THE OUTCOME OF CARDIOEMBOLIC STROKE IS IMPROVED WITH ANTIOXIDANT TREATMENT: A CLINICAL STUDY

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ABSTRACT

Oxidative stress has a major role in the pathogenesis of ischemic and mainly of reperfusion injuries in many organs, including the brain. This is the reason why several researches were dedicated to finding efficient antioxidant therapies capable of reducing the extent of the lesions. The present paper shows that alpha-lipoic acid administered early in cardioembolic strokes can improve the functional outcome of the patients by reducing the oxidative stress.
Key words: cardioembolic stroke, oxidative stress, MDA, SOD, alpha-lipoic acid

Selected abbreviations: CT – computerized tomography; EKG – electrocardiogram; eNOS – endothelial nitric oxide synthase; HMG-CoA reductase – hydroxi methyl glutaryl CoA reductase; iNOS – inducible nitric oxide synthase; MDA – malondialdehyde; MRI/MRA – magnetic resonance imaging/ magnetic resonance angiography; NADH – reduced nicotinamide dinucleotide; NADPH – reduced nicotinamide dinucleotide phosphate; NIHSS – National Institute of Health Stroke Scale; NF-?B – nuclear transcription factor; ROS – reactive oxygen species; SOD – superoxide dismutase.

INTRODUCTION

Cardiogenic cerebral embolism is recognized increasingly as an important cause of stroke. Approximately 15-31% of strokes come from (or through) the heart (1, 2). The clinical features of cardioembolic stroke include the following:

- Decreased level of consciousness at onset of stroke
- The symptoms and signs are maximal at onset
- The major hemispheric deficits recover rapidly („spectacular shrinking deficit") due to early lysis of the embolus and reperfusion of the ischemic brain tissue
- Frequently the onset of symptoms follows a Valsalva-provoking activity
- The symptoms usually reflect involvement of different vascular territories of the brain
- The cortical deficits (aphasia or visual field defects) indicate the involvement mainly of the middle cerebral arteries and the posterior cerebral arteries or their branches

The diagnosis of cardioembolic stroke is based on:

a. identification of a cardiac source of emboli
b. absence of other likely causes of focal cerebral ischemia and
c. supportive clinical features that have been described above

The cardiac disorders that may lead to brain embolism carry distinct embolic risk, so dividing the cardiopathies into major- and minor-risk categories is clinically useful when deciding the patient's treatment (in the acute setting, as well as secondary prevention) (3).

Clinically and pathologically, cardioembolic strokes are characterized by larger areas of infarcted tissue (due to the considerable size of the embolus), a more serious clinical picture, a 1.5-1.7 times higher mortality rate as compared to non-embolic strokes (4), and a high rate of spontaneous recanalisation. Angiographic controlled studies in humans have shown that spontaneous recanalisation can occur in around 17% of the cases within the first 6 to 8 hours of stroke and that approximately half of the vessels will reopen in 3 to 4 days (5).

Several research papers highlighted the importance of the excessive free radical generation in the penumbral area of the infarcted tissue, the oxidative stress being much increased following reperfusion (6, 7). Free radicals are produced in high concentration from partially damaged mitochondria that are exposed to oxygen (8), from the activation of phospholipase A_2 by lipid peroxidation and the breakdown of lipid membranes, as well as from the free fatty acid metabolism via the ciclooxygenase pathway and the metabolism of adenine nucleotides and purines via the xanthine oxidase pathway (9). Moreover, moderate

increases in intracellular calcium lead to induction of inducible nitric oxide and subsequent peroxynitrate production (9, 10), while free radicals also directly affect proteins, especially membrane channels, leading to exacerbation of high intracellular calcium and inhibition of mitochondrial respiratory chain enzymes. Finally, ischemia also damages the brain's capillaries and endothelium and incites an inflammatory response whereby white blood cells infiltrate regions of infarct. Neutrophils are neurotoxic in several ways, including generation of free radicals from NADPH oxidase, nitric oxide production from iNOS within neutrophils, and formation of arachidonic acid leading to more free radical formation (11). The generated free radicals cause a loss of cell membrane fluidity (9), disturb the electrolyte balance mainly by altering the membrane channels (9), interfere with the regulation of vascular smooth muscle cells (12), and may inactivate a series of enzymes through peroxidation, as well as cause structural DNA lesions (13).

