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Red cell superoxide dismutase and catalase activity in multiple sclerosis

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Multiple sclerosis (MS) is a chronic demyelinating disease that affects the central nervous system. Its characteristic pathological feature is the breakdown of myelin sheath, with relative sparing of axons.

At present there is much interest in the role of oxygen free radicals in the myelin breakdown characteristic of MS [1]. Superoxide radical ($O_2^{\cdot-}$) is a common intermediate of oxygen reduction [2]. A number of reactions have been shown to generate superoxide radical [3 - 6]. A variety of enzymes have been also shown to produce this radical [7].

Superoxide radical undergoes the dismutation reaction giving O_2 and hydrogen peroxide (H_2O_2) which, in presence of transition metal ions and $O_2^{\cdot-}$ can interact to form hydroxyl radical ($^{\bullet}OH$) by the Haber - Weiss reaction. In the iron-dependent Fenton reaction hydrogen peroxide can also yield the hydroxyl radical, and possibly singlet oxygen. Oxygen free radicals are able to initiate lipid peroxidation [8] which can lead to multiple consequences, first of all to membrane damage [9].

The antioxidant enzymes: superoxide dismutase (SOD)[1] and hydroperoxidases (catalase and glutathione peroxidase) form the first line of cell defence by efficient elimination of both $O_2^{\cdot-}$ and H_2O_2 thus preventing formation of $^{\bullet}OH$ by the above described iron-catalyzed reactions [10, 11]. Mickel [12] and Palo *et al.* [13] proposed that disturbed lipid peroxidation plays a role in MS pathogenesis. The purpose of this work was to determine the SOD and catalase activities in erythrocytes of patients with multiple sclerosis.

Our study involved 29 patients (17 females and 12 males) with a definite diagnosis of MS. Their age ranged from 21 to 61 years (mean: 37 years). The diagnosis was made by neurologists on the basis of clinical criteria [14 - 16]. The duration of the disease ranged from 1 year to 31 years; in 9 patients it did not exceed 3 years. Five patients had relapses, 5 were in the state of remission, and 19 were characterized as chronically progressive. All patients had been treated with antispastic drugs; patients treated with steroids or immunosuppressive drugs were excluded from the study.

The control group comprised healthy volunteers (19 women and 40 men, aged 19 - 55, mean 32.5 years) with no family background of demyelination and other diseases of the central nervous system.

Blood was collected on heparin as anticoagulant. The red cells separated by centrifugation, were washed twice with an excess of 0.15 M NaCl. The cells were hemolyzed by two freezing and thawing cycles and centrifuged again to remove debris. Catalase activity was determined in the supernatant [17] and expressed in International Units per gram hemoglobin. The International Unit of catalase is defined as micromole of substrate converted per min at 25°C. The substrate (H_2O_2) concentration should be approx. 1.25×10^{-2} M. The concentration of hemoglobin was determined with Drab-

[1]Abbreviations: PUFA, polyunsaturated fatty acid residues; MS, multiple sclerosis; SOD, superoxide dismutase

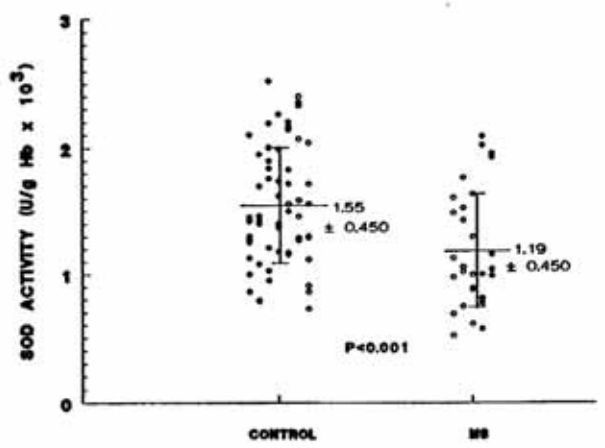

Fig. 1. Distribution of SOD activity of red blood cells of patients with multiple sclerosis and healthy persons (men, ● ; women, ○)

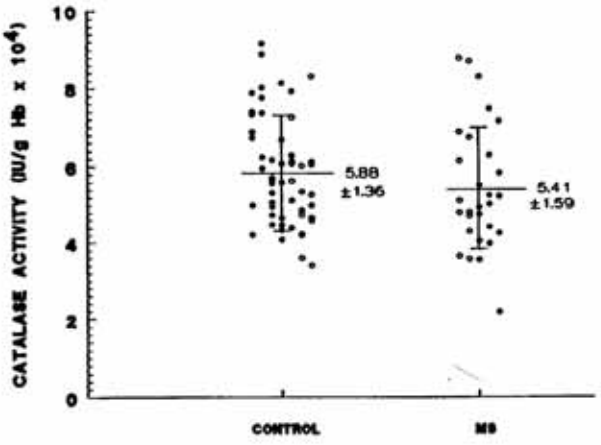

Fig. 2. Distribution of catalase activity of red blood cells of patients with multiple sclerosis and healthy persons (men, ●; women, ○)

kin's reagent. The SOD activity was determined by the method of Misra & Fridovich [4], which is based on inhibition of the superoxide radical mediated conversion of adrenalin to adrenochrome by SOD at pH 10.2. One unit of SOD activity is defined as the amount of the enzyme which inhibits by 50% adrenalin oxidation. The enzyme activity was expressed in units per gram of hemoglobin. Student's *t*-test was used to analyze the results.

