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Antioxidant Status in Acute Stroke Patients and Patients at Stroke Risk

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Key Words

Acute stroke · Stroke risk · Antioxidants · Superoxide dismutase · Glutathione · Glutathione peroxidase

Abstract

Background and Purpose: Antioxidant enzymes like copper/zinc superoxide dismutase (SOD), catalase and glutathione peroxidase (GSHPx) are part of intracellular protection mechanisms to overcome oxidative stress and are known to be activated in vascular diseases and acute stroke. We investigated the differences of antioxidant capacity in acute stroke and stroke risk patients to elucidate whether the differences are a result of chronic low availability in arteriosclerosis and stroke risk or due to changes during acute infarction. ***Methods:*** Antioxidant enzymes were examined in 11 patients within the first hours and days after acute ischemic stroke and compared to risk- and age-matched patients with a history of stroke in the past 12 months (n = 17). Antioxidant profile was determined by measurement of glutathione (GSH), malondialdehyde (MDA), SOD, GSHPx and minerals known to be involved in antioxidant enzyme activation like selenium, iron, copper and zinc. ***Results:*** In comparison to stroke risk patients, patients with acute ischemic stroke had significant changes of the GSH system during the first hours and days after the event: GSH was significantly elevated in the first hours ($p < 0.01$) and GSHPx was elevated 1 day after the acute stroke ($p < 0.05$). Selenium, a cofactor of GSHPx, was decreased ($p < 0.01$). GSHPx levels were negatively correlated with National Institutes of Health Stroke Scale (NIHSS) scores on admission ($r = -0.84$, $p < 0.001$) and NIHSS scores after 7 days ($r = -0.63$, $p < 0.05$). MDA levels showed a trend for elevation in the first 6 h after the acute stroke ($p = 0.07$). No significant differences of SOD, iron, copper nor zinc levels could be identified. ***Conclusions:*** Differences of antioxidant capacity were found for the GSH system with elevation of GSH and GSHPx after acute stroke, but not for other markers. The findings support the hypothesis that changes of antioxidant capacity are part of acute adaptive mechanisms during acute stroke.

Free radicals and reactive oxygen species (ROS) are believed to cause biological cell damage. A sensitive balance between generation and neutralization of oxidants by different intra- and extracellular defense mechanisms helps to protect vital cell components. Circulating scavenging antioxidants with a high redox potential like ascorbic acid, tocopherols, β-carotene and ubiquinone, intrinsic antioxidants like bilirubin, urate and albumin as well as intracellular antioxidant enzymes like gluthatione peroxidase (GSHPx), superoxide dismutase (SOD) and catalase maintain this equilibrium.

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Arteriosclerotic vascular damage is believed to be one of the consequences of increased oxidative stress including excessive generation of ROS, oxidation of lipoproteins and formation of arteriosclerotic plaques. Oxidative stress is also one of the mechanisms involved in neuronal damage induced by ischemia/reperfusion [1, 2]. The antioxidant activity of plasma may be an important factor providing protection from neurological damage caused by stroke-associated oxidative stress [3, 4]. Acute ischemia leads to increased production of free radicals and ROS in tissue and plasma [1]. During the early phase of ischemia and reperfusion, antioxidants can be mobilized, though antioxidant capacity might be limited by chronically low availability, consumption of neutralizing scavenging antioxidants or excessive oxidative stress [3, 5]. This study was designed to examine the differences and changes of antioxidant enzymes in acute stroke to elucidate whether there are acute adaptive changes to overcome oxidative stress.

Subjects and Methods

Patient Selection

Antioxidant markers were examined in two groups of patients: (a) in patients within the first hours and days after acute stroke (group A, n = 11) and (b) in age- and risk-matched patients with a history of stroke in the past 12 months (group B, n = 17). Group A consisted of 11 patients with symptoms of acute stroke admitted within 6 h after onset of neurologic deficit to the emergency room of Krankenhaus Munich-Harlaching. Blood was taken within 6 h after onset of symptoms and cranial CT scan was performed to exclude intracranial hemorrhage and to determine the size of infarction. Patients also had blood controls 1, 3 and 7 days after acute stroke. Neurologic examination was recorded by the National Institutes of Health Stroke Scale (NIHSS) [6] on admission and day 7 and a numeric risk score was calculated according to the Copenhagen City Heart Study (CCHS) risk score for stroke risk patients [7]. Type and etiology of infarction were determined by examination results of cranial CT, duplex ultrasonography, ECG, echocardiography and clinical situation.

