Acta Biochimica Polonica

Vol. 48 No. 1/2001

283–285

QUARTERLY

Communication

Antioxidant status in erythrocytes of cystic fibrosis children

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Received: 31 July, 2000; revised: 18 December, 2000; accepted: 19 February, 2001

Key words: catalase, glutathione peroxidase, superoxide dismutase, erythrocytes, cystic fibrosis, vitamin E

Activities of superoxide dismutase, catalase and glutathione peroxidase in erythrocytes of cystic fibrosis children were studied in order to estimate the severity of their deficiency. Our results point to increased susceptibility of erythrocytes of cystic fibrosis subjects to oxidative injury and indicate that the antioxidant status of patients should be carefully monitored.

Cystic fibrosis (CF) is the most frequent genetic disease affecting Caucasian populations. In cystic fibrosis, as a result of chronic pulmonary infections and digestive malabsorption, an imbalance between the production of reactive oxygen species and their inactivation by protective systems is observed [1, 2]. It is commonly accepted that some plasma as well as erythrocyte biomarkers serve for the evaluation involved in the total antioxidant capacity of blood. Several studies demonstrated lower values of total radical-trapping antioxidants parameters (TRAP) in CF patients than in healthy subjects, the lower values being accompanied by low concentration of vitamin E, A and β-carotene in plasma [3, 4]. Only a few informations are available on antioxidants in erythrocytes of children with cystic fibrosis. A low level of vitamin E was previously reported by Peters & Kelly [5] and Laskowska-Klita *et al.* [6]. Studies of Benabdeslam *et al.* [4] and Niki *et al.* [7] indicated that oxidation processes in erythrocytes and their membranes due to oxidative stress may result in haemolysis. Therefore some markers of the antioxidant defence system of red blood cells were studied to estimate its deficiency in the population of CF children treated at our Institute.

MATERIALS AND METHODS

One hundred CF children, patients of Department of Pediatrics, National Research Institute of

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Abbreviations: CF, cystic fibrosis; TRAP, total radical-trapping antioxidants parameters; RBC, red blood cells; SOD, superoxide dismutase; GPx, glutathione peroxidase; PS, patients with pancreatic sufficiency; PIS, patients with pancreatic insufficiency.

Mother and Child, aged between 1 and 18 years were divided into two groups: PS – patients with pancreatic sufficiency (n = 13) and PIS – patients with pancreatic insufficiency (n = 87). Patients were classified according to clinical symptoms and genetic and biochemical parameters. Twenty five healthy children in the same age range were selected as control subjects. The institutional review board has approved the studies. Informed consent was obtained from parents.

The activities of superoxidase dismutase (SOD, EC 1.15.1.1) and glutathione peroxidase (GPx, EC 1.11.1.9) were measured by commercially available kits (Randox Laboratories Ltd. G.B.). The activity of catalase (EC 1.11.1.6) was determined according to Beers & Sizer [8]. Activities of the enzymes were expressed as U/g Hb. Haemoglobin determination was carried out by the haemoglobin cyanide method (kit, Technoplastyka, Poland).

Vitamin E in erythrocytes was determined by HPLC (Knauer, Germany) equipped with UV detector and its concentration was expressed as μmol/L [9].

All data were compared by Student's *t*-test. Differences were regarded as statistically significant at $P < 0.05$.

RESULTS

The concentration of vitamin E in erythrocytes of CF patients was half that found in healthy children (2.9 μmol/L). It amounted to 1.36 μmol/L and 1.74 μmol/L in the PIS and PS group, respectively ($P < 0.0001$). These results indicate that the deficiency of vitamin E in CF patients especially

