CLINICAL STUDY

THE ROLE OF ANTIOXIDANT TREATMENT IN ACUTE ISCHEMIC STROKE: A CLINICAL STUDY

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Selected abbreviations:
BI – Barthel index; BMI – body mass index; CSF – cerebrospinal fluid; CT – computerized tomography; ECSOD – extracellular superoxid dismutase; HDL – high density lipoproteins; LDL – low density lipoproteins; MDA – malondialdehyde; MRI/MRA – magnetic resonance imaging/magnetic resonance angiography; NIHSS – National Institute of Health Stroke Scale; ROS – reactive oxygen species; SOD – superoxid dismutase; TBA – tiobarbituric acid; TG – triglycerides; VLDL – very low density lipoproteins;

ABSTRACT

Experimental studies provide evidence of the important role of oxidative stress in the development of cerebral lesions caused by focal ischemia, but data in humans are still limited and controversial. The present clinical study demonstrates a characteristic course of the markers of oxidative stress in each main stroke subtype as well as an improvement of the outcome of patients with cardioembolic stroke and with stroke caused by large vessel disease, who received antioxidants.
Key words: ischemic stroke, oxidative stress, antioxidants

INTRODUCTION

There is to date a large body of evidence, from experimental as well as from clinical studies, to implicate oxidative stress in the pathophysiology of acute ischemic stroke.

Researchers have found an increase in plasmatic lipid peroxides and oxidized LDL, as well as an increase of malondialdehyde (MDA) and other TBA reactive molecules (1,2,3,4) at the onset of ischemic stroke, while in the course of the stroke, measurements of the oxidative stress markers yielded divergent results. Some researchers found persistently elevated concentrations of MDA up to 6 months after the stroke (5), while others could not demonstrate significant alterations of the currently available markers of oxidative stress in the plasma or CSF of stroke patients (1).

The study of the total plasmatic antioxidant capacity offered also contradicting results: some studies did not find significant alterations of plasmatic superoxide dismutase (SOD) activity in acute stroke patients (3,6), while others showed increased SOD activity in plasma as well as in CSF (7) or, on the contrary, lower serum SOD activities (8).

After providing evidence for the involvement of oxidative stress in the pathogenesis of tissue damage in acute stroke, the next step was an attempt to diminish the deleterious effects of reactive oxygen species (ROS) with exogenously administered antioxidants, an approach that would likely improve the outcome and prognosis of stroke patients. There were several substances investigated, but the results were rather disappointing. On the other hand, all these studies focused mainly on identifying a most accurate marker of oxidative stress and not on the selection of stroke cases. The strokes were, at most, classified into lacunar and non-lacunar ones.

In view of these facts, the present study attempts to characterize the oxidative stress and to evaluate the usefulness of reaching currently available and clinically approved antioxidant drugs for therapeutic purposes in the main stroke subtypes.

SUBJECTS AND METHODS

The study was conducted on a series of consecutive ischemic stroke patients admitted to the Neuro IC and IIIB wards of the Clinical Hospital of Neurology and Psychiatry Oradea between March 1st and June 15th 2007 who received antioxidants in addition to the conventional treatment. The control group consisted of a series of consecutive

ischemic stroke patients hospitalized in the Neuro IIIB ward of the same hospital between May 1st and August 31st 2006. Each patient had the demographic data (age, gender), the time elapsed between onset of symptoms and admittance, and the prior medication recorded. The neurological deficit was scored on the NIHSS scale and the functional status evaluated based on the Barthel index at admittance and at discharge. Each patient gave informed consent.

The evaluation protocol was similar in both groups, and consisted of electrocardiography, transthoracic echocardiography, duplex examination of the carotid and vertebral arteries, and emergency CT scan after which selected patients underwent MRI/MRA. As for laboratory evaluation, each patient had the following blood tests performed:

- the total serum cholesterol was determined photocolorimetrically, after hydrolysis and enzymatic oxidation (9)
- HDL-cholesterol was assessed colorimetrically (10)
- seric triglyceridemia was measured colorimatrically (11)
- the seric LDL-cholesterol was calculated with the Friedewald formula (12): LDL-cholesterol = total Cholesterol total – (HDL-cholesterol + VLDL-cholesterol); VLDL-cholesterol = TG/5
- the fibrinogenemia was measured with the Vincent method (13)
- the serum malondialdehyde was colorimetrically assessed on days 1, 3, and 7 from stroke onset (Punchard and Kelly, cited by 14).
- the serum SOD activity was assessed only in the antioxidant-treated group of patients on days 1, 3, and 7 from stroke onset, with the method described by Winterbourn and coworkers (cited by 15).

