Plasma Vitamin C Levels Are Decreased and Correlated With Brain Damage in Patients With Intracranial Hemorrhage or Head Trauma

Maria Cristina Polidori, MD; Patrizia Mecocci, MD, PhD; Balz Frei, PhD

Background and Purpose—Free radical hyperproduction may play an important role in brain hemorrhage and ischemia/reperfusion injury. The aims of this study were to assess whether antioxidant depletion occurs after intracranial hemorrhage (ICH) and head trauma (HT) and to evaluate the relation between the diameter of the brain lesion, the degree of the neurological impairment, and any observed antioxidant changes.

Methods—We measured plasma levels of vitamin C (ascorbic acid, AA), uric acid (UA), vitamin E (α-tocopherol), and ubiquinol-10 in 13 patients with ICH and 15 patients with HT on the day of the brain injury and subsequently every other day up to 1 week. Patients were compared with 40 healthy control subjects.

Results—ICH and HT patients had significantly lower plasma levels of AA compared with healthy subjects, in contrast to plasma levels of UA, α-tocopherol, and ubiquinol-10. AA levels were significantly inversely correlated with the severity of the neurological impairment as assessed by the Glasgow Coma Scale and the National Institutes of Health Stroke Scale. AA levels were also significantly inversely correlated with the major diameter of the lesion. In addition, mean plasma AA levels were lower in jugular compared with peripheral blood samples obtained from 5 patients.

Conclusions—These findings suggest that a condition of oxidative stress occurs in patients with head trauma and hemorrhagic stroke of recent onset. The consequences of early vitamin C depletion on brain injury as well as the effects of vitamin C supplementation in ICH and HT patients remain to be addressed in further studies. (Stroke. 2001;32:898-902.)

Key Words: antioxidants ■ brain hemorrhage ■ head trauma ■ oxidative stress ■ vitamin C

Increased production of free radicals and reactive oxygen species (ROS) leading to oxidative stress appears to play an important role in the pathogenesis of ischemic, hemorrhagic, and traumatic brain injury. Studies in animal models of focal cerebral ischemia and reperfusion have demonstrated the presence of increased levels of ROS. ROS also probably contribute to brain injury in head trauma (HT) and intracranial hemorrhage (ICH) in humans, because hemorrhage is associated with the release of hemoglobin-bound heme iron, which can participate in free radical reactions to produce ROS.

One possible consequence of excess ROS formation is lipid peroxidation. The brain appears to be particularly vulnerable to oxidative lipid damage because of its high content of polyunsaturated fatty acids. Lipid peroxidation may alter the fluidity and permeability of neuronal membranes and thus, cellular functioning, or damage membrane-bound receptors and enzymes. In brain hemorrhage, the presence of “free” iron may favor the conversion of lipid hydroperoxides to lipid alkoxyl radicals, which can “reinitiate” lipid peroxidation and hence further expand the radical chain reaction. In addition, tissue lactic acidosis can dramatically enhance ROS formation and lipid peroxidation in brain tissue, which in turn can increase the dissociation of catalytic iron from proteins.

Because ROS are short-lived and usually present at low concentrations, they are difficult to measure in biological samples. However, there are indirect indexes that can be used to examine sequelae of ROS production, such as oxidatively modified macromolecules and changes in the concentration of endogenous antioxidants, such as vitamin C (ascorbic acid, AA), uric acid (UA), vitamin E (α-tocopherol), and ubiquinol-10. Vitamin C appears to be particularly important in limiting oxidative lipid damage in biological systems. Numerous studies have demonstrated that under many different types of oxidizing conditions, AA forms the first line of antioxidant defense and effectively protects the lipids in plasma and lipoproteins against detectable peroxidative damage, even in the presence of free, redox-active iron.
Therefore, the aims of this study were to (1) determine whether there is evidence of plasma antioxidant depletion in patients with ICH or HT; (2) examine the time course of any observed changes in antioxidant levels; and (3) correlate those changes with the clinical severity of the disease and/or the extent of the brain injury.

Subjects and Methods

Thirteen patients with ICH (6 women and 7 men, 62.4±6.1 years of age), 15 patients with HT (1 woman and 14 men, 44.6±6.9 years of age), and 40 healthy, nonsmoking, normolipemic subjects, including 20 “young control subjects” (<45 years of age; 10 women and 10 men, 30.7±7.1 years of age) and 20 “adult control subjects” (>45 years of age; 10 women and 10 men, 58.3±5.8 years of age), were studied. Patients were consecutively recruited from the Neurological/Neurosurgical Intensive Care Unit of the Massachusetts General Hospital after approval was obtained from the ethics committee of the hospital and informed consent was obtained from patients or their relatives. In all patients, clinical examination was performed every day, and a CT scan was performed. The National Institutes of Health (NIH) Stroke Scale and Glasgow Coma Scale were assessed on admission and daily until discharge.