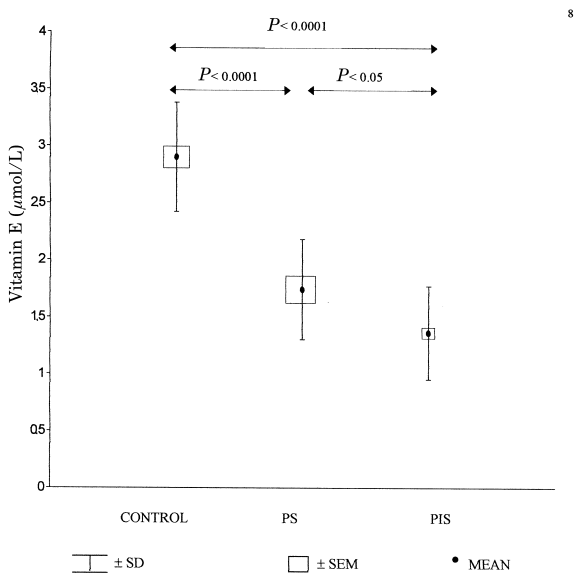

Figure 1. Concentration of vitamin E (μmol/L) in erythrocytes of control group and cystic fibrosis patients (for details see Methods).

those with PIS are at the level of risk of oxidative injury to lipophilic cell fractions (Fig. 1). Among antioxidant enzymatic activities in RBC, that of GPx remained unaffected. Catalase activity was lower in the PS and PIS groups than in control. Activity of SOD was higher both in PIS group and in PS group as compared with the control. The differences were significant at a level of 0.0001 and 0.01, respectively (Table 1).

DISCUSSION

It is well known that CF patients, due to impaired ability to absorb fat from the gastrointesti-

Table 1. Activities of superoxide dismutase, catalase and glutathione peroxidase in erythrocytes of cystic fibrosis and healthy children

Children	Activities (U/g Hb)		
	Superoxide dismutase	Catalase	Glutathione peroxidase
Control (n = 25)	1116.2 ± 168.9	18.6 ± 4.3	31.1 ± 8.1
PS (n = 13)	1465.6 ± 374.4*	12.5 ± 2.3**	30.7 ± 11.3
PIS (n = 87)	1380.6 ± 360.8***	14.3 ± 5.7*	30.2 ± 11.2

Data are means ± S.D.; *$P < 0.01$; **$P < 0.001$; ***$P < 0.0001$; Enzymic activities were determined as described in Methods.

nal tract, show a tendency to have vitamin E deficiency [1]. The content of vitamin E in plasma represents a small fraction of this vitamin, which is known to be located mainly in the cell membrane. Vitamin E as an antioxidant protects the lipid fraction of cell membrane from a free radical oxidative injury. Since it has been suggested that erythrocytes with lowered vitamin E concentration have a shortened survival time [10], red blood cells of CF patients are especially at the risk of oxidative damage. Low level of vitamin E in erythrocytes of CF patients was found by Peters & Kelly [5]. We have confirmed their observation.

Literature data concerning the activities of enzymatic antioxidants in RBC of CF patients are contradictory. Matkovics *et al.* [11] reported a significantly higher activity of SOD and catalase, but no modification of these enzymes was observed by Carmagnol *et al.* [12]. In our CF patients SOD activity in erythrocytes was significantly higher ($P < 0.0001$), but that of catalase was significantly lower ($P < 0.001$) than in controls. Similarly to our findings, only slightly decreased GPx activity in erythrocytes of CF children was observed by Portal *et al.* [13] and Winklhofer-Roob [3].

Oxidant–antioxidant imbalance of erythrocytes in cystic fibrosis suggested previously by Benabdeslam *et al.* [4], Sokol *et al.* [14] and Brown & Kelly [2] was confirmed in our studies.

Elevated SOD activity, may expresses an adaptive response of RBC to excess of superoxide anion in erythrocytes of CF patients. Dismutation of this radical to hydrogen peroxide gives the substrate for GPx and catalase. Activities of these enzymes at the level observed in this study seem to be insufficient to neutralize $H_2O_2^-$ and oxygen radicals.

The obtained results point to increased susceptibility of erythrocytes of CF children to oxidative injury and indicate that the antioxidant status of patients should be carefully monitored to consider if necessary application of some antioxidant therapy.

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