Oxidative Stress in the Context of Acute Cerebrovascular Stroke

Mohsen Muhammad Hussein El Kossi, MBBch, MSc, MD; Madeha Mahrous Zakhary, MSc, PhD

Background and Purpose—Free radical generation and consequent oxidative stress in thrombotic cerebrovascular stroke have a distinctive role in the pathogenesis of ischemic brain injury. One of the potential injurious effects of homocyst(e)ine in occlusive vascular diseases is free radical generation. In the current study, we investigated the status of oxidant stress in the acute phase of thrombotic cerebrovascular stroke and the possible role of homocyst(e)ine.

Methods—We determined levels of plasma homocyst(e)ine, lipid peroxide, ascorbic acid, superoxide dismutase, and nitric oxide in 30 patients with thrombotic cerebrovascular stroke within 2 days of the onset of the attack as well as in 22 healthy volunteers of comparable age and gender.

Results—Statistically significant elevation of homocyst(e)ine (P<0.001), lipid peroxide (P<0.001), and nitric oxide (P<0.001) plasma levels were observed in stroke patients compared with healthy controls. On the other hand, the antioxidant ascorbic acid plasma levels were significantly lower in the patient group compared with healthy control subjects (P<0.001). Meanwhile, superoxide dismutase plasma levels were not statistically different in either groups. The study also revealed a significant and strong positive correlation between homocyst(e)ine and lipid peroxide (r=0.85, P<0.001). Ascorbic acid plasma levels were significantly negatively correlated with both homocyst(e)ine (r=−0.875, P<0.001) and lipid peroxide (r=−0.576, P<0.001). The nitric oxide level was positively correlated with superoxide dismutase (r=0.396, P<0.05).

Conclusions—We conclude that hyperhomocyst(e)inemia is a possible causal factor in free radical generation during the acute phase of thrombotic cerebrovascular stroke. Pharmacological intervention could potentially be beneficial in this setting and warrants further evaluation. (Stroke. 2000;31:1889-1892.)

Key Words: free radicals ■ homocyst(e)ine ■ nitric oxide ■ stroke, ischemic

In the past few years, the identification of many molecules that participate in neuronal death and particularly in apoptosis, has shed light on the pathogenesis of ischemic brain injury. Oxidative stress is probably one of the mechanisms involved in neuronal damage induced by ischemia-reperfusion, and the antioxidant activity of plasma may be an important factor providing protection from neurological damage caused by stroke-associated oxidative stress.1 The mechanisms that participate in the development of oxidative stress in such cases are not completely defined. Homocystinuria, since its discovery in 1962, has highlighted the significance of homocyst(e)ine (Hcy) in vascular disease in general. Hyperhomocyst(e)inemia (Ht Hcy) is now recognized as a common risk factor for thrombotic events such as myocardial infarction and venous thrombosis.2–5 The exact mechanism(s) responsible for this association is still under investigation. Endothelial cell injury through oxidative stress might be one of these mechanisms,6,7 and the vasodilator nitric oxide (NO) is a possible target.8

This study was designed to evaluate the potential relationship between plasma levels of Hcy and those of lipid peroxide, NO, and the antioxidants superoxide dismutase (SOD) and ascorbic acid in patients who sustained thrombotic cerebrovascular stroke.

Subjects and Methods

Thirty patients with cerebral infarction were enrolled in this study in the Department of Internal Medicine, Assiut University Hospital, Assiut, Egypt. Patient characteristics are presented in Table 1. Patients with diabetes mellitus, renal impairment, or embolic cerebral infarction were excluded from the study. Each participant was subjected to thorough history taking, clinical examination, transeophageal echocardiographic imaging to exclude cardiac sources of embolization, as well as cranial CT scan to establish the radiological diagnosis of brain infarction. Serum urea, creatinine, and blood sugar were evaluated for all participants (Table 2).