After the complete work-up, the strokes were classified into subtypes according to the TOAST criteria (16).

The treatment of the patients was concordant with the currently available guidelines (17); cases with a progressive course not due to cerebral edema, hemorrhagic transformation, or aggressive lowering of the blood pressure in the acute setting, were anticoagulated with full dose intravenous unfractionated heparin. The antioxidant treatment consisted in the intravenous administration of 600 mg alpha-lipoic acid for 7 days to patients with cardioembolic stroke and/or with diabetes, or of a combination of antioxidant vitamins and selenium (10 mg beta-carotene, 40 mg DL-alpha-tocopherol, 100 mg ascorbic acid, 50 micrograms selenium) orally for 7 days to the remainder of patients.

The statistical analyses were performed with the SPSS (Statistical Package for Social Sciences) for Windows, version 10.0 (18). The means of the different parameters of the groups were compared with the independent samples t test, and correlation analyses were performed by means of the Pearson test. Statistical significance was defined as $p < 0.05$.

RESULTS

The control group consisted of 52 patients, of which 35% had strokes caused by large-vessel disease, 34% had cardioembolic strokes, and 31% lacunar strokes. 71 patients were included in the antioxidant-treated group, 53% of which had strokes judged to be caused by macroangiopathy, 24% cardioembolisms, and 23% lacunar strokes.

The antioxidant treatment was well tolerated, without any significant side-effects.

1. For the strokes caused by large vessel disease, the NIHSS score at admission was significantly correlated with the fibrinogenemia ($r = 0.275$, $p = 0.041$) and inversely correlated with the HDL-cholesterol ($r = - 0,293$, $p = 0,031$). The malondialdehyde levels on day 1, as an indicator of the „baseline oxidative stress", were correlated with the triglycerides ($r = 0.363$, $p = 0.008$), the BMI ($p = 0.03$), and negatively correlated with the SOD activity ($p = 0.01$).

In the course of the stroke (Fig. 1), in the conventionally treated patients' sera the MDA levels rose significantly on day 3, followed by a declining tendency on day 7, while in the sera of stroke patients treated with antioxidants a constant decline of the MDA was found, paralleling the SOD activity (Table 1). Statistically, the difference in the mean MDA values between the two groups was highly significant on day 3 ($p = 0.002$), while on day 7, although the antioxidant-treated group had lower MDA levels, the difference did not reach statistical significance ($p = 0.07$).

The NIHSS score at discharge was found to be influenced by the LDL-cholesterolemia ($r = 0.295$, $p = 0.03$), the fibrinogenemia ($r = 0.340$, $p = 0.01$), and inversely correlated with the HDL-cholesterolemia ($r = -0.263$, $p = 0.05$). The difference in the NIHSS scores of the two groups (Table 1, Fig. 2) supports the efficacy of the antioxidant treatment: non-significant differences between the two groups on admission, and significantly lower scores at discharge in the antioxidant-treated group ($p = 0.03$).

