Q J Med 2002; **95**:685–690

Antioxidant capacity after acute ischaemic stroke

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Received 2 May 2002 and in revised form 4 July 2002

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Summary

Background: Experimental studies have reported a rapid increase in the production of markers of oxidative damage following acute stroke due to the reperfusion event following ischaemia, and that endogenous antioxidant defences are rapidly depleted, permitting further tissue damage.
Aim: To measure changes in antioxidant capacity (individual and total) in stroke disease within a known time period post infarct.
Design: Observational cohort study.
Methods: We studied 31 acute ischaemic stroke patients; 26 hospitalized non-stroke patients and 23 community-based healthy controls. Non-fasting venous blood was obtained within 24 h, at 48–72 h and at 7 days after stroke onset (after hospitalization for non-stroke patients) and at baseline for community controls. Vitamins E and C, total plasma glutathione, total antioxidant capacity (TAC), uric acid, thiobarbituric-acid-reactive substances (TBARS), serum albumin, transferrin and C-reactive protein (CRP) were measured.
Results: Baseline glutathione concentrations were non-significantly lowest and TBARS significantly highest in ischaemic stroke patients compared with controls. Serum TAC strongly correlated with serum uric acid. Under multivariate analysis, serum uric acid explained most of the variance in TAC during the study period. Despite increased concentrations of uric acid, TAC was reduced in stroke patients compared with controls. Serum vitamin C concentrations deteriorated significantly in stroke patients, and differences between the cumulative changes between strokes and hospital controls were also statistically significant ($p = 0.013$).
Discussion: There was some evidence of reduction in TAC, despite increased uric acid concentrations, and deterioration in serum vitamin C levels in ischaemic stroke patients compared with controls.

Introduction

Stroke is the third most common cause of death in most western populations after coronary heart disease and cancer.[1] It is thus the most common life-threatening neurological disorder, and the resulting disability is the most important single cause of severe disability among Western people living in their own homes.[2]

There is strong evidence that lipid peroxidation, with accumulation of both conjugated dienes and thiobarbiturate-reactive material, is consistently found when cerebral ischaemia is followed by reperfusion,[3] and there is some evidence that this effect is enhanced by poor α-tocopherol and vitamin C status, and can be mitigated by supplementation with these vitamins.[4,5]

A recent uncontrolled study reported an association between the plasma concentrations of ascorbic acid and alpha tocopherol, and the degree of neurological impairment after ischaemic stroke.[6] Cherubini *et al*. also found evidence of reduced

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antioxidants concentrations in ischaemic stroke patients, and that higher vitamin A and uric acid concentrations, and lower vitamin C levels and erythrocyte superoxide dismutase activity, were associated with poor early clinical outcome.[7] Enhanced antioxidant capacity (individually and total) after acute stroke therefore may protect against the adverse effects of free radical production during ischaemia and reperfusion. Measurement of total antioxidant capacity (TAC) of biological fluids, however, is regarded as more physiologically representative, in certain settings, than individual antioxidants, and is believed to be a useful measure of how much the antioxidants present can protect against oxidative damage to membranes and other cellular components.[8]

The aim of this study was therefore to measure changes in markers of antioxidant capacity (measured individually and total) following acute stroke within a known period of time post infarct.

Methods

Patients and controls

For a 6-month period, all patients admitted to our University Teaching Hospital with a diagnosis of ischaemic stroke ($n = 31$) of < 24 h duration according to the World Health Organization criteria were identified prospectively. All had computerized tomography of the head which showed cerebral infarction and their mean time of presentation was 11 h (SD 7.3) after onset of stroke. Stroke patients were divided into groups according to the clinical subtypes of cerebral infarction (anterior circulation, posterior circulation and lacunar infarcts).[9] We excluded patients with cerebral haemorrhage, language disorders, swallowing difficulties, cognitive impairment, diagnosed malignancy or sepsis. Consecutive age-matched (within 3 years) hospitalized non-stroke control patients ($n = 26$) free of clinically diagnosed cerebrovascular, ischaemic heart disease or peripheral vascular disease were also recruited. Community-based healthy controls ($n = 23$), defined as being free of major medical or surgical illness within 5 years and leading an active and independent life, were recruited through advertisements for volunteers from elderly community groups. All patients and controls had demographic and medical data collected including history of hypertension, smoking, alcohol and drug intake, diabetes mellitus, and cardiovascular diseases. Stroke and non-stroke patients and healthy controls were excluded if they had active gastrointestinal disease, severe medical or psychiatric illness, serum creatinine of > 150 μmol/l, a history of gout or renal failure, or took antioxidant vitamins. The study was approved by Local Health Ethics Committee, and all subjects or their carers gave written informed consent.