Heparinized fasting venous blood samples were taken on the second day of the attack for determination of plasma levels of Hcy, lipid peroxide, SOD, ascorbic acid, and NO. Plasma was separated and kept in aliquots at −70°C. Results from the patient group were compared with those obtained from 22 healthy subjects of comparable age and gender. Characteristics of the control group are shown in Table 1. Informed consent was obtained from all participants or their relatives.

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Homocyst(e)ine was estimated as described in Dudman et al, whereas ascorbic acid was determined by the 2,4 dinitrophenylhydrazine method. Lipid peroxide was evaluated as the thiobarbituric acid reactive substances, according to the method of Satoh. Plasma SOD was measured according to the method of Misra and Fridovich, which depends on the ability of the enzyme to inhibit autooxidation of epinephrine in alkaline medium. Nitric oxide was evaluated through its oxidation products nitrates and nitrites, because it has a very short half-life and its overflow in the brain circuits renders it rapidly oxidizable by hemoglobin.

Results are expressed as mean±SD. For statistical analysis, the Student’s t test and Pearson correlation coefficient were utilized for normally distributed values and the Mann-Whitney test and Spearman rank correlation coefficient for skewed ones. A value of P<0.05 was considered significant.

### Results

As shown in Table 3, Hcy lipid peroxide and NO plasma levels were significantly higher, while ascorbic acid plasma levels were significantly lower among stroke patients compared with control subjects.

Superoxide dismutase did not reveal any significant difference between stroke patients and healthy controls (P>0.05; Table 3).

A statistically significant negative correlation was observed between Hcy and ascorbic acid levels (r=-0.7855, P<0.001). The latter also revealed statistically significant negative correlation with lipid peroxide plasma levels (r=-0.576, P<0.001). Lipid peroxide and Hcy plasma levels were significantly positively correlated (r=0.85, P<0.001). A statistically significant positive correlation, albeit weaker, was also found between NO and SOD (r=0.3964, P<0.05).

There was no statistically significant correlation between duration of hypertension, patient age, and smoking index (duration of smoking x number of cigarettes/d) with Hcy plasma levels.

### Discussion

A large body of experimental research indicates that the generation of free radicals leading to oxidative stress plays an important role in the pathogenesis of ischemic brain injury. Brain tissues may be especially prone to the deleterious effects of free radicals for a number of reasons. The brain cellular membrane lipids are very rich in polyunsaturated fatty acid side chains, which are especially sensitive to free radical attack. In addition, the brain is poor in catalase activity and has only moderate amounts of SOD and glutathione peroxidase. Hyperhomocyst(e)inemia has been implicated as a contributory factor in the pathogenesis of such oxidative stress. The detrimental effect of Ht Hcy in the setting of acute thrombotic stroke seems to be multifactorial, involving the coagulation system rendering it more coagulable and the elaboration of excitotoxic neurotransmitters such as homocysteic acid and cysteine sulfenic acid, leading to neuronal death.

Our study revealed a significant increase in fasting plasma levels of Hcy in patients compared with control subjects. This finding concords with previous reports. On the other hand, Delport et al denied the role of Ht Hcy as a primary initiating factor in a restricted group of stroke patients (black individuals). They reported that Ht Hcy might be partially caused by renal insufficiency. Of note, our patients had normal renal function. Such observation cannot be generalized, as their patients were a pure ethnic group with their own specific genetic pattern and differing etiology, including a high percentage with hypertensive strokes. The timing of their specimen collection relative to ischemic attack is a factor that should be taken into consideration during interpretation of the result. Lindgren et al reported raised plasma Hcy levels in the convalescent phase compared with the acute phase in stroke patients. They did not find a significant difference in plasma Hcy in the acute phase of stroke (mean, 2 days after stroke onset) compared with healthy controls.