Group B consisted of 17 age- and risk-matched patients with similar cardiovascular risk factors having suffered from minor stroke in the past 12 months and presenting at the outpatient clinic for check-up visits. None of the patients was suffering from another inflammatory, degenerative or malignant disease. All patients gave their written informed consent. They underwent neurological examination, cardiovascular risk factors were checked by patient history and clinical parameters, and CCHS risk score was calculated.

Analysis of Blood Samples

Venous blood samples were taken, serum and whole EDTA blood was frozen immediately and stored at –70°C for a maximum of 6 months until determination of antioxidant enzymes (GSHPx, SOD), markers of peroxidation [malondialdehyde (MDA), glutathione (GSH)] and minerals (selenium, copper, zinc, iron) involved in the regulation of antioxidant enzymes.

Serum GSHPx was determined by the method described by Paglia and Valentine [8] coupling the peroxidase reaction with the reduction of oxidized GSH reductase and NADPH. Whole blood SOD activity was measured from lysate by a chemiluminometric method using the RanSOD 125 kit (Randox Laboratories, UK) and serum MDA was determined by condensation with thiobarbituric acid and photometric determination of the pink chromogen [9]. Whole blood GSH was measured by fluometric reaction with Ellmanns reagent described by Mergel et al. [10]. For determination of minerals, flame and flameless atomic absorption spectroscopy was used.

Normal values were based on laboratory reference values including 101 healthy subjects analyzed under the same conditions (20 female, 81 male). Normal values were 1.6–2.4 μmol/l for MDA, 68–97 μmol/l for GSH, 96–150 U/l for GSHPx, 15–22 U/μmol hemoglobin for SOD, 0.72–1.33 μmol/l for selenium in serum, 9–26 μmol/l for copper, 62–102 μmol/l for zinc and 51.7–95.7 μmol/l for iron in whole blood [11].

Results are expressed as means ± SD. For statistical analysis, the Mann-Whitney U test and Spearman rank correlation coefficient were used. A value of $p < 0.05$ was considered significant.

Results

A description of the patients and their risk profile is presented in table 1. In patients with acute stroke, MDA levels showed a trend for elevation in the first 6 h after stroke (2.1 ± 0.6 μmol/l, $p = 0.07$), which decreased to 1.7 ± 0.2 μmol/l after 7 days similar to the levels of stroke risk patients (1.7 ± 0.5 μmol/l, table 2). In more than half of the patients of group B, GSH levels were below the normal range. Compared to them, patients with acute stroke showed significantly elevated GSH levels on admission ($p < 0.01$, table 2).

Levels of GSHPx were elevated during the first days after acute stroke as well, showing significant elevation 1 day after the acute event (table 2, $p < 0.05$). GSHPx levels during the first 6 h after acute stroke were negatively correlated with the NIHSS score on admission ($r = –0.84$, $p < 0.001$) and the NIHSS score after 7 days ($r = –0.63$, $p < 0.05$, fig. 1).

Selenium in serum, known to be a cofactor for GSHPx, was significantly lower in acute stroke compared to stroke risk patients ($p < 0.05$, table 2).

Whole blood SOD activity was similar in both groups of patients (table 2). There were no differences of copper or zinc – cofactors of SOD – between the groups (copper: 13.7 ± 1.4 μmol/l versus 14.0 ± 1.7 μmol/l; zinc: 90 ± 11 versus 89 ± 12 μmol/l). No difference in whole blood iron was found.

