Antioxidant Profile and Early Outcome in Stroke Patients

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Background and Purpose—Experimental studies provide evidence of an association between ischemic stroke and increased oxidative stress, but data in humans are still limited and controversial. The purpose of this study was to investigate the time course of plasma antioxidant changes in ischemic stroke patients.

Methods—Plasma antioxidants, including water-soluble (vitamin C and uric acid) and lipid-soluble (vitamins A and E) compounds as well as antioxidant enzyme activities in plasma (superoxide dismutase [SOD] and glutathione peroxidase) and erythrocytes (SOD), were measured by high-performance liquid chromatography (antioxidant vitamins) and by spectrophotometry (antioxidant enzymes) in 38 subjects (25 men and 13 women aged 77.2±7.9 years) with acute ischemic stroke of recent onset (<24 hours) on admission, after 6 and 24 hours, and on days 3, 5, and 7. Antioxidant levels in patients on admission were compared with those of age- and sex-matched controls.

Results—Mean antioxidant levels and activities in patients on admission were lower than those of controls and showed a gradual increase over time. Patients with the worst early outcome (death or functional decline) had higher vitamin A and uric acid plasma levels and lower vitamin C levels and erythrocyte SOD activity than those who remained functionally stable.

Conclusions—These results suggest that the majority of antioxidants are reduced immediately after an acute ischemic stroke, possibly as a consequence of increased oxidative stress. A specific antioxidant profile is associated with a poor early outcome. (Stroke. 2000;31:2295-2300.)

Key Words: antioxidants ■ outcome ■ oxidative stress ■ stroke

Ischemic stroke is a leading cause of mortality and disability in Western countries, particularly in the elderly. Many studies have assessed or are in the process of evaluating neuroprotective therapies in the acute phase of stroke, but there is a substantial need for further research on agents able to significantly reduce the cerebral damage related to both ischemia and reperfusion. These agents might be particularly important not only in those patients who cannot receive thrombolysis but also in those who, undergoing this type of treatment, are at risk for so-called reperfusion injury.1

Antioxidants have been evaluated as neuroprotective agents in stroke2 since there is evidence supporting the occurrence of a condition of oxidative stress in the brain during ischemia. In experimental studies, an increased free radical generation during cerebral ischemia/reperfusion injury has been shown in vivo using several techniques such as microdialysis, salicylate spin trapping, and electron paramagnetic resonance.3–6 An increase of lipid peroxidation products7,8 and a decrease in tissue antioxidant levels in the brain during ischemia9 have been reported as indirect evidence of oxidative stress. Pharmacological studies in animals showed that antioxidant molecules able to cross the blood-brain barrier, such as polyethylene glycol–conjugated superoxide dismutase (SOD) and catalase10 and lazaroids,11 reduce ischemic cerebral damage. Finally, transgenic mice overexpressing SOD have reduced infarct size compared with wild-type mice,12 while SOD knockout mice have an increased infarct size compared with controls.13

Human studies on stroke and oxidative stress in the brain are still lacking, mainly because of the methodological difficulties in measuring free radical production in the cerebral tissue. Research aimed at evaluating oxidatively modified molecules or antioxidants in blood, urine, or cerebrospinal fluid, however, revealed the evidence of lower plasma vitamin C and vitamin E levels in patients with stroke14,15 as well as a decrease in vitamin C and an increase in thiobarbituric acid reactive substances 2 days after the onset of cerebral ischemia.16 Lower serum SOD activity has been found in acute stroke patients.17 Recently, it has been shown that total peroxyl radical trapping potential of plasma as well as ascorbic acid, α-tocopherol, and protein thiol plasma levels are inversely correlated with neurological impairment after

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Stroke is available at http://www.strokeaha.org
cerebral infarction.\textsuperscript{18} In other studies, however, no differences in vitamin C\textsuperscript{19} or in vitamin A or E\textsuperscript{20} concentrations were found between stroke patients and controls.

With respect to byproducts of lipid peroxidation, malondialdehyde and 4-hydroxynonenal were found to have increased in stroke patients with cardioembolic source,\textsuperscript{21} and higher levels of malondialdehyde were found in subjects with ischemic stroke than in controls.\textsuperscript{18} In another study, however, the urinary excretion of F2-isoprostanes, a specific in vivo byproduct of lipid peroxidation, was found to be increased in stroke patients with cardioembolic source,\textsuperscript{21} and higher levels of malondialdehyde were found in subjects with ischemic stroke than in controls.\textsuperscript{18}

We measured levels and activities of several antioxidants in plasma and red blood cells (RBC) during the first week after acute ischemic stroke to characterize longitudinal changes of antioxidant levels and to verify whether they were associated with stroke severity and early outcome.