Patients with HT and ICH were divided into subgroups according to the neuroradiological size (CT scan) of the hemorrhage or contusion. The CT scan used for this purpose was performed between the second and the fourth days after the hemorrhage in all patients (mean time, 2.6 days). Groups A, B, and C, respectively, consisted of patients with small, medium, or massive hemorrhage or contusion with major diameter ≤2 cm, between 2 and 4 cm, or >4 cm. Figure 1 shows an example of measurement of the major diameter of an ICH.

In 4 HT patients and 1 ICH patient, simultaneous blood samples were obtained from central (jugular) and peripheral lines.

All patients were enrolled in the study within 24 hours from the onset of the injury. A 10-mL tube of heparinized blood was obtained on admission and every other day up to 1 week. Blood was immediately centrifuged, and plasma was separated and stored frozen at −80°C until analysis, which was performed within 1 week.

Blood from healthy control subjects was obtained in a single setting at 8 AM after an overnight fast. In all brain-injured patients, blood pressure, white blood cell count, temperature, inflated oxygen flow (FiO₂) (if intubated), medications before and during the hospitalization, nutritional status, smoking habit, and alcohol abuse were assessed. Subjects with multiple and/or major organ failure, other neurological or psychiatric disorders, or taking antioxidant vitamin supplements were excluded from the study. In addition, we excluded patients with multiple brain hemorrhages and other traumas.

The determination of plasma AA and UA levels was performed by high-performance liquid chromatography (HPLC) with electrochemical detection. A 100-μL aliquot of plasma was extracted with an equal volume of 5% metaphosphoric acid containing 1 mmol/L of the metal chelator diethylenetriaminepentaaetatic acid, then vortexed and centrifuged. Twenty microliters of the supernatant was mixed with 6 μL of 2.58 mol/L potassium phosphate (pH 9.8) and 74 μL of the mobile phase (40 mmol/L sodium acetate, 0.54 mmol/L Na₂EDTA, 1.5 mmol/L dodecyl triethylammonium phosphate, 7.5% methanol, pH 4.75). AA was detected at an applied potential of +0.6 V.²⁰

Ubiquinol-10 was quantified by HPLC with chemiluminescence detection. A 250-μL aliquot of plasma was extracted with 1 mL of ice-cold methanol and 5 mL of ice-cold hexane. The sample was vortexed and centrifuged. The hexane extract was dried under N₂, resuspended in 450 μL of methanol/ butanol (50:50), and analyzed with reversed-phase HPLC with chemiluminescence detection. The eluate was mixed with a reaction solution containing microperoxidase and isoluminol, and chemiluminescence produced by ubiquinol was measured at 0.01 mA. UV absorbance at λ=210 nm was monitored in series for quantification of α-tocopherol.²⁰

All values are presented as mean±SD. Plasma concentrations of AA, UA, α-tocopherol, and ubiquinol-10 were compared between the groups of patients by 1-way ANOVA for the values on day 1 and by 2-way ANOVA for the values over time. Correlations of AA, UA, α-tocopherol, and ubiquinol-10 plasma levels with the NIH Stroke Scale, Glasgow Coma Scale, or the diameter of the lesions were examined by linear regression or by Spearman’s correlation as appropriate. Significance was accepted if the null hypothesis was rejected at the level of P<0.05.

Results

Eleven ICH patients (84%) and 1 HT patient (6%) were hypertensive, and 2 ICH patients (15%) had diabetes. None of the subjects studied had history of alcohol abuse. Nine HT patients (60%) and 6 ICH patients (46%) were intubated, with a mean FiO₂ of 45.5±3.3% and 49.3±2.5%, respectively. The mean white blood cell count in the patients with ICH and HT was 10 220±550 and 11 320±465/mL, respectively. There were no differences of body temperature between patients, and none showed evidence of viral or bacterial infection.

<table>
<thead>
<tr>
<th>TABLE 1. Plasma Levels (μmol/L) of Ascorbic Acid, Uric Acid, α-Tocopherol, and Ubiquinol-10 in Patients With Intracranial Hemorrhage or Head Trauma on Day 1 and in Healthy Control Subjects</th>
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</thead>
<tbody>
<tr>
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<tr>
<td>------------------</td>
</tr>
<tr>
<td>AA</td>
</tr>
<tr>
<td>UA</td>
</tr>
<tr>
<td>α-Tocopherol</td>
</tr>
<tr>
<td>Ubiquinol-10</td>
</tr>
</tbody>
</table>

Values are mean±SD.