Table 1. Patient data and risk profile

	Patients with acute stroke	Patients with history of stroke
Patients	11	17
Female/male	6/5	3/14
Age, years	62.2 ± 8.4	63.4 ± 7.8
Systolic blood pressure, mm Hg	153 ± 12	143 ± 20
Diastolic blood pressure, mm Hg	87 ± 7	81 ± 10
CCHS stroke risk score[1]	15.7 ± 9.4 (8–38)	16.1 ± 7.9 (3–30)
NIHSS score on admission	7.6 ± 6.6 (0–20)	
NIHSS score on day 7	3.9 ± 5.3 (0–18)	
Type of ischemia	PRIND/TIA: 7 pat. (64) stroke: 4 pat. (36)	PRIND/TIA: 11 pat. (65) stroke: 6 pat. (35)
Size of infarction	4 pat.: small lesion[2] (36) 3 pat.: moderate lesion[3] (27) 4 pat.: large lesion[4] (36)	not available
Type of infarction	4 atherothrombotic (36) 3 cardioembolic (27) 4 microangiopathic (36)	9 atherothrombotic (53) 4 cardioembolic (24) 4 microangiopathic (24)
Stroke prevention	5 pat.: ASS (45) 2 pat.: clopidogrel (18) 4 pat.: anticoagulation (36)	7 pat.: ASS (41) 5 pat.: clopidogrel (29) 5 pat.: anticoagulation (29)

PRIND = Prolonged ischemic neurological deficit; TIA = transient ischemic attack; pat. = patients. Figures in parentheses indicate ranges or percentages.

[1] CCHS prospective stroke risk score: a risk evaluation by age, blood pressure, atrial fibrillation, smoking, left ventricular hypertrophy, treatment of hypertension, cardiovascular disease and diabetes mellitus.

[2] Small lesion: < 100 mm^2 as maximal hypodense area on the cranial CT scan with the largest demarcation.

[3] Moderate lesion: 100–500 mm^2.

[4] Large lesion with hypodense area: > 500 mm^2.

Table 2. Antioxidant markers in patients with acute ischemic stroke in comparison to patients with a history of stroke

	Laboratory reference values	Patients with a history of stroke (n = 17)	Patients with acute stroke (n = 11)			
			admission	day 1	day 3	day 7
MDA i.S., µmol/l	1.6–2.4	1.7 ± 0.5	2.1 ± 0.6	1.9 ± 0.4	1.9 ± 0.3	1.7 ± 0.2
GSHPx i.S., U/l	96–150	113 ± 36	128 ± 44	153 ± 37[b]	165 ± 55[c]	147 ± 61
GSH i.B., µmol/l	68–97	58 ± 23	84 ± 26[a]	78 ± 39	81 ± 42	77 ± 34
SOD i.B., U/µmol Hb	15–22	12.2 ± 2.1	12.2 ± 2.9	12.7 ± 4.3	10.7 ± 1.2	12.8 ± 2.6
Se i.S., µmol/l	0.72–1.33	0.93 ± 0.14	0.78 ± 0.12[a]	0.78 ± 0.14	0.77 ± 0.16	0.82 ± 0.13
Fe i.B., µmol/l	52–96	73 ± 9	66 ± 13	73 ± 12	69 ± 13	72 ± 9

i.S. = In serum; i.B. = in whole blood/lysate.

[a] $p < 0.01$ compared to patients at stroke risk, [b] $p < 0.05$ compared to patients at stroke risk, [c] $p = 0.07$ compared to patients at stroke risk. Laboratory reference values have been established before from 101 healthy subjects.