### Subjects and Methods

#### Subject Selection

Patients older than 65 years with acute ischemic stroke admitted within 24 hours from the onset of symptoms to the acute geriatric ward of the Perugia University Hospital were consecutively enrolled.

A CT scan of the brain was performed in all patients. Subjects with hemorrhagic stroke, with other neurological diseases, or taking iron or antioxidant vitamins during the months preceding the enrollment were excluded. Control subjects were age- and sex-matched healthy relatives of hospital employees. None of the controls was undergoing pharmacological treatment. All subjects gave informed consent to participate in the study.

On admission, all patients underwent full physical and neurological examinations. Body temperature was measured on admission and daily until discharge. Vascular risk factors, including hypertension, diabetes, and smoking habits, were recorded. Caloric intake in the week before admission and during hospitalization was assessed by a food frequency questionnaire.\textsuperscript{23} The functional status of patients on admission based on Barthel Index (BI)\textsuperscript{24} score was compared with that preceding (2 weeks) and following (1 week) the admission.

Proxy respondents were questioned when the patient was unable to provide all the requested information. On the basis of clinical\textsuperscript{25} and neuroradiological criteria, it was possible to distinguish patients as having lacunar or nonlacunar syndromes (including total anterior, partial anterior, and posterior syndromes). The severity of the neurological deficit was measured by means of the Canadian Neurological Scale (CNS),\textsuperscript{26} administered on admission and after 3, 5, and 7 days.

Patients were divided into 2 groups according to the outcome 1 week after the acute cerebrovascular event: those who died or experienced a decline in functional status measured on the basis of a decrease in the BI score (group W [worse]) and those who did not undergo functional decline, as indicated by a stable BI score (group S [stable]).

In the patient groups, an initial sample of blood was collected in a sodium heparin tube on admission (T1), then after 6 hours (T2), 24 hours (T3), 3 days (T4), 5 days (T5), and 7 days (T6). In the control group, blood was obtained in the morning after an overnight fast. All samples were centrifuged at 1500\textsuperscript{g} for 15 minutes at 4°C, and the plasma and pelleted RBC were stored at \textminus80°C until analysis.

#### Antioxidant Measurements

To preserve vitamin C, an aliquot of plasma was deproteinized with 10% metaphosphoric acid, and the supernatant was kept at \textminus80°C. Vitamin C and uric acid were detected by high-performance liquid chromatography with electrochemical detection according to Kutnik at al\textsuperscript{27} with a Supelco C18 column (250\times4.6 mm ID) and a Supelco C18 guard column (20\times4.6 mm ID).

Vitamin A and vitamin E were measured, after extraction with ethanol and hexane, by high-performance liquid chromatography with UV detection at 280 nm\textsuperscript{28} with a Waters Symmetry C8 column (150\times4.6 mm ID). The levels of the vitamins and of uric acid are expressed as micromoles per liter. Since total, HDL, and LDL cholesterol and triglyceride levels were similar in stroke patients and controls, the concentrations of vitamins A and E were not adjusted for lipids.

SOD (U/mL) and glutathione peroxidase (GPX) (\mu mol/L NADPH/min/mL) activities were measured in plasma according to the methods of L’Abbe and Fisher\textsuperscript{29} and Flohe and Günzler,\textsuperscript{30} respectively. To measure SOD activity in erythrocytes, RBC were hemolyzed with cold distilled water, and extraction was performed.

### Table 1. Demographic Characteristics and Antioxidant Levels (on Admission [T1] and After 1 Week From Stroke Onset [T6]) in Stroke Patients and Controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Stroke Patients (n=38)</th>
<th>Controls (n=37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>77.2±7.9</td>
<td>77.8±8.1</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>25/13</td>
<td>23/14</td>
</tr>
<tr>
<td>Vitamin E, (\mu)mol/L</td>
<td>45.0±8.8 (T1)</td>
<td>45.3±7.6</td>
</tr>
<tr>
<td></td>
<td>54.0±10.3* (T6)</td>
<td></td>
</tr>
<tr>
<td>Vitamin A, (\mu)mol/L</td>
<td>1.9±0.4* (T1)</td>
<td>2.4±0.3</td>
</tr>
<tr>
<td></td>
<td>2.3±0.56 (T6)</td>
<td></td>
</tr>
<tr>
<td>Uric acid, (\mu)mol/L</td>
<td>195.7±67.5* (T1)</td>
<td>276.6±69.0</td>
</tr>
<tr>
<td></td>
<td>244.5±72.38 (T6)</td>
<td></td>
</tr>
<tr>
<td>Vitamin C, (\mu)mol/L</td>
<td>21.4±7.0* (T1)</td>
<td>50.2±16.0</td>
</tr>
<tr>
<td></td>
<td>23.1±9.5* (T6)</td>
<td></td>
</tr>
<tr>
<td>Plasma SOD, U/mL</td>
<td>25.9±7.0* (T1)</td>
<td>31.8±1.0</td>
</tr>
<tr>
<td></td>
<td>28.0±6.21 (T6)</td>
<td></td>
</tr>
<tr>
<td>Plasma GPX, (\mu)mol NADPH/min/mL</td>
<td>0.117±0.03 (T1)</td>
<td>0.125±0.02</td>
</tr>
<tr>
<td></td>
<td>0.135±0.03 (T6)</td>
<td></td>
</tr>
<tr>
<td>RBC SOD, U/g hemoglobin</td>
<td>2289.7±576.3 (T1)</td>
<td>3124.1±251.7</td>
</tr>
<tr>
<td></td>
<td>3514.6±499.5* (T6)</td>
<td></td>
</tr>
</tbody>
</table>