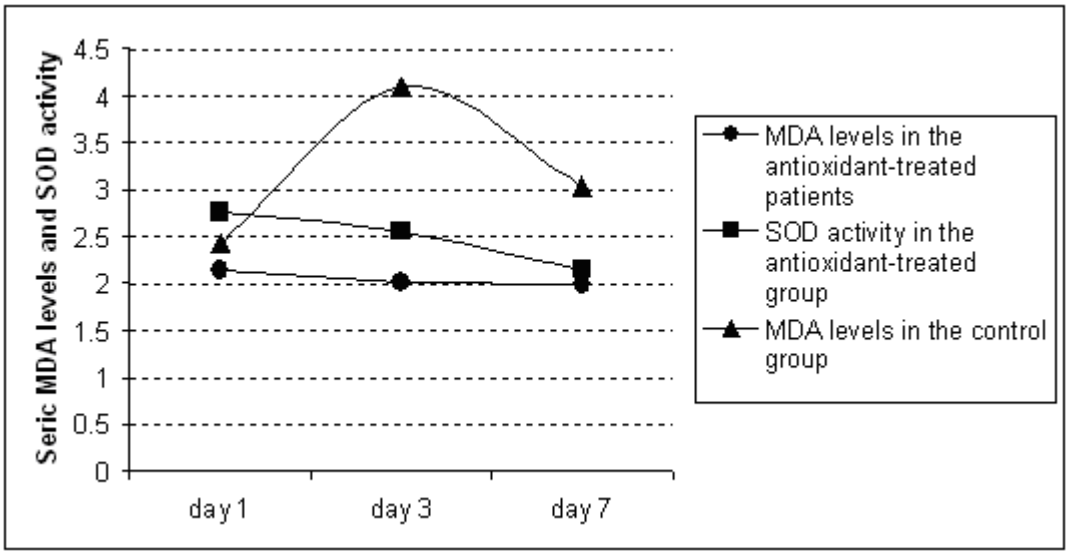

Figure 1
The mean serum MDA levels (in ng/mL) and SOD activity (in μg/mL), in dynamics, in patients with macroangiopathic stroke.

Table 1
The clinical course and the parameters of oxidative stress in patients with macroangiopathic stroke

Group		Minimum	Maximum	Mean	Standard deviation
Antioxidant-treated patients	NIHSS on admission	3	24	7.97	4.09
	NIHSS at discharge	0	12	2.92	2.88
	Barthel index on admission	10	100	67.368	23.067
	Barthel index at discharge	35	100	87.97	16.48
	Serum MDA on day 1	.50	4.20	2.1371	.9030
	Serum MDA on day 3	.20	6.70	2.0222	1.1379
	Serum MDA on day 7	.60	7.80	1.9944	1.3193
	SOD activity on day 1	.60	9.10	2.7656	2.3222
	SOD activity on day 3	.60	8.90	2.5515	2.1296
	SOD activity on day 7	.20	6.20	2.1485	1.5512
Conventionally treated patients (control group)	NIHSS on admission	3	25	9.50	6.45
	NIHSS at discharge	0	18	5.24	4.88
	Barthel index on admission	25	90	72.647	17.331
	Barthel index at discharge	0	100	81.11	24.83
	Serum MDA on day 1	.80	7.90	2.4333	1.9363
	Serum MDA on day 3	.80	7.80	4.0944	2.3000
	Serum MDA on day 7	.70	7.90	3.0438	2.0442

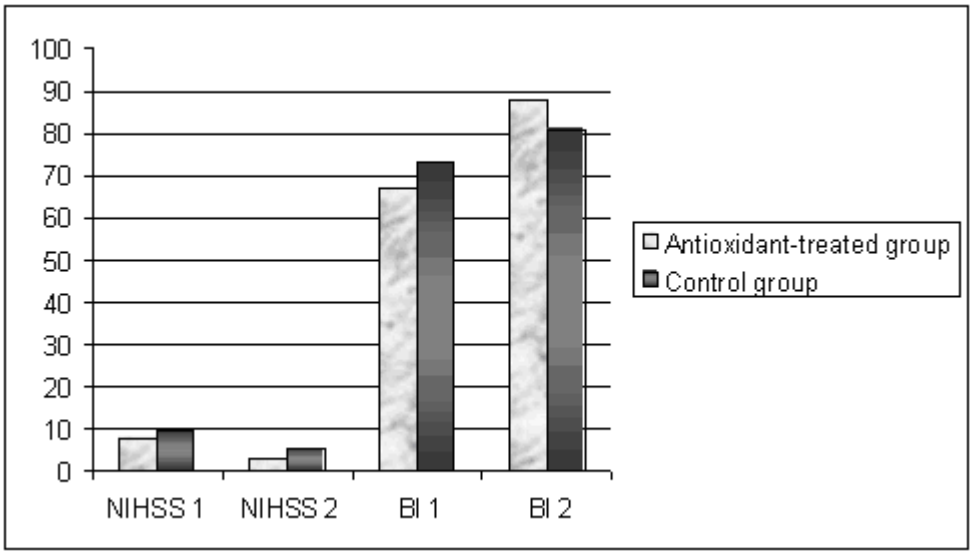

Figure 2
The outcome of the two groups of patients with macroangiopathic stroke. NIHSS 1 = mean NIHSS score on admittance; NIHSS 2 = mean NIHSS score at discharge. BI 1 = Mean Barthel index on admittance; BI 2 = Mean Barthel index at discharge.