*Plasma AA levels were significantly lower (P<0.002) in ICH and HT patients compared with control subjects; no other significant differences were observed.
during the time of the study. No clinical evidence of muscle and tissue injury was observed, and LDH serum levels were in range in all patients. Four ICH patients died, 1 on day 3 and the other 3 on day 7. None of the HT patients died.

All brain-injured patients had significantly (P, 0.002) lower plasma AA levels on day 1 compared with healthy control subjects (Table 1). Plasma concentrations of UA, α-tocopherol, and ubiquinol-10 were not different in patients compared with control subjects (Table 1). In addition, plasma antioxidant levels did not significantly change over time in ICH and HT patients (Figure 2).

Patients of group A (small hemorrhage or contusion) had higher mean plasma levels of AA, UA, and α-tocopherol than patients of group B (medium hemorrhage or contusion), who had higher levels than patients of group C (massive hemorrhage or large contusion) (Table 2). However, only AA levels between groups A and C were significantly different (P<0.05).

Plasma AA levels in all patients but not any of the other antioxidants were significantly inversely correlated with the major diameter of the hemorrhage or contusion (r = −0.47, P=0.002, and r = −0.54, P=0.002, respectively) (Figure 3). Similarly, only plasma AA levels were significantly negatively correlated with the NIH Stroke Scale (r=−0.12, P<0.03) and significantly positively correlated with the Glasgow Coma Scale (r=+0.21, P<0.02). No significant correlations between antioxidant levels and smoking habit, hypertension, diabetes, caloric intake, FIO2, serum cholesterol levels (total, HDL, and LDL), or white blood cell count were observed, both in ICH and HT patients.

In the 5 patients from whom both peripheral and central blood was obtained, central (jugular) plasma exhibited lower AA concentrations than peripheral plasma on 3 of the 4 days examined, although none of the differences were significant (Table 3). Two-way ANOVA for values over time also showed no significant differences.

**Figure 2.** Plasma levels (μmol/L) of AA (circles) and UA (squares) (A) and α-tocopherol (circles) and ubiquinol-10 (squares) (B) in patients with ICH (n=13; open symbols) or head trauma (n=15; closed symbols) (mean±SD). Two-way ANOVA for values over time showed no significant differences.

**Figure 3.** Plasma levels (μmol/L) of AA in all patients are significantly inversely correlated with major diameter of hemorrhage (closed circles; r=−0.47, P=0.002) and contusion (open circles; r=−0.54, P=0.002).

**TABLE 2.** Plasma Levels (μmol/L) of Ascorbic Acid, Uric Acid, α-Tocopherol, and Ubiquinol-10 in Patients With Intracranial Hemorrhage or Head Trauma on Day 1

<table>
<thead>
<tr>
<th></th>
<th>ICH A (n=4)</th>
<th>ICH B (n=4)</th>
<th>ICH C (n=5)</th>
<th>HT A (n=7)</th>
<th>HT B (n=4)</th>
<th>HT C (n=4)</th>
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</thead>
<tbody>
<tr>
<td>AA</td>
<td>39.4±2.8</td>
<td>30.1±6.2</td>
<td>19.9±5.8*</td>
<td>34.6±9.9</td>
<td>32.3±10.1</td>
<td>24.7±12.3*</td>
</tr>
<tr>
<td>UA</td>
<td>268.3±58.6</td>
<td>251.7±56.3</td>
<td>245.7±49.6</td>
<td>281.2±55.3</td>
<td>276.9±47.9</td>
<td>264.3±55.3</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>28.1±5.8</td>
<td>25.7±6.1</td>
<td>24.5±7.9</td>
<td>30.5±7.5</td>
<td>28.9±7.9</td>
<td>27.8±8.3</td>
</tr>
<tr>
<td>Ubiquinol-10</td>
<td>1.55±0.95</td>
<td>1.40±0.96</td>
<td>1.35±0.86</td>
<td>1.51±0.94</td>
<td>1.55±0.94</td>
<td>1.63±1.02</td>
</tr>
</tbody>
</table>

Values are mean±SD.

Groups A, B, and C, respectively, consisted of patients with small, medium, or massive hemorrhage or contusion with major diameter <2 cm, between 2 and 4 cm, or >4 cm.

*Plasma AA levels were significantly lower (P<0.05) in group C compared with group A, both in ICH and HT patients; no other significant differences were observed.
TABLE 3. Plasma Levels (μmol/L) of Ascorbic Acid in Blood Samples Obtained From Both Jugular and Peripheral Lines in 4 Head Trauma patients and 1 Intracranial Hemorrhage Patient

<table>
<thead>
<tr>
<th>Time, d</th>
<th>Jugular Line (n=5)</th>
<th>Peripheral Line (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25.5±10.6</td>
<td>28.6±12.3</td>
</tr>
<tr>
<td>3</td>
<td>23.2±10.5</td>
<td>30.2±12.4</td>
</tr>
<tr>
<td>5</td>
<td>32.2±9.9</td>
<td>31.2±12.1</td>
</tr>
<tr>
<td>7</td>
<td>16.5±10.2</td>
<td>27.3±9.9</td>
</tr>
</tbody>
</table>

Values are mean±SD.