Methods

Following recruitment to the study, a non-fasting blood sample was collected within 24 h, at 48–72 h and at day 7 following stroke onset, or following recruitment of the controls. Blood was collected in appropriate tubes and centrifuged in a Sorvall refrigerated centrifuge (RT6000), at 3000 *g* for 15 min to separate plasma from red blood cells. The supernatant was stored at -70 °C, and analyses were performed within one week. Serum vitamin E and vitamin C were analysed by high performance liquid chromatography (HPLC); TAC was analysed by enhanced chemiluminescence (ECL).[10–12] Total plasma glutathione was analysed using the method of Beuttler and Gelbart.[13] Thiobarbituric acid reactive substances (TBARS), markers of damage to membrane lipids, were measured by a standard curve prepared using tetraethoxy-propane.[14] C-reactive protein (CRP) concentrations were measured by latex enhanced method (normal range < 10), and uric acid levels were measured by a standard enzymic uricase method (normal range 160–400 μmol/l). Although CRPs have not been directly linked to oxidative stress, recent reports suggest that high CRP concentrations may reflect the degree of stroke severity, as the degree of inflammation may correspond to the extent of cerebral infarction, underlying unstable atherosclerotic lesions and/or secondary complications of stroke at the time of sampling.[15]

Statistical analysis

A repeated-measures analysis of variance (ANOVA) test was used to test within and between subject differences and $p < 0.05$ was considered significant. Differences between groups at baseline were adjusted for history of smoking and alcohol consumption, chronic illness, drug intake including aspirin, angiotensin-converting enzyme inhibitors, beta-blockers, calcium channel blockers, diuretics and nitrates and CRPs. Partial correlation and the Mann-Whitney U test were also used.

A forward stepwise multiple regression analysis was done to identify individual antioxidants (vitamins E and C, total plasma glutathione, serum albumin, transferrin, bilirubin and uric acid) and CRPs, which together account for the TAC as measured by ECL. Adjusted R^2 values were then

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used to measure the extent to which the TAC concentration can be explained by individual antioxidants and other clinical variables included in the model.

This study had the power to detect a true difference of 4 μmol/l and 1.38 μmol in the population means of plasma vitamin C and TBARS (a measure of lipid peroxidation) at 80% power, and type 1 error probability of $\leqslant 0.05$ given that the within-group standard deviations for independent design and two controls per case were 5.5 μmol and 2 μmol, respectively.

Results

Table 1 shows baseline characteristics of stroke patients and controls. Twenty-one of the stroke patients included had large-vessel disease (partial anterior circulation) and 10 had lacunar infracts. The underlying diagnoses in the hospitalized control group were chronic obstructive pulmonary disease (4), arthritis (3), deep vein thrombosis (3), abominal pain (2), leg cellulitis (2), falls (1), anaemia (1), peptic ulcer (1), paracetamol overdose (1), congestive heart failure (1), thyrotoxicosis (1), muscle pain (1), and miscellaneous (5).