2. As for the 32 lacunar strokes (15 in the antioxidant-treated group and 17 in the control group), the NIHSS score on admission was influenced only by the plasmatic fibrinogen (r = 0.489; p = 0.005), while the oxidative stress, evaluated by the MDA levels on day 1, was inversely correlated also with the fibrinogenemia (r = - 0.355; p = 0.04).

In the following 7 days after stroke onset, the control patients showed a significant rise of the MDA levels on day 3 with the maintenance of similar values on day 7, while in the antioxidant-treated group of patients there was a decline of the MDA levels on day 3 with similar levels measured on day 7 (Table 2, Fig. 3). The differences between the two groups were found to be statistically significant (p = 0.02). The serum SOD activity exhibited a similar dynamics with the serum MDA levels.

The NIHSS score at discharge was also significantly correlated with the fibrinogen levels (r = 0.372; p = 0.04).

As for the efficacy of the antioxidants in acute microangiopathic strokes, the outcome of the 2 patient groups did not show statistically significant differences (mean NIHSS scores of 5.44 on admission and 1.33 at discharge in the antioxidant-treated group, as compared to 6.31 and 2.75 in the control group, respectively) (Fig. 4, 5).

3. The degree of the neurological impairment of cardioembolic strokes on admission, mirrorred by the NIHSS score, was not significantly tied to any of the tested parameters.

Antioxidant treatment in cardioembolic strokes clearly diminished the rising tendency of serum MDA on days 3 and 7 (Table 3, Fig. 5). The

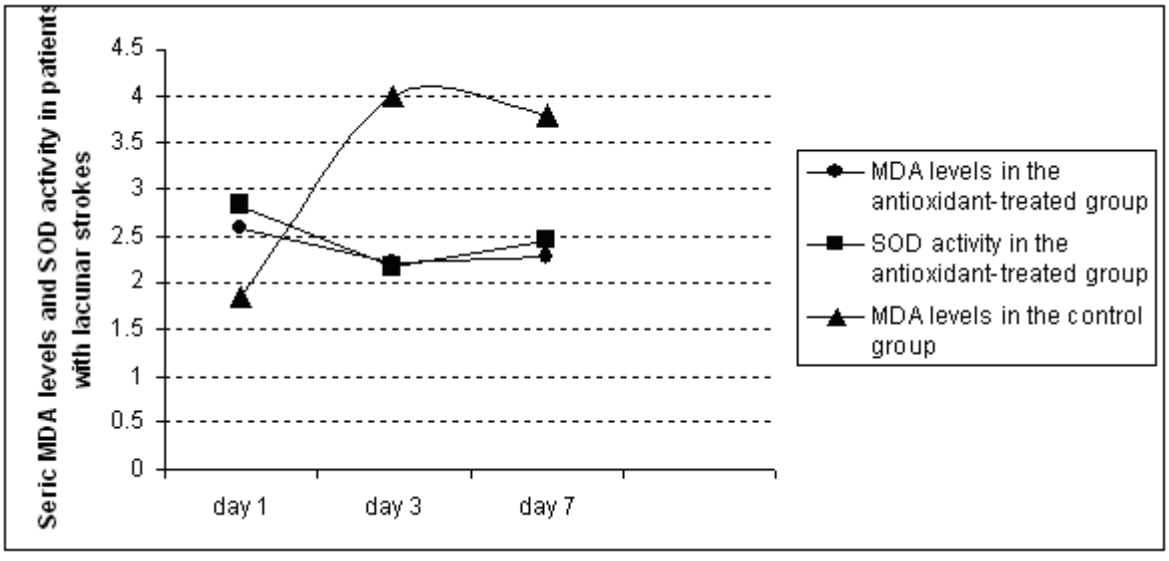

Figure 3
The mean serum MDA levels (in ng/mL) and SOD activity (in µg/mL), in dynamics, in patients with lacunar strokes.