Therapeutic strategies aimed at decreasing brain injury have included the use of antioxidant drugs like vitamins, coenzyme Q, ebselen, or spin-trapping agents (e.g. NXY – 059). Moreover, drugs that are already recommended by guidelines to be used in the treatment of acute stroke, like the HMG-reductase inhibitors (14, 15), the angiotensin conversion enzyme inhibitors, and the angiotensin receptor blocking agents (16, 17), have also antioxidant effects described.

SUBJECTS AND METHODS

The study was performed on two series of consecutive ischemic stroke patients admitted to the Clinical Hospital of Neurology and Psychiatry Oradea, of which the patients with cardioembolic stroke were selected. The first group of patients hospitalized between May 1st and August 31st 2006 in the Neuro IIIB ward received only the conventional treatment and made up the control group, while the second group of patients, admitted between March 1st and May 31st 2007 to the Neuro IC and IIIB wards received antioxidants in addition to the current treatment. Strokes of other determined etiology as well as those of undetermined etiology (two or more causes identified, negative or incomplete evaluation) were excluded. Written informed consent was obtained from each patient.

Demographic data (age, gender), the time frame from symptom onset to hospital admittance, data regarding prior medication were recorded for each patient. The neurological deficit was scored on the NIHS scale and the functional status evaluated based on the Barthel index on admittance and at discharge. The evaluation protocol included a 12-channel electrocardiography, transthoracic echocardiography, an emergency CT scan based on which selected cases underwent MRI/MRA, carotid and vertebral artery ultrasound evaluation, and several hematological and biochemical tests. Each patient had serum samples collected on days 1, 3, and 7 from stroke onset which were frozen and stored until the malondialdehyde was measured. The patients who received antioxidant treatment had also samples for the evaluation of SOD activity collected. The MDA and SOD measurements were performed by researchers blinded to the stroke subtype and clinical status of the patients.

Patients were treated with full-dose, unfractionated, intravenous heparin or low molecular weight heparin, followed by oral anticoagulants, HMG-CoA reductase inhibitors, a means of 10 mL of a peptide porcine brain extract (Cerebrolysin®, Ebewe Austria) given intravenously for 10 days in view of its proven neuroprotective-neuroplastic properties, angiotensin conversion enzyme inhibitors ± other classes of blood pressure lowering drugs, cardiotonics, antiarrhythmics, and nitrates as needed, and the antioxidant-treated group also received daily 600 mg alpha-lipoic acid in i.v. infusion for 7 days.

Statistical analyses were performed with the SPSS (Statistical Package for Social Sciences) for Windows version 10.0 soft (18), using the t student test, and correlation analyses were run by means of the Pearson test. Statistical significance was defined as $p < 0.05$.

RESULTS

In the control group cerebral embolisms occurred in 18 cases (34.6%), while in the antioxidant-treated group we found 15 cases of cardioembolic strokes (23%).

Patients in the control group were aged between 47 and 85 years (mean age – 69.9 years) and had a NIHSS score and Barthel index on admittance varying between 2 and 24 (mean 9.8) and 20 and 95 (mean 71.9) respectively. They were discharged with NIHSS scores between 0 and 15 (mean 4.5) and Barthel indices varying between 40 and 100 (mean 86.9).

The patients in the antioxidant-treated group were slightly older, their age varying between 46 and 96 years (mean 72.6 years), and had more serious conditions on admission (NIHSS scores varied between 4 and 25, and Barthel indices between 10 and 95) (Table 1), but because of the wide range of these parameters, the differences did not reach statistical significance. On discharge, this group of patients had similar neurological and functional status, with NIHSS scores between 0 and 17, and Barthel indices between 20 and 100 (Figure 1, 2). Alpha-lipoic acid was well

tolerated by all patients, without any significant side effects.

Comparing these data, it can easily be observed that patients who received also antioxidants, although in worse condition on admission, showed a better neurological and functional outcome than the conventionally-treated group, dropping 8.9 points on the NIHS Scale and gaining 28.9 points on the Barthel index as compared to 5.2 and 15 points respectively, a statistical significant difference, $p < 0.05$ (Table 1, Figure 3, 4).