\(\ast P<0.001, \dagger P<0.02 \) vs controls.

\(\ast P<0.001, \S P<0.05, \| P<0.01 \) vs T1.
with an ethanol/chloroform mixture (1:1). SOD activity (U/g hemoglobin) was measured in the supernatant according to the method of Winterbourn and colleagues.31

**Statistical Analysis**

Data are presented as mean±SD, unless otherwise specified. Statistics were performed with the program SigmaStat (version 2.03, 1997; SPSS Inc). Continuous variables were compared by the unpaired t test or the Mann-Whitney test, as appropriate. Prevalences were compared by the χ² test. Correlation analyses were performed by means of the Pearson test. Plasma concentrations of nonenzymatic antioxidants and plasma and RBC activities of antioxidant enzymes over time in groups W and S were compared by 2-way ANOVA. The Tukey test was used for post hoc analyses. Statistical significance was defined as P<0.05.

**Results**

The study population consisted of 75 subjects: 38 patients with acute ischemic stroke admitted within 24 hours from stroke onset (mean time, 8.0±5.7 hours) (25 men and 13 women aged 77.2±7.9 years) and 37 controls (23 men and 14 women aged 77.8±8.1 years). According to the evaluation of stroke outcome, 25 patients were included in group W (10 with a lacunar syndrome) and 13 in group S (12 with a lacunar syndrome). Four patients died, 3 of them with a nonlacunar syndrome. Two of them had a deteriorating stroke, 1 suffered from pulmonary embolism, and another died as a result of worsening of cardiac failure.

The demographic characteristics and antioxidant levels (on admission and on day 7) of stroke subjects and controls are reported in Table 1. Demographic and clinical characteristics of the W and S stroke groups are shown in Table 2. Indices of renal and hepatic function, as well as lipid profile, were within the normal range in all subjects. There were no differences in body temperature between groups. On admission, mean antioxidant levels were lower in patients than in controls. Significance was reached for vitamin C, vitamin A, and uric acid concentrations and plasma SOD activity (P<0.001; Table 1). During the days after the acute event, all antioxidants tended to increase. After 1 week, antioxidant levels and activities were not significantly correlated with CNS or BI scores at any time (Table 3). Patients of group W had a higher degree of neurological impairment than group S patients on admission (CNS score, 4.4 versus 7.9; P<0.001) and at every time thereafter (data not shown). Age, sex, and vascular risk factors did not differ between W and S groups. With respect to antioxidants, subjects in group W had significantly higher levels of vitamin A (Figure 1) and uric acid (Figure 2) and a lower concentration of vitamin C (Figure 2) with lower RBC SOD activity (Figure 3). No differences were observed in vitamin E levels (Figure 1), plasma SOD (Figure 3), and GPX (Figure 4) activities. Basal antioxidant levels and activities were not significantly correlated with early outcome as assessed by BI score after 1 week, with the exception of vitamin A (r=−0.39, P<0.02).

**Discussion**

To our knowledge, this is the first study evaluating the time course of levels and activities of several enzymatic and nonenzymatic antioxidants in stroke patients during the first week after the occurrence of a cerebral infarct. Mean plasma levels of nonenzymatic antioxidants and antioxidant enzyme activities were lower in patients than in healthy controls on admission. These levels gradually increased in the following

![Figure 1. Vitamin E (circles) and vitamin A (triangles) plasma levels (mean±SEM) in patients who worsened (group W, closed symbols) or remained stable (group S, open symbols) in the first week after stroke. Plasma vitamin A levels were significantly higher (P<0.001; 2-way ANOVA) in group W than in group S patients.](image-url)
days, although vitamin C and plasma SOD activity remained lower in patients than in controls, while vitamin E and erythrocyte SOD activity became higher. Moreover, patients with the worst outcome (death or increasing disability after stroke) were characterized by a peculiar antioxidant profile, showing consistently lower RBC SOD activity and vitamin C levels and higher vitamin A and uric acid levels than patients who survived the stroke without experiencing any functional decline.