Table 1 Baseline characteristics of stroke patients and control groups

	Strokes ($n = 31$)	Hospital controls ($n = 26$)	Community controls ($n = 23$)
Age (years)	73.5 (10.5)	70.0 (12)	73.5 (5)
Sex (female)	19 (61%)	18 (69%)	14 (63%)
Chronic diseases			
Hypertension	9 (29%)	4 (15%)	3 (14%)
IHD	9 (29%)	2 (8%)	3 (14%)
CVA/TIA	9 (29%)	0	0
Diabetes mellitus	7 (23%)	1 (4%)	1 (5%)
Smoking			
Current	6 (19%)	6 (23%)	3 (14%)
Ex-smokers	10 (32%)	7 (27%)	1 (5%)
Alcohol			
$\geqslant 21$ units/week	5 (16%)	3 (11%)	3 (28%)
Drugs			
Aspirin	16 (52%)	1 (4%)	3 (14%)
ACE-I	3 (10%)	1 (4%)	2 (9%)
Ca-channel blocker	3 (10%)	1 (4%)	3 (14)
Beta-blockers	3 (10%)	1 (4%)	1 (5)
Urea (3–8 mmol/l)	7 (3.2)	6.6 (3)	
Creatinine (73–133 μmol/l)	101 (24.7)	93 (19)	

Where no percentage is shown, data are means (SD). CVA, cerebrovascular accident; TIA, transient ischaemic attack; ACE-I, angiotensin-converting-enzyme inhibitor; IHD, ischaemic heart disease. Ex-smoker, gave up smoking $\geqslant 3$ months ago.

Despite the significantly higher levels of serum uric acid in stroke patients, TAC levels were lowest in stroke patients, and deteriorated further during the study period compared with both control groups. Baseline total plasma glutathione concentrations were non-significantly lowest, and TBARS highest, in stroke patients compared with both control groups (Table 2). Serum vitamin C concentrations deteriorated significantly in stroke patients, and differences between the cumulative changes in serum vitamin C between stroke patients and hospital controls were also statistically significant ($p = 0.013$), (Table 2). TBARS concentrations were higher, and remained so, in stroke patients compared with control groups. Baseline CRPs were significantly higher among hospital controls compared with the stroke patients, but continued to rise in stroke patients during the study period (Table 2). We found no correlation between the antioxidant capacity (individual or total) and size or type of the stroke.

There were significant correlations between TAC and serum uric acid concentrations for the groups, both separately and combined, throughout the study period (Table 3 and Figures 1–2). Under multivariate analysis, serum uric acid explained most of the variance in TAC during the study period. Of the other analytes included in the multivariate model, only baseline serum vitamin C showed significant correlation with TAC in stroke patients (Table 4).

Discussion

The main findings of this study were that TAC was reduced, despite increased concentrations of uric acid in stroke patients compared with both control groups and that serum vitamin C concentrations deteriorated significantly in ischaemic stroke patients during the study period. There were also some changes, albeit non-significant, in other markers of antioxidant capacity and oxidative damage between ischaemic stroke patients and control groups. Serum uric acid concentrations were significantly higher in stroke patients compared with controls, and TAC measured using ECL test is mostly accounted for by uric acid.

There is strong indirect evidence that free radical production appears to be an important mechanism

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Table 2 Baseline and follow up markers of antioxidant capacity and oxidative stress in strokes and control groups [mean (SE)]

Markers	Stroke patients ($n = 31$)			Hospital controls ($n = 26$)			Community controls ($n = 23$)
	Baseline (24 h)	48–72 h	7 days	Baseline (24 h)	48–72 h	7 days	Baseline
Serum vitamin C* (μmol/l)	39.0 (6)	33.3 (4)	32.1 (5)**	31.2 (4.9)	28.6 (5.8)	30.4 (6.3)**	66.6 (5.9)
Serum vitamin E (μmol/l)	17.4 (1.7)	16.7 (1.5)	15.1 (1.0)	15.0 (0.9)	13.6 (1.3)	12.9 (0.8)	14.1 (1.1)
Plasma glutathione (μmol/l)	0.18 (0.02)	0.17 (0.02)	0.19 (0.03)	0.22 (0.03)	0.23 (0.06)	0.21 (0.05	0.35 (0.08)
TAC (mmol/l)	540 (38)	516 (28)	487 (39)	550 (38)	545 (70)	603 (113)	568 (35)
TBARS* (μmol/l)	5.64 (0.52)	5.08 (0.43)	5.90 (0.53)	5.27 (0.27)	5.57 (0.44)	4.52 (0.37)	4.26 (0.16)
Urate* (μmol/l)	398 (20)	362 (21)	330 (22)	330 (25)	345 (47)	328 (50)	303 (35)
C-reactive protein* (mg/l)	18.3 (5)	22.9 (7)	39.7 (10)**	63.4 (17)	70 (22)	33.1 (11)**	3.5 (0.7)