The successive measurements of malondialdehyde on days 1, 3, and 7 showed a steady rise of this compound in embolic strokes. Antioxidants attenuated

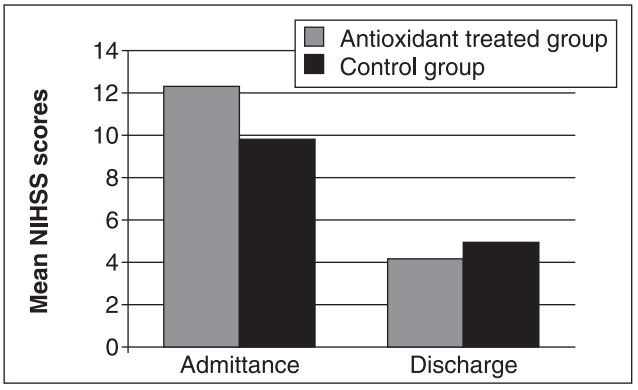

Figure 1

The NIHSS scores of the two groups of patients with cardioembolic strokes on admittance (NIHSS 1) and at discharge (NIHSS 2).

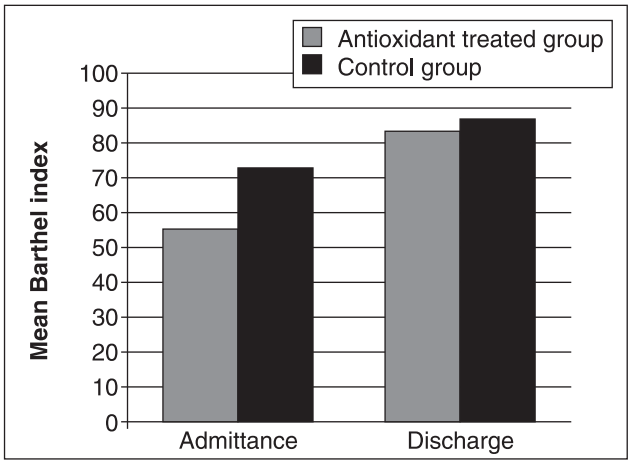

Figure 2

The Barthel index of the two groups of patients with cardioembolic strokes on admittance (BI 1) and at discharge (BI 2)

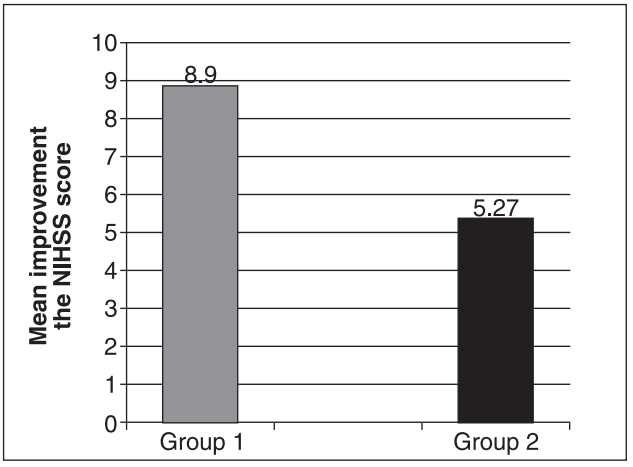

Figure 3

The mean difference of the NIHSS scores in the two groups

Table 1

Descriptive statistics of the parameters recorded in the two groups of patients with cardioembolic strokes

Parameters	Group	Mean	Standard deviation	Standard error of mean
Age	patient	72.67	13.05	3.37
	control	69.61	11.12	2.62
Time from stroke onset to admittance	patient	9.733	17.549	4.531
	control	9.167	8.198	1.932
NIHSS score on admission	patient	12.27	6.52	1.68
	control	9.83	6.62	1.56
NIHSS score at discharge	patient	4.07	4.95	1.32
	control	4.56	4.13	.97
Barthel index on admission	patient	57.333	28.213	7.284
	control	71.944	18.242	4.300
Barthel index at discharge	patient	85.00	22.68	5.86
	control	86.94	15.45	3.64
Serum MDA on day 1	patient	1.7786	.8116	.2169
	control	2.1941	1.7862	.4332
Serum MDA on day 3	patient	2.0929	.8983	.2401
	control	2.9444	1.8072	.4260
Serum MDA on day 7	patient	2.7500	.8980	.2592
	control	4.7118	1.9163	.4648
Improvement on the NIHS Scale	patient	8.9231	4.7163	1.3081
	control	5.2778	4.2538	1.0026
Improvement on the Barthel index	patient	28.9286	21.5887	5.7698
	control	15.0000	5.9409	1.4003