Discussion

This study provides evidence for oxidative stress-induced antioxidant depletion in human ICH and HT, specifically AA depletion. Importantly, the decreased plasma levels of AA were significantly inversely correlated with the major diameter of the brain lesion and with indexes of injury-related clinical impairment, suggesting the presence of oxidative stress in brain trauma and hemorrhage. We believe that the loss of vitamin C may be due to the brain injury for a number of reasons. First of all, vitamin C depletion was independent from the presence of smoking habit, hypertension, diabetes, and from dietary or FIO2 intake. Second, plasma vitamin C levels were not correlated with serum cholesterol levels, body temperature, or white blood cell count both in ICH and HT patients. These findings, along with the observation of lower AA concentrations in the jugular blood plasma as compared with the peripheral blood plasma of brain-injured patients, do not support the idea of a systemic cause alone of vitamin C depletion.

The present data are consistent with observations in experimental animals of antioxidant depletion in brain injury. For example, decreased brain concentrations of AA were observed in animal models of focal cerebral ischemia, and tissue levels of AA and α-tocopherol were decreased after spinal cord impact trauma in several species. In rats, transient cerebral ischemia and reperfusion caused ROS production associated with consumption of ubiquinol-9 and ubiquinol-10. Similarly, decreases in tissue levels of ubiquinol-9 and ubiquinol-10, AA, and α-tocopherol were observed after spinal cord impact trauma in rats. Studies of oral supplementation with antioxidants in animal models showed that treatment with vitamin E and selenium before traumatic brain injury significantly protected the nervous tissue from progressive declines in white matter blood flow. Treatment with AA before the trauma also significantly decreased posttraumatic spinal cord hypoperfusion.

Finally, several studies found that a low vitamin C status in humans is associated with increased mortality rates from stroke and critically ill patients. These data on antioxidant depletion suggest that there is significant oxidative stress associated with brain injury. In agreement with this notion, it has been reported that ischemia/reperfusion injury, global ischemia, and head trauma in experimental animals are associated with oxidative damage to proteins and lipids.

ROS released during reperfusion of ischemic brain tissue may also contribute to cerebral edema, vascular wall injury, and hemorrhage. Hemorrhage is a frequent complication of reperfusion in ischemic brain tissue, and hemorrhagic transformation of an ischemic cerebral area can occur spontaneously. Hemorrhage is associated with the release of heme iron normally bound to hemoglobin, and as a result, Fenton-type reactions may ensue with production of hydroxyl radicals and consequent initiation of lipid peroxidation. The time course and intensity of brain hydroxyl radical generation, as measured by the salicylate-trapping method, were studied in animal models of moderate or severe head injury. A dramatic increase in the indexes of hydroxyl radical formation was observed immediately after head trauma, and this increase was prevented by the administration of tirilazad mesylate. In addition, a significant increase in the levels of prostaglandin F2α, thromboxane A2, and leukotriene C4 was observed after head trauma. Methylprednisolone, which can inhibit lipid peroxidation, has shown beneficial therapeutic effects in experimental models and patients with acute spinal cord injury. Interestingly, iron-induced and trauma-induced injuries to neural tissue are similar in nature, supporting a role of ROS produced during the conversion of arachidonate to prostaglandins in trauma-associated microvascular damage.

Some limitations to this study should be acknowledged. Although this appears to be the first study regarding plasma antioxidant longitudinal changes after brain hemorrhage, antioxidant concentrations before the occurrence of the disease were unknown. This information would facilitate a clearer interpretation of data. Furthermore, the exact relation between plasma and cerebral tissue antioxidant levels in brain hemorrhage is still to be addressed. Finally, the achievement of a conclusion regarding the role of vitamin C loss may be reached through the analysis, in a larger sample of patients, of the relations existing between plasma vitamin C levels and (1) the enlargement of the hemorrhage over time, (2) the functional outcome after 1 week, and (3) the loss of cerebral tissue after 3 months.

In summary, the present study shows that plasma AA levels are significantly lower in brain-injured patients compared with healthy control subjects and are inversely correlated with the major diameter of the brain lesion. The consequences of early vitamin C depletion on brain injury, as well as the effects of vitamin C supplementation in ICH and HT patients, remain to be addressed in further studies.

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