*One-way ANOVA for differences in baseline values between three groups and after adjusting for smoking, drug intake and CRP (TBARS, $p = 0.04$; urate, $p = 0.05$; CRP, $p = 0.001$). **Mann-Whitney U test for differences between the cumulative changes in stroke and hospital controls (vitamin C, $p = 0.01$; CRP, $p = 0.01$)

Table 3 Correlations between the total antioxidant capacity (TAC) and serum uric acid concentration in serum of stroke patients and controls

	Stroke patients ($n = 31$)			Hospital controls ($n = 26$)			Community controls ($n = 23$)
	Baseline (24 h)	48–72 h	7 days	Baseline (24 h)	48–72 h	7 days	Baseline
TAC (μmol/l)	540 (38)	516 (28)	487 (39)	550 (38)	545 (70)	603 (113)	568 (35)
Uric acid (160–400 mmol/l)	398 (20)	362 (21)	330 (22)	330 (25)	345 (47)	328 (50)	303 (35)
Correlation coefficient [r]	0.78*	0.79*	0.74*	0.87*	0.74*	0.86[1]	0.53**

Data are means (SE). *$p < 0.001$; **$p < 0.05$. Results adjusted for CRP.

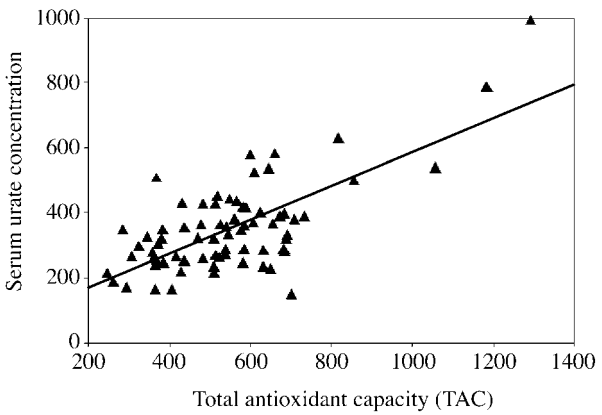

Figure 1. Correlation between baseline serum TAC and urate concentration for all patients and controls combined.

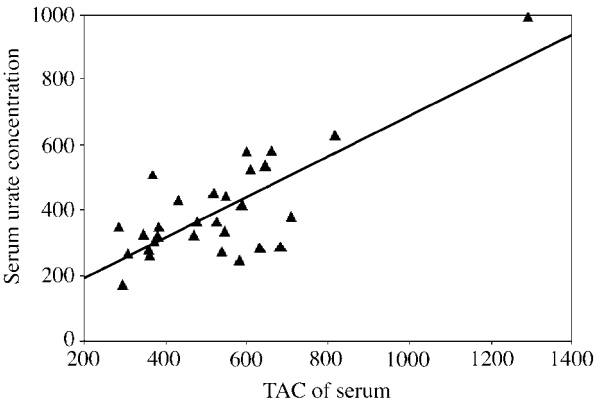

Figure 2. Correlation between baseline serum TAC and urate concentration for stroke patients.

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Table 4 Multiple regression result for all subjects compared with stroke patients, with baseline total antioxidant capacity as the dependent variable

Variable	R^2		ΔR^2		p	
	All	Strokes	All	Strokes	All	Strokes
Vitamin C	0.032	0.160	0.032	0.160	0.135	0.029*
Vitamin E	0.032	0.224	0.000	0.064	0.898	0.146
Glutathione	0.039	0.247	0.007	0.023	0.484	0.384
Uric acid	0.528	0.679	0.489	0.432	<0.0001*	<0.0001*
CRP	0.533	0.719	0.005	0.040	0.401	0.079
Albumin	0.533	0.725	0.000	0.007	0.824	0.453
Transferrin	0.533	0.730	0.000	0.005	0.962	0.548
Age	0.538	0.731	0.004	0.000	0.448	0.845