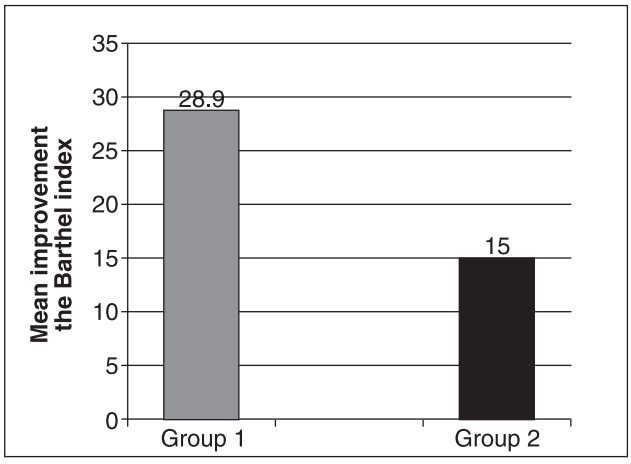

Figure 4
The mean improvement of the functional status of the two groups, evaluated based on the Barthel index

this incremental trend: the levels recorded on day 1, indicating the „baseline oxidative stress" associated with the various risk factors, did not differ between the two groups, on day 3 the antioxidant-treated group had non-significantly lower levels of MDA ($p = 0.093$), while and on day 7 the difference became highly significant ($p = 0.003$) (Table 2, Figure 5).

The serum SOD activity exhibited an opposite trend, dropping on the successive measurements in the group of patients receiving antioxidants (Table 2). The correlation analysis revealed a strong inverse relation between the MDA levels and SOD activity on day 3 (Pearson coefficient $= -0.567$, $p = 0.043$), and a weaker association on day 7 (Figure 6).

As for the therapeutic window of the antioxidant, the small number of cases analyzed did not permit to draw definite conclusions, although a tendency toward a better outcome was identified in those patients receiving the treatment in the first 12-hour time frame.

DISCUSSIONS

Concordant with previous studies, which showed a higher incidence of cardioembolisms in the young (< 50 years), due mainly to valvular diseases, and in the very old (> 75 years) individuals, due to the escalating incidence of atrial fibrillation, in the present study patients with cardioembolic strokes had a high mean age and important neurological deficits on admittance, but with early treatment some patients made a remarkable recovery.

As already mentioned, embolic strokes have a high rate of spontaneous recanalisation, and, although oxidative stress has been shown to be involved in causing ischemia-reperfusion injuries, this pathogenic pathway is to date not covered by the current treatment guidelines in spite of some antioxidant properties described for drugs used in acute stroke treatment. For example, the HMG-CoA reductase inhibitors activate the phosphatydyl- inosytol-3-kinase pathway, which activates eNOS, they increase eNOS activity, diminish the NAD(P)H oxidase activity (possibly due to diminishing NADPH oxidase isoprenilation), and reduce the generation of superoxide and other free radicals (15, 19, 20). Another group of drugs shown to exhibit „vascular protective" actions are the angiotensin converting enzyme inhibitors, which also decrease NAD(P)H oxidase activity (21) and stimulate the endothelial production of nitric oxide (22).

Alpha-lipoic acid diminishes lipid peroxidation (23), illustrated in the present study by the decreased levels of MDA in the sera of patients who received this antioxidant. Moreover, alpha-lipoate scavenges the hydroxyl radicals, recycles other antioxidants, like vitamin C and E, increases the intracellular glutathione concentration, and regulates the activity of the nuclear transcription factor NF-ηB (24). Early administration of alpha-lipoic acid reduces oxidative stress and contributes to a more favorable neurological and functional outcome of embolic stroke patients, as shown in the present study. Since the antioxidant-treated group of patients had a higher mean age and the rest of the treatment was similar in both groups, the most plausible explanation for the better outcome is the beneficial effect of the antioxidant treatment.

