

Superoxide dismutase and lipid peroxidation in cerebrospinal fluid of patients with ischemic brain stroke

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Ischemic stroke is often complicated by local edema [1, 2]. The mechanism underlying ischemic brain edema has not been fully elucidated, despite its serious consequences on the course of ischemic brain injury [2]. It has been suggested that brain destruction in cerebral ischemia is, at least partly, due to oxidative damage [3]. Highly reactive oxygen-derived free radicals such as superoxide anions ($O_2^{\bullet-}$) and hydroxyl radicals (OH^{\bullet}) can damage endothelial cells, disrupting the blood-brain barrier; they can also injure the brain directly, causing brain edema and structural changes in neurons and glia [4, 5]. It is likely that membrane phospholipids of the brain, because of their high content of polyunsaturated fatty acids, undergo peroxidation and degradation induced by free radicals [6-9]. Several authors have provided evidence that lipid peroxidation occurs *in vivo* either during or after brain ischemia and reperfusion [2, 3, 10]. However, its occurrence is still controversial due to different methodologies used for detection and analysis of lipid peroxidation or to the use of different models of brain ischemia [3]. *In vivo*, all experiments were performed on animal models, and to our knowledge, no data are available on the antioxidant activity and lipid peroxidation in patients with ischemic brain injury.

The aim of our study was to investigate superoxide dismutase (SOD¹) activity and lipid peroxide concentration in cerebrospinal fluid of patients with ischemic brain stroke.

Samples of cerebrospinal fluid (CSF) were obtained during routine diagnostic lumbar puncture of 11 patients with ischemic brain stroke (either sex, aged 41-84), in whom no clinical signs of brain edema were observed before lumbar puncture, and the cerebrospinal fluid pressure was normal. On the basis of computer tomography performed 2 days after lumbar puncture, patients were divided into two groups: i. with evident brain edema (n = 4), and ii. without evident brain edema (n = 7). Control samples of cerebrospinal fluid were obtained during lumbar anesthesia of 8 patients with lower limb operations (either sex, aged 40-68).

Superoxide dismutase activity was assayed by the method of Misra & Fridovich [11]. Evaluation of lipid peroxidation was based on the level of conjugated dienes (CD) according to Lunec & Dormandy [12]. The CD level was measured spectrophotometrically at 242 nm [13] and expressed in absorbance units. Routine parameters such as: total protein, glucose and chloride anion concentrations in CSF were also analyzed, using standard laboratory tests (Aqua-Med, Łódź, Poland). The statistical significance of differences was estimated by Stu-

¹Abbreviations: CD, conjugated dienes; CSF, cerebrospinal fluid; SOD, superoxide dismutase.

dent's *t*-test, and $P < 0.05$ was considered significant.

The results obtained are shown in Table 1. SOD activity in both groups of patients was higher than in the control group, but only in the individuals without brain edema this increase was statistically significant. No differences in CD level between the patients without brain edema and the control group were observed. However, a highly significant ($P < 0.0001$) increase in CD level was found in the patients with brain edema as compared to those without brain edema and controls. Differences in SOD activity and CD level in ischemic patients in comparison with control ones, are presented in Fig. 1. Total protein and glucose concentrations

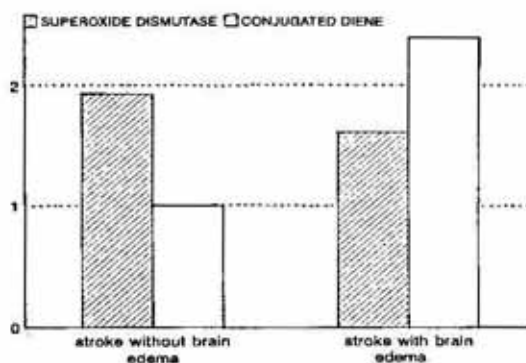


Fig. 1. Changes in superoxide dismutase activity and conjugated diene level in cerebrospinal fluid of patients with ischemic stroke referred to control value taken as 1.

in both patient groups were higher, than in control. No significant differences in the above mentioned parameters were observed between the two stroke groups. Furthermore, we have not found any change in chloride anion concentration. No correlations between SOD activity, CD level and total protein and glucose content were found.

Stroke characteristically results in deep ischemia in only part of the tissue (the focus) which is surrounded by a rim of less affected ischemic tissue, often called the penumbra [1, 14]. Attention has been directed to penumbra for two main reasons: it is considered to be slowly recruited in the infarction process, and it is believed to be the target of successful pharmacological intervention [14]. Lipid peroxidation caused by free radicals formed during post-ischemic reoxygenation of penumbra is believed to be one of the most important factors in ischemic brain damage [2, 4, 15]. It is worth noting that the enhancement of lipid peroxidation, was observed only in patients with brain edema, and that this increase was found before any other clinical signs of brain edema could be observed. In our opinion this is a very strong argument confirming the role of the lipid peroxidation process in brain edema development. An increase in lipid peroxidation can result either from an overproduction of free radicals or from a loss of efficacy of the scavenging systems [8, 16, 17]. Bromont *et al.* [3]

Table 1

Superoxide dismutase activity, conjugated diene level, and concentrations of total protein, glucose and chloride anion in cerebrospinal fluid of patients with ischemic stroke and controls.

The results are expressed as mean values \pm S.D.

Group Parameter	Stroke with brain edema	Stroke without brain edema	Control group
	n = 4	n = 7	n = 8
Superoxide dismutase activity (units/l)	6.23 \pm 3.41	7.49 ^a \pm 1.32	4.06 \pm 1.89
Conjugated diene (A ₂₄₂ units)	0.970 ^{b,c} \pm 0.132	0.405 \pm 0.034	0.405 \pm 0.104
Total protein (g/l)	0.512 ^a \pm 0.189	0.601 ^a \pm 0.232	0.328 \pm 0.107
Glucose (mM)	4.467 ^a \pm 0.628	4.200 ^a \pm 0.841	3.410 \pm 0.525
Chloride anion (mM)	124.0 \pm 3.7	123.5 \pm 1.6	124.4 \pm 1.4

Statistical significance: ^a $P < 0.05$; ^b $P < 0.001$ referring to control group; ^c $P < 0.001$ referring to stroke without brain edema.

tried to explain increased lipid peroxidation by reduced SOD activity in ischemic brain tissue. Our results are inconsistent with this suggestion, because SOD activity was elevated in both stroke groups. This increase was more evident in patients without brain edema, in whom the level of lipid peroxides was not elevated. These data suggest that the enhancement of lipid peroxidation in ischemic tissue is caused by insufficient antioxidant activity. A high superoxide dismutase activity, found in the individuals without brain edema, suggests that this enzyme prevents secondary brain injury in the stroke patients. This confirms also previous suggestions that SOD may play a beneficial role in ischemic injury and its application may be of value in treating brain edema [4, 5, 13].

Our results confirm the hypothesis that free radicals reactions including lipid peroxidation play an important role in ischemic brain injury and edema. A high lipid peroxide level found in patients with brain edema implies that determinations of CD in cerebrospinal fluid may be a valuable prognostic parameter in the course of the ischemic stroke.

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