Group		Minimum	Maximum	Mean	Standard deviation
Antioxidant-treated patients	NIHSS on admission	0	14	5.44	3.10
	NIHSS at discharge	0	5	1.33	1.50
	Barthel index on admission	55.0	100.0	79.063	13.689
	Barthel index at discharge	80	100	96.33	6.11
	Serum MDA on day 1	1.00	7.30	2.5813	1.8770
	Serum MDA on day 3	.80	4.20	2.2125	.8973
	Serum MDA on day 7	.80	4.10	2.2733	.8163
	SOD activity on day 1	.50	8.20	2.8286	2.5578
	SOD activity on day 3	.90	5.20	2.1615	1.3991
	SOD activity on day 7	.30	5.50	2.4417	1.6522
Control patients	NIHSS on admission	3	12	6.31	2.77
	NIHSS at discharge	0	10	2.75	2.98
	Barthel index on admission	60.0	90.0	79.688	9.031
	Barthel index at discharge	60	100	91.88	12.09
	Serum MDA on day 1	.50	6.20	1.8500	1.3736
	Serum MDA on day 3	1.40	8.90	4.0063	2.6032
	Serum MDA on day 7	1.60	8.30	3.8083	1.9552

Table 2
Evaluation of the clinical course and parameters of oxidative stress in patients with lacunar stroke.

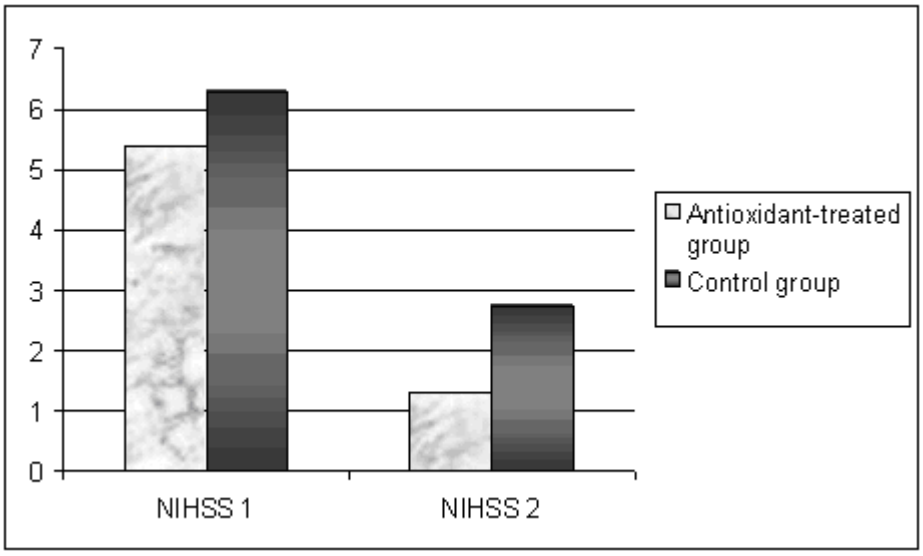

Figure 4
The neurological deficit, measured on the NIHS scale, on admission (NIHSS 1) and at discharge (NIHSS 2) in the two groups of patients with lacunar stroke.