The oxidative stress in our work has been suggested by the significant elevation of lipid peroxide that derives from the autooxidation of membrane polyunsaturated fatty acids, the significant elevation of NO as well as the significant reduction of the antioxidant ascorbic acid in patients compared with controls. Such an observed increase in lipid peroxide in our patients matches the observation of Sharp et al. Imre et al have reported an increase of the lipid peroxidation capacity of stroke patients’ erythrocytes that might explain the hemorheological disturbances observed in the microcirculation of these patients. The observed positive

### Tables

**Table 1. Demographic and Clinical Data of Patients With Thrombotic Strokes and Controls**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (n=22)</th>
<th>Patients (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean±SD, y</td>
<td>57.5±6.5</td>
<td>59.13±11.7</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>14/8</td>
<td>19/11</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>0</td>
<td>11</td>
</tr>
</tbody>
</table>
correlation between Hcy and lipid peroxide in our study may suggest a possible role of Hcy in the release of reactive oxygen species. On the other hand, we cannot exclude that the acute oxidative stress has contributed to increased Hcy levels.

The significant reduction in patients’ plasma ascorbic acid matches that reported by Sharp et al.27 Many epidemiological studies have suggested that increased ascorbic acid intake may be protective against stroke.29,30 Moreover, ascorbic acid status was reported to be related to the risk of death from stroke.31 Mortality from stroke in elderly people was highest in those with the lowest vitamin C status, whether measured by dietary intake or plasma concentration of vitamin C.31 On the other hand, Keli et al32 did not reveal any association between vitamin C intake and stroke risk.

Ascorbate is considered one of the most important antioxidants in human plasma,29 and it is the first antioxidant to be utilized during lipid peroxidation.34 The significant reduction of ascorbic acid plasma levels in our patients seems to be related to the exhaustion of this antioxidant by the challenge of free radical stress. This possibility is reinforced by the fact that humans are unable to synthesize ascorbic acid from glucose. The observed significant inverse correlation between ascorbic acid and both Hcy and lipid peroxide in our patients gives further evidence of an overwhelming effect of oxidant stress on the ascorbic acid pool.

In keeping with previous studies by Gruener et al35 and Adachi et al,36 we did not find a significant difference between SOD plasma levels of patients and those of controls. On the other hand, Spranger et al37 observed significant reduction in SOD activity within 5 days after a stroke, which reverted to control levels later on.

Unlike ascorbic acid that depends exclusively on an exogenous supply, SOD is regenerated endogenously. This might explain the insignificant difference of patients’ plasma levels compared with those of controls. Moreover, the intracellular fraction of SOD may also participate to a larger extent in the process of free radical scavenging, maintaining the extracellular fraction of SOD without remarkable change.

The significant NO elevation of our patients compared with controls is most probably due to the induction of NO synthase by the effect of thrombotic attack. In accordance with our hypothesis is the observation of Wei and Quast,38 who found an excessive glutamate excitotoxicity, leading to enhanced generation of hydroxyl radicals via a NO-mediated mechanism and resulting in severe ischemia/reperfusion injury. This could be attenuated by the administration of a NO synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME).39 The neurotoxic effect of NO may be mediated by inhibiting mitochondrial metabolism40 or accelerating programmed cell death (apoptosis).40 The vasodilator effect of NO is attenuated in the presence of Ht Hcy. Zhang et al8 observed, in an experimental rat model, that the infusion of Hcy with copper inhibits NO-related vasodilator responses by scavenging of NO. This may be one of the mechanisms by which Ht Hcy predisposes to cerebrovascular diseases. The significant increase in NO levels observed in our study could be a protective mechanism against Ht Hcy to produce s-nitrosohomocysteine, which is less injurious than Hcy in terms of neurotoxicity.41 Another proposed mechanism is that the significant elevation of NO might reflect the elaboration of the angiopathic free radical peroxynitrite through the generation of superoxide anions and hydrogen peroxide.42 On the other hand, Chow and colleagues43 denied any effect of Hcy on constitutive or inducible NO synthase. In conclusion; oxidative stress is an outstanding event in the setting of thrombotic stroke, and it may have a detrimental effect on stroke outcome. Hyperhomocysteinemia is probably one of the causal factors in the evolution of free radical stress. In this study, we have reported association between Hcy and mediation of oxidative stress that may point in that direction. Attention should be paid for possible pharmacological intervention for Ht Hcy in the acute phase of stroke that may shut down the cascade of free radical generation and its deleterious effects.

References


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