In our study, the activity of plasma extracellular SOD as well as that of RBC SOD was lower in stroke patients at the time of admission than in controls. Only RBC SOD activity increased after stroke, becoming, after 1 week, even higher than that of controls. Interestingly, RBC SOD activity was lower in patients who died or experienced functional decline after stroke.

Experimental studies performed in transgenic mice over-expressing SOD and in rats treated with exogenous SOD showed that this enzyme plays a protective role toward cerebral damage induced by ischemia. However, reports concerning endogenous SOD level or activity in cerebrovascular ischemia are not consistent: SOD activity and concentration in brain tissue after ischemia/reperfusion have been found to be both decreased and increased, while SOD concentration after stroke was unchanged in serum, increased in cerebrospinal fluid, and increased in both cerebrospinal fluid and plasma in other studies. RBC SOD activity has been found to be unchanged in stroke patients. More recently, extracellular SOD activity was found to be lower in acute stroke patients but increased to the level of controls within 5 days from the cerebrovascular accident. Although the regulation of SOD by oxidative stress is not yet completely defined, it has been demonstrated in rat neurons that conditions that increase oxidative stress do not induce the enzyme production. Oxidative stress reduces extracellular SOD expression in human dermal fibroblasts, likely as a result of a general toxic effect on the cell. Moreover, free radicals can directly damage the enzyme, reducing its activity.

Low vitamin C intake or levels were associated with a higher risk of stroke in epidemiological studies. Vitamin C is a cofactor in several metabolic activities and represents the major water-soluble antioxidant in the human body. In classic experiments it was shown that until all the vitamin is completely consumed, there is neither a significant loss of other antioxidants nor an increase in lipid peroxidation in human plasma. Since our study is cross-sectional, we do not know whether plasma antioxidants were already lower before stroke or decreased after it. However, the antioxidant increase after stroke, mainly observed in functionally stable patients, suggests that cerebral infarction is followed by a reduction of vitamin C levels, perhaps because of increased free radical production. Vitamin C was consistently lower in patients who had the worst outcome, suggesting that it might represent both a prognostic marker in acute ischemic stroke and a possible therapeutic agent in this disease.
Uric acid is an end product of purine metabolism that has significant antioxidant activity. Recently, it has been shown that high uric acid blood levels predict the occurrence of stroke in diabetic patients and that it has a neuroprotective role in both in vitro and in vivo models of cerebral ischemia. High uric acid levels were associated with a worse outcome 1 week after stroke. This might reflect either prestroke levels or an increase after a cerebrovascular event that may be due to an increased purine catabolism occurring in severely ill patients.

Finally, we found that stroke patients had lower levels of vitamin A than controls on admission, reaching plasma concentrations similar to those of controls 1 week after stroke. Moreover, those patients who died or suffered a functional decline had consistently higher levels than those who remained stable. The difference between patient subgroups in vitamin A plasma levels is not due to differences of lipid profile or renal or hepatic function since patients were homogeneous in terms of these parameters. There are very few data concerning vitamin A levels in acute stroke patients. In a previous report, vitamin A plasma levels did not differ between stroke patients and controls, but in another report significantly reduced levels were found in patients. Vitamin A is a lipid-soluble antioxidant that can protect cell membranes from oxidative damage. It is carried in plasma by the retinol binding protein, which is synthesized by the liver and has a short half-life. Retinol binding protein levels decrease during the acute phase response to tissue injury, and this may account for the lower values of vitamin A in the hyperacute phase of stroke. However, since we did not measure retinol binding protein levels in our study, this hypothesis remains to be investigated.

Some limitations of this study should be acknowledged. The lack of data regarding antioxidant levels before stroke onset hinders the possibility of ascertaining whether low antioxidants are a cause or a consequence of stroke. Several epidemiological studies have shown that low levels of vitamin C, vitamin E, and carotenoids in the diet or in blood are associated with an increased risk of stroke. However, since almost all antioxidant levels increased in the days after the stroke, it seems reasonable to assume that at least in part they declined after the cerebral infarct. We did not observe significant changes in dietary intake during hospitalization in patients, thereby excluding the possible influence of diet on differences in antioxidant levels between patients and controls. Moreover, after excluding from the analysis the patients who died and those whose clinical conditions were worse, we obtained identical results.

In conclusion, our longitudinal study of antioxidant levels during the first week after acute ischemic stroke reveals that almost all antioxidants are reduced immediately after a cerebrovascular accident and increase over the following days, suggesting the presence of a condition of oxidative stress in this setting. Furthermore, the finding of a relationship between antioxidant profile and early outcome of the cerebral infarct might provide new insights into the pathogenesis of ischemic stroke as well as open new therapeutic possibilities.

References

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