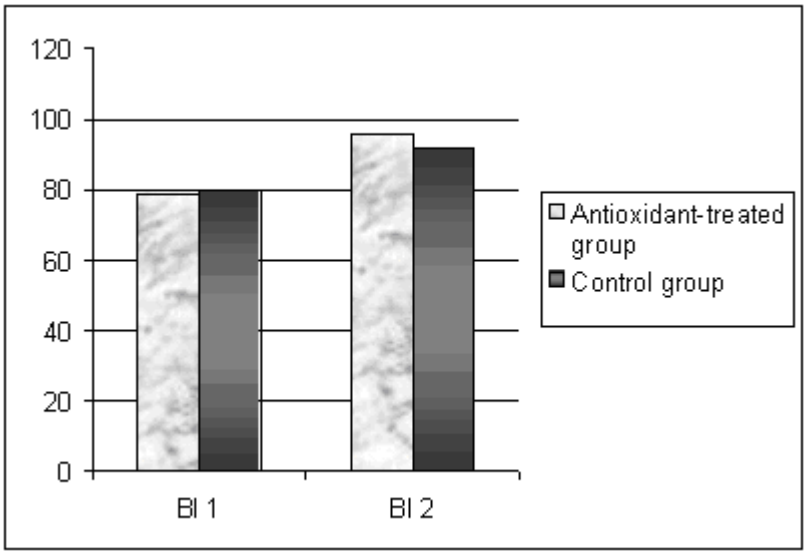

Figure 5
The functional status, evaluated based on the Barthel index, on admission (BI 1) and at discharge (BI 2) in the two groups of patients with lacunar stroke.

differences between the two groups were statistically significant (1.98 ng/ml on day 3 and 2.67 ng/ml on day 7 in the antioxidant-treated group as compared to 2.94 ng/ml and 4.71 ng/ml respectively in the control group; p = 0.05, and p = 0.001 respectively). The serum SOD activity exhibited a declining trend on the three successive measurements.

In terms of clinical course, although the antioxidant-treated group of patients had more severe neurologic deficits on admission (table III), they showed a more favorable outcome, being discharged with a mean NIHSS score of 3.9 and BI of 85 as compared to 4.5 and 86,9 respectively in the conventionally treated patients. Even if the differences between these scores taken separately did not reach statistical significance, the drop on the NIHSS scale and the improvement of the Barthel index in the two groups of patients were statistically significant (p = 0.037 and, respectively, p = 0.014) (Fig. 7 and 8).

DISCUSSION

A number of papers emphasize the important role free radicals play in the pathogenesis of ischemia-reperfusion injuries (19). The nervous system is particularly vulnerable to oxidative stress and ROS mediated injury for a number of biochemical, physiological, and anatomical reasons (20,21,22,23):

- a high rate of oxidative metabolic activity;
- high concentration of oxidisable substrate, in particular membrane lipid polyunsaturated fatty acids
- large area of cell membranes compared to the cytoplasmic volume

Group		Minimum	Maximum	Mean	Standard deviation
Antioxidant-treated group	NIHSS on admission	4	25	11.65	6.38
	NIHSS at discharge	0	17	3.93	4.80
	Barthel index on admission	10	100	60.294	27.809
	Barthel index at discharge	20	100	85.00	21.91
	Serum MDA on day 1	.30	3.20	1.7000	.8025
	Serum MDA on day 3	.40	3.90	1.9813	.8901
	Serum MDA on day 7	1.40	4.60	2.6769	.8992
	SOD activity on day 1	.80	8.70	3.4188	1.7562
	SOD activity on day 3	.80	5.60	2.7562	1.3952
	SOD activity on day 7	.90	5.00	2.3077	1.2066
Control group	NIHSS on admission	2	24	9.83	6.62
	NIHSS at discharge	0	15	4.56	4.13
	Barthel index on admission	20	95	71.944	18.242
	Barthel index at discharge	40	100	86.94	15.45
	Serum MDA on day 1	.40	8.20	2.1941	1.7862
	Serum MDA on day 3	.80	7.70	2.9444	1.8072
	Serum MDA on day 7	1.40	8.50	4.7118	1.9163

Table 3
The parameters of the clinical course and oxidative stress in the 2 groups of patients with cardioembolic stroke.