The mean SOD activity in erythrocytes of the MS patients (Fig. 1 and Table 1) was $(1.19 \pm 0.45) \times 10^3$ U/g Hb and in the control group $(1.55 \pm 0.45) \times 10^3$ U/g Hb. The difference was statistically significant ($P < 0.001$).

The mean catalase activity (Fig. 2 and Table 1) in erythrocytes of the MS patients was $(5.41 \pm 1.59) \times 10^4$ IU/g Hb, while in the control group $(5.88 \pm 1.36) \times 10^4$ IU/g Hb. The difference was not significant.

Table 1 presents the particular SOD and catalase activities, age, duration and stage of MS activity of the patients.

In recent years several authors described increased [18, 19] or decreased [20, 21] activity of SOD in the red blood cells of MS patients. In our group the red cell SOD activity was by about 22% lower than in the control individuals.

The question how to explain the decrease SOD activity in our patients cannot be answered directly. The hypothesis of Mickel [10] which presupposes that the increased lipid peroxidation may cause MS may offer an explanation.

Cunnane *et al.* [22] found that phosphatidylinositol content was lower, and that of cholesterol significantly lower in erythrocytes from MS patients as compared to healthy control subjects. The authors studied also long-chain fatty acids, and found a lower content of the ω-3 fatty acids in plasma from MS patients and a decreased content of linoleic acid in erythrocyte ghosts. Ford [23] and Navarro [24] obtained similar results. The above effects correlated with the duration of the disease and the degree of disability [24]. Mickel [10] suggested that the decreased linoleic acid content in MS might be a result of its peroxidation. The cellular source of free radicals in MS is unknown. However, it is important to note that the brain itself is a rich source of free radical activity [25]. This activity may originate from glial cells, which in culture show a considerable quantity of free radical production [26]. The first evidence of increased lipid peroxidation in multiple sclerosis during a clinical exacerbation was provided by Toshniwal & Zarling [27].

The decrease of SOD activity caused by either inhibition or decreased synthesis leads to an increase in oxygen free radicals concentration and this, as a further consequence, may increase lipid peroxidation.

The observed changes in the structure of red blood cells membrane in MS patients [28] may be connected with polyunsaturated fatty acid

Table 1

Superoxide dismutase and catalase activity in red blood cells of patients with multiple sclerosis

Patients						Enzyme activity	
No.	Code	Age	Sex	Duration of disease (years)	Clinical state[a]	SOD[b]	Catalase[c]
1	Z.H.	28	F	7	R	0.69	6.16
2	W.H.	27	F	8	AE	1.13	8.80
3	T.E.	46	F	10	CP	1.61	6.89
4	S.K.	36	F	4	CP	1.49	3.65
5	S.B.	46	F	3	CP	0.52	4.83
6	P.B.	20	F	1	AE	0.98	4.82
7	O.D.	31	F	2	CP	1.02	8.72
8	M.G.	37	F	3	R	1.06	4.05
9	K.H.	37	F	1	AE	0.75	4.72
10	K.W.	34	F	7	R	1.77	6.75
11	K.K.	21	F	1	R	1.43	4.32
12	K.A.	25	F	1	AE	1.53	5.14
13	G.A.	38	F	10	CP	1.30	3.57
14	G.J.	43	F	14	CP	1.00	5.53
15	D.A.	54	F	9	CP	1.64	4.96
16	Ch.L.	28	F	7	CP	0.61	4.77
17	B.Z.	36	F	5	CP	0.88	8.32
18	Z.A.	25	M	2	CP	0.89	3.55
19	U.S.	45	M	15	CP	0.78	6.30
20	U.S.	44	M	17	CP	0.57	5.27
21	W.J.	39	M	1	CP	0.81	7.48
22	Sz.H.	51	M	12	CP	1.00	4.43
23	S.A.	36	M	10	CP	2.09	5.04
24	S.K.	36	M	17	AE	2.02	3.98
25	P.A.	33	M	15	CP	1.93	5.85
26	J.W.	61	M	31	CP	1.16	7.16
27	H.M.	35	M	2	CP	0.99	5.25
28	G.J.	52	M	21	R	1.04	2.18
29	G.J.	35	M	13	CP	1.96	4.27
Mean ± S.D.		37 ± 9.8		8.59 ± 7.12		1.19 ± 0.45	5.41± 1.59

[a]AE, acute exacerbation; R, remission; CP, chronic progression

[b]U/g Hb × 10^3

[c]IU/g Hb × 10^4

residues (PUFA) peroxidation. The size of this peroxidation is related, first of all, to the activity of SOD. Decreased activity of SOD may depend on clinical state of MS patients (in some cases it could be increased).

Catalase and glutathione peroxidase protect erythrocytes against the harmful effect of H_2O_2, which is produced from O_2^- in the reaction catalysed by SOD. The activity of catalase in red blood cells is high [29, 30]. The decrease of its activity found in our group of MS patients was not statistically important as compared to that in the control group. Similar results were described previously by Hunter *et al.* [21]. When the activity of SOD is decreased, the concentration of H_2O_2 would also be decreased and not utilized excess of O_2^- may cause, among other damages, peroxidation of lipids.

Both, our and literature data indicate that the activities of SOD, catalase and glutathione peroxidase are decreased in red blood cells of MS patients. These results suggest that the antioxidative defence in MS patients is decreased.

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