The rise of the MDA levels in the sera of patients with cardioembolic strokes demonstrates the

Table 2
The serum MDA levels and SOD activity recorded in the two groups of patients

Group	Parameter	Minimum	Maximum	Mean	Standard deviation
pacient	Serum SOD seric on day 1 (in μg/mL)	2.20	5.00	3.4385	.7709
	Serum SOD seric on day 3	1.40	5.10	2.9077	1.0210
	Serum SOD seric on day 7	.90	5.00	2.1500	1.0353
	Serum MDA on day 1 (in ng/ml).	.30	3.20	1.7786	.8116
	Serum MDA on day 3	.40	3.90	2.0929	.8983
	Serum MDA on day 7	1.40	4.60	2.7500	.8980
control	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

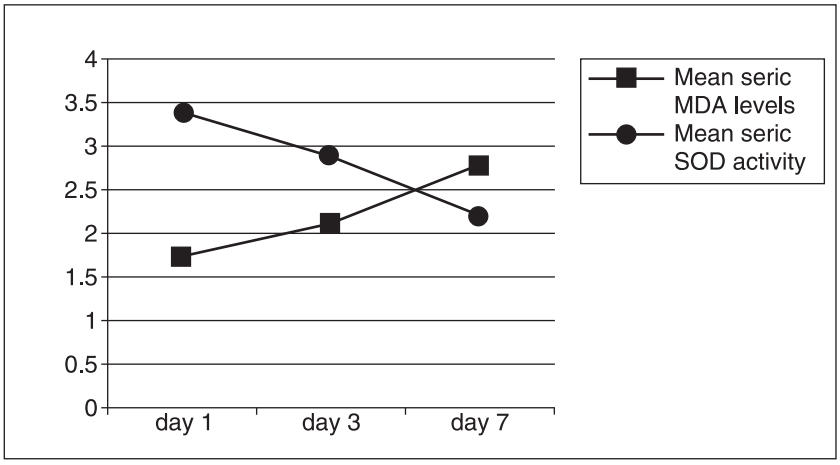

Figure 5
The 2 groups of patients with cardioembolic strokes show distinct trends of the MDA levels (in ng/mL), supporting the reduction of the oxidative stress with alpha-lipoic acid.

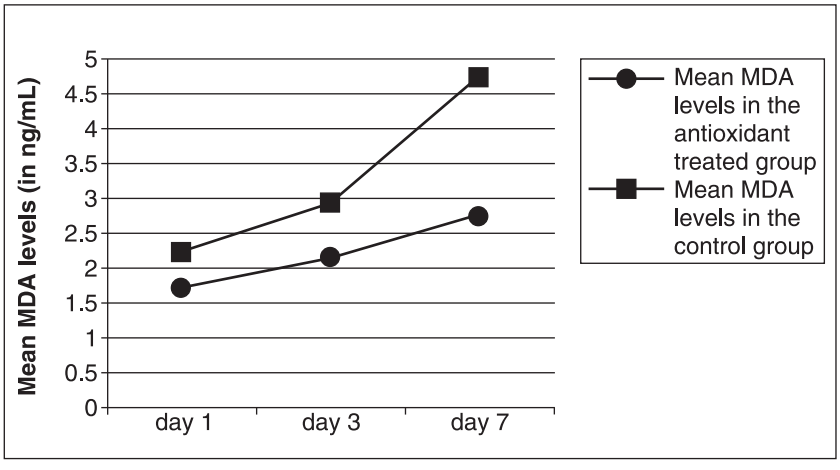

Figure 6
The dynamics of the serum MDA levels (in ng/mL) and serum SOD activity (in µg/mL) in the antioxidant-treated group of patients with cardioembolic strokes.

„respiratory burst" which follows reperfusion and which is insufficiently controlled with our current therapeutic means. The decremental trend of SOD activity may be due to the excessive turn-over in the attempt to neutralize the free radicals, as well as to the down-regulation of SOD by the generated ROS (25).

Studies of SOD activity in acute stroke either did not show any significant disturbances (26), or offered divergent results: 2-3-fold increases in SOD activity as compared to controls (27), or lower serum activities as compared to healthy individuals (28). Similarly, in patients with acute coronary syndromes, the SOD activity was higher than in controls and inversely related to the extent of the infarction (28). In the present series of cases no significant relation between the clinical status and plasmatic SOD activity could be demonstrated, presumably because of the limited number of patients and the therapeutic manipulation of oxidative stress.

In conclusion, alpha-lipoic acid may improve the outcome and functional prognosis of patients with embolic strokes, presumably due to its antioxidant properties.

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