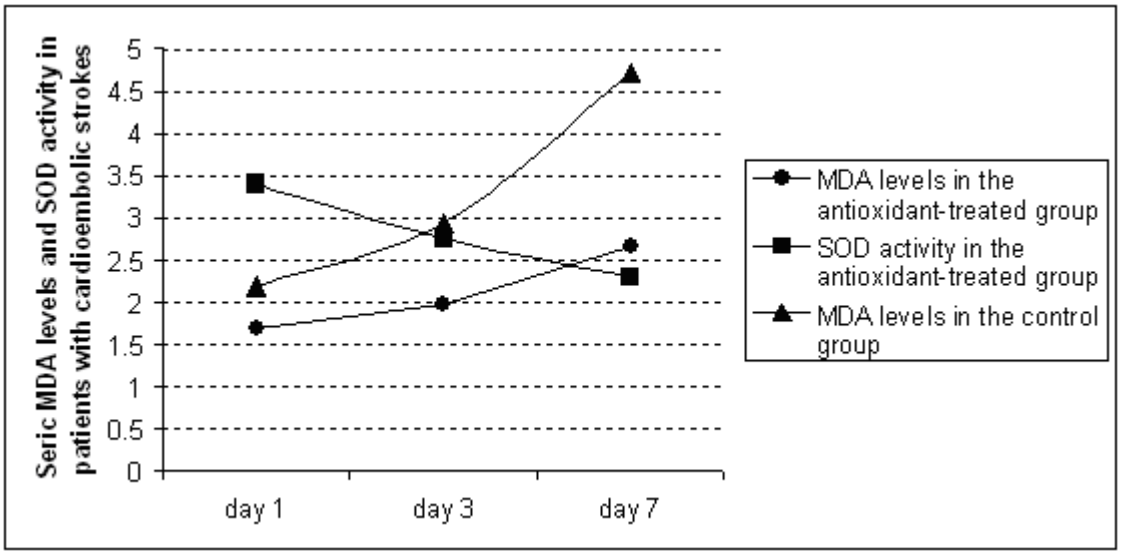

Figure 6
The dynamics of serum MDA levels (in ng/mL) and SOD activity (in µg/mL) in patients with cardioembolic stroke treated with antioxidants.

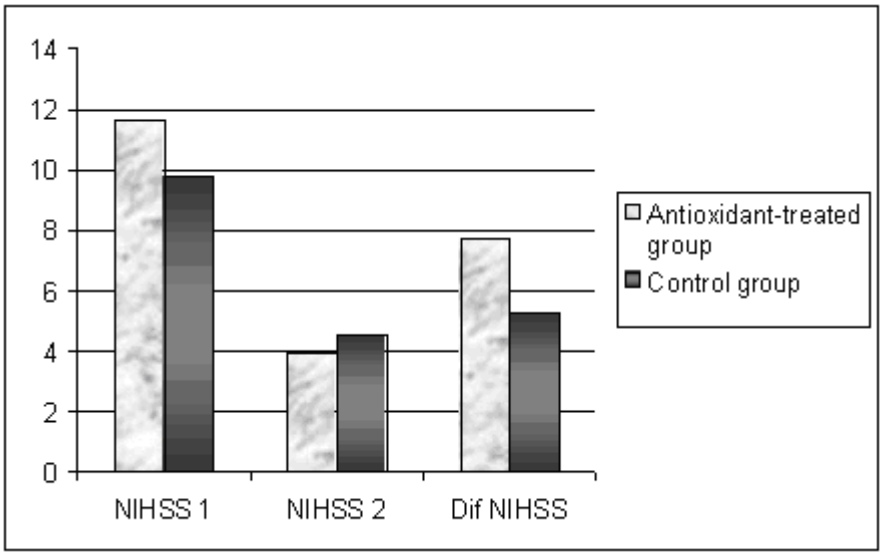

Figure 7
The outcome of patients with cardioembolic stroke treated conventionally and with antioxidants, assessed with the Barthel index. BI 1 = Barthel index on admission; BI 2 = Barthel index at discharge; Dif BI = mean improvement on the Barthel index scale.

