine and human enzymes are analysed, one unit in the present assay corresponds to 0.024 units CuZn superoxide dismutase and 0.24 units Mn superoxide dismutase in the ‘xanthine oxidase’ assay, respectively. The present assay is thus about 10 times more sensitive for CuZn superoxide dismutase activity than for Mn superoxide dismutase activity.

Catalase
The rate of H2O2 disproportionation was studied at 240 nm in a cuvette containing 10 mm H2O2 in 3 ml 10 mm K phosphate, pH 7.4 + 0.1 mm diethylenetriamine penta¬acetic acid. One unit was defined as the activity that brought about a disproportionation at a rate of 10−3 s−1.

Glutathione peroxidase
Glutathione peroxidase was assayed by the method of Güzler et al. (1974), with some modifications. The haemolysates were treated with 1 mm KCN and 8.7 mm NaCN in order to inhibit the peroxidase activity of haemoglobin. A haemolysate of 25 µl was added to 500 µl 50 mm potassium phosphate + 2 mm diethylenetriamine
penta-acetic acid, pH 7.0 with 0.16 mM NADPH, 2 mM glutathione, 0.50 units glutathione reductase and 0.6 mM tert-butyl-hydroperoxide. One unit glutathione peroxidase is defined as the activity that brings about the oxidation of 1 µmol NADPH per min.

**Protein**

Protein was determined as described by Bradford (1976). The assay was standardized with human albumin.

**HbA₁**

HbA₁ was analysed with a microcolumn chromatographic procedure. A commercial kit was supplied by Biocod laboratories, Richmond, USA. The procedure was performed according to the manufactures instruction manual. The individual minor components (HbA₁ab, and c) were not separated from one another.

**Results**

The results of enzyme analysis on erythrocytes are given in Table 1. The mean CuZn superoxide dismutase activity was slightly lower in the IDDM children (P < 0.001) but almost all IDDM results fell within the mean ± 2 SD limits of the controls. The lowest control was as low as the lowest IDDM patient (47 U/mg Hb). The catalase activity was not different from the controls. The mean glutathione peroxidase activity was slightly lower in the IDDM patients (P < 0.05). However, all patients here fell within the mean ± 2 SD limits of the controls.

We found no statistical difference in lymphocyte CuZn superoxide dismutase and in Mn superoxide dismutase between the controls and the IDDM children (Table 2). There was no association between the degree of metabolic control as measured by HbA₁ and the different enzymic activities.

**Discussion**

Catalase and glutathione peroxidase remove hydrogen peroxide. Superoxide dismutase disproportionates the superoxide anion radical O₂⁻ + O₂ 2H⁺ → H₂O₂ + O₂ (McCord & Fridovich 1969). There are two types of superoxide dismutase in the cells. One, found both in the cytoplasm and in the mitochondria, contains 2 Cu and 2 Zn atoms. The other type has Mn as prosthetic metal and is found in the matrix space in the mitochondria (Weisiger & Fridovich 1973). In primates, it is possibly also found in the cytoplasm (McCord et al. 1977). Erythrocytes contain only CuZn superoxide dismutase. In order to study Mn superoxide dismutase, a cell type with mitochondria had to be investigated. Because of their availability, lymphocytes were chosen in the present study.

The erythrocyte catalase activity of the IDDM patients was not different from that of the controls.
whereas the mean CuZn superoxide dismutase activity and the mean glutathione peroxidase activity were slightly lower. However, the differences from the controls were small, and the findings can hardly be assigned biological significance. Furthermore, in lymphocytes from IDDM patients the superoxide dismutase activities were not statistically different from the controls.

As mentioned in the introduction there are indications that toxic oxygen reduction products are involved in several types of B-cell damage, and that these cells inherently possess low activities of the protective enzymes. The present investigation does not support the idea that a hereditary deficiency of such enzymes in B-cells is related to the development of diabetes in IDDM patients, at least as far as such a deficiency is reflected in blood cells.

IDDM patients show a relative hypozincemia and hyperzincuria (Hägglof et al. 1983). As judged from the present results this does not lead to a clinically significant deficient synthesis of CuZn superoxide dismutase in the patients.

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