*$p < 0.05$.

of brain injury after exposure to ischaemia and reperfusion.[3] Indirect information on the impact of free radicals may be obtained by comparisons of the antioxidant concentrations, because serious damage by free radicals implies insufficiency of the body's multilevel defence systems against radicals. A number of components present in serum have been shown to possess chain breaking antioxidant capacity, including vitamins C and E, albumin, urate, bilirubin and protein thiols in aqueous or organic solution *in vitro*.[16] The TAC of biological fluids is believed to be a useful measure of the ability of antioxidant present in the fluids to protect against oxidative damage to membranes and other cellular components. The large variety of components of serum with potential antioxidant capacity has led to the development and widespread use of assays which measure TAC, thereby providing a clinically useful global measurement.[8]

Ryan *et al*. have previously reported that in healthy subjects, TAC measured by ECL is almost completely accounted for by uric acid.[8] Uric acid, which is the end product of purine metabolism, has long been regarded as a potent endogenous water-soluble antioxidant and radical scavenger in humans.[17] However, its therapuetic benefit in diseases in which free radicals are thought to be involved is yet to be proven. There is some evidence that increased oxidative stress is associated with high circulating uric acid levels and that uric acid may protect against oxidative modification of endothelial enzymes and preserves the ability of endothelium to mediate vascular dilatation in the face of oxidative stress.[17] On the other hand there is some evidence that uric acid may have a direct role in the atherosclerotic process, because human atherosclerotic plaques contains more uric acid than do control arteries.[18] Following cerebral ischaemia and reperfusion, however, the metabolism of nucleosides and purine bases to inosine and hypoxanthine via the xanthine oxidase pathway results in production of oxygen free radicals.[3] Because xanthine oxidase, an enzyme that controls the formation of uric acid, may be important in formation of oxygen radicals during reperfusion in the brain, blocking the activity enzyme may improve recovery from cerebral ischaemia and reperfusion.[19]

Animal models of transient ischaemia/reperfusion in the brain suggest that brain lipids are vulnerable to oxidation during this period.[4,20] There is some evidence that this effect is enhanced by poor α-tocopherol (vitamin E) status and can be mitigated by supplementation with this vitamin.[4] A recent uncontrolled study reported an association between the plasma concentrations of ascorbic acid and α-tocopherol, and the degree of neurological impairment after ischaemic stroke.[6] The same study demonstrated an association between plasma total antioxidant activity and the volume of ischaemic cerebral infarction and the degree of neurological impairment that followed.[6] Polidori *et al*. also reported a significant positive correlation of lipid hydroperoxides with NIH stroke scale, and a significant negative correlation with the Glasgow coma scale.[21] The size and type of the stroke is no doubt an important factor in the overall antioxidant capacity and oxidative stress, but the lack of correlation between antioxidant capacity and the size or type of the stroke in our this study may be due to the small sample size.

The main limitations of our study lie in the weakness of parameters used for measurement of oxidative stress. TBARS are not the best available markers of lipid peroxidation, and we have not included measures of red-cell antioxidant enzymes.

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Another weakness is the lack of data on dietary intake during the study period; however, in a previous study, we measured the daily in-hospital energy and protein intakes of 24 acute stroke and non-stroke age- and sex-matched patients during the first 2 weeks of the hospital stay. We found no significant difference in average daily energy intake between the two groups.[22]

Nevertheless, accepting these limitations, our results show a reduction in TAC, despite increased uric acid concentrations, and deterioration in serum vitamin C levels in ischaemic stroke patients compared with controls. Larger clinical studies in this area are needed to clarify the temporal relationships between antioxidant capacity and oxidative damage following ischemia and reperfusion in man, and to form the basis of appropriate antioxidant intervention strategies to minimize long-term brain injury following cerebral ischemia. Further work is also needed to explore the physiological role of uric acid following acute ischaemic stroke.

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