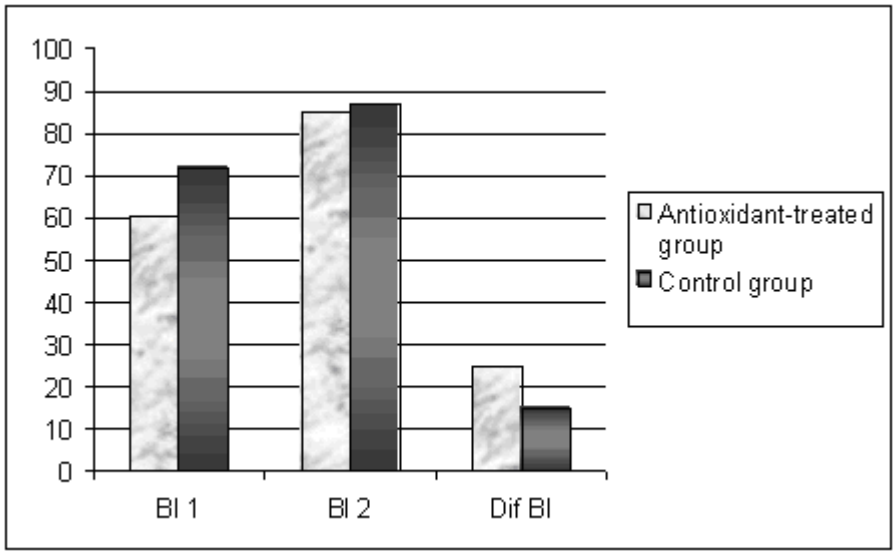

Figure 8
The improvement of the neurologic deficit, measured on the NIHS scale, in the two groups of patients with cardioembolic stroke. NIHSS 1 = NIHSS score on admission; NIHSS 2 = NIHSS score at discharge; Dif NIHSS = mean improvement of the neurological deficit on the NIHS Scale.

– endogenous generation of ROS by specific neurochemical reactions, for example, dopamine oxidation
– low level of antioxidant enzymes, mainly catalase and glutathione peroxidase
– high levels of iron and ascorbate, and low concentrations of transferrin and ceruloplasmine

In the present series of stroke patients, the increasing oxidative stress caused by acute cerebral ischemia was demonstrated by the rise in the serum MDA levels on days 3 and 7 after stroke onset, a finding consistent with other reports (3,24). In addition to these findings, a distinct course of the MDA as a marker of oxidative stress characterized each stroke subtype (25). The most spectacular and constant rise of the MDA levels was found in cardioembolisms, probably in relation with the high rate of spontaneous recanalisation and reperfusion that characterize this stroke subtype. In the other etiopathogenic subtypes of acute focal cerebral ischemia the generated ROS could contribute to the increase in the hemorrheological disturbances described in the microcirculation of these patients (26), especially since the severity of the macroangiopathic and lacunar strokes was found to be related to fibrinogenemia, which is known to increase the plasma viscosity. Further, the correlation of the NIHSS score on admission with the HDL- and LDL-cholesterol levels may reflect the role of these lipids in the generation of the atherosclerotic lesions that promote macroangiopathic strokes.

The currently available antioxidant treatment, well tolerated by all patients, improved recovery mainly in cardioembolic strokes, but also in strokes caused by large vessel disease. In these patients, the clinical improvement was probably due to the reduction of oxidative stress because a separate and distinct course of the MDA levels was also recorded. In lacunar strokes, although the antioxidant treatment reduced the oxidative stress, it did not influence significantly the clinical course. This is presumably due to the limited amount of cerebral tissue involved in the ischemic damage. Still, in this setting, antioxidants in long-term use might prevent recurrences and clinically silent infarcts.

In analyzing the plasmatic activity of SOD, strokes caused by macroangiopathy and small vessel disease exhibited lower values at the onset of symptoms than cardioembolisms. This might be due to increased oxidative stress related to the vascular risk factors associated with the first two stroke subtypes. In the first 7 days following stroke onset, the ECSOD activity dropped in all stroke subtypes in spite of exogenously administered antioxidants. Although the regulation of SOD by oxidative stress is not yet completely defined, it has been demonstrated that conditions that increase oxidative stress do not induce the enzyme production (27) but rather they reduce extracellular SOD expression as part of a general cytotoxic effect, at least in human dermal fibroblasts (28). Furthermore, SOD activity might decrease because of excessive consumption while neutralizing the free radicals, or the enzyme

might be directly damaged and inactivated by the reactive oxygen species (29).

In conclusion, antioxidant treatment may be an efficient therapeutic option for cardioembolisms and macroangiopathic strokes, contributing to the improvement of the neurological deficits and functional status of the patients through the reduction of oxidative stress following ischemia and/or reperfusion. In view of these effects, it might also prove beneficial in thrombolysed patients.

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