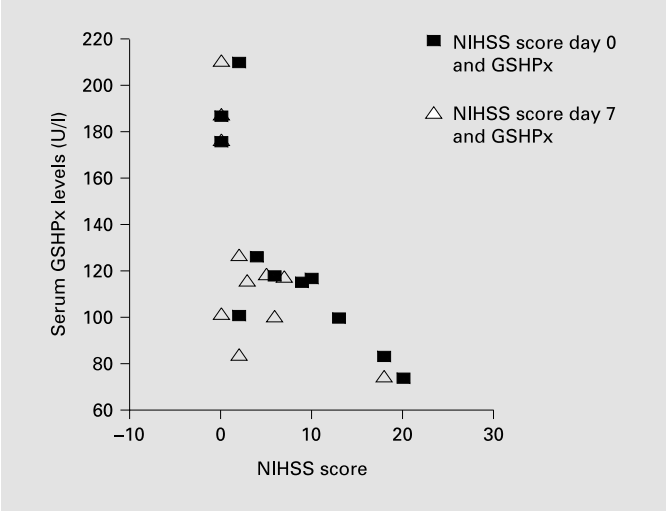

Fig. 1. Negative correlation of the NIHSS score and serum GSHPx levels on day 0 ($r = -0.84$, $p < 0.001$) and day 7 ($r = -0.63$, $p < 0.05$).

Discussion

We could identify some differences of antioxidant markers and enzymes between patients with acute stroke in comparison to age- and risk-matched patients with a history of ischemic stroke: most significant differences could be found for the GSH system, while MDA levels showed only a trend for elevation in the first hours after acute stroke.

The role of endogenous defense mechanisms including antioxidant enzymes in stroke risk and arteriosclerosis is controversial [3]. On the one hand, decreased availability of antioxidant defense mechanisms is a chronic problem in arteriosclerosis. On the other hand, the amount of oxidative stress and acute changes of antioxidant capacity might influence the prognosis of cerebral ischemia [2].

MDA levels are widely accepted as markers of lipid peroxidation in states of increased oxidative stress. Some but not all authors have found increased MDA levels after acute stroke [12–14]. Our patients with acute stroke showed a trend for MDA elevation during the first hours after the event decreasing to levels similar to those of stroke risk patients after 7 days.

GSH is part of the intracellular nonenzymatic small-molecule antioxidant defense system. It is a free radical scavenger and a proton donor for GSHPx and known to play a neuroprotective role [15, 16]. Depleted GSH levels have been found in a number of neurodegenerative diseases with states of oxidative stress as well as in the process of normal aging [15]. Nearly two thirds of our patients with a stroke in the past showed decreased GSH levels, possibly associated with increased oxidative stress and arteriosclerosis. In animal models, acute increase in GSH by activation of GSH synthase has been found during acute oxidative stress [17]. Our patients had elevated GSH levels during the first hours after acute stroke. This could be part of a first-line defense mechanism against oxidative stress, providing neuroprotection against amino acid excitotoxicity in stroke [16].

Changes in enzymatic antioxidative defense mechanisms after acute stroke are controversial. Some authors found reduced SOD activity in red blood cells and serum of patients with acute stroke [5, 13]. Reduced SOD levels led to an enhancement and progression of cerebral infarctions [5, 18]. Others have found increased levels of SOD in red blood cells after cerebrovascular events [19]. We did not find any significant differences of erythrocyte SOD activity or its cofactors copper and zinc between the two groups and there was no correlation between erythrocyte SOD activity and infarct size or NIHSS score in our patients. Anyhow, the patient number might be too small to draw negative conclusions and infarctions might not have been large enough to have obvious influence on whole blood SOD levels.

In our study, acute stroke was associated with increased serum levels of GSHPx during the first and third day after the acute event compared to patients with a history of stroke. Zachara et al. [20] found increased plasma GSHPx levels up to 48 h after myocardial infarction and Cherubini et al. [18] a significant increase in plasma GSHPx activity between day 1 and 6 after acute stroke. On the other hand, autopsied human brain tissue was GSHPx positive only 6 days after acute stroke [21] and red blood cell GSHPx activity even decreased in patients with acute ischemic stroke [13]. Differences could be explained by the fact that plasma GSHPx mainly originates from hepatocytes [22], while glial cells were found to be the major source of brain GSHPx [21].

Several animal studies have found that GSHPx has protective effects on brain damage and that reduced GSHPx levels are associated with an increased stroke risk [23, 24]. Data from our patients at stroke risk fit this concept, showing that high GSHPx levels correlated with low neurologic deficit (low NIHSS score on admission) as well as good outcome (low NIHSS score on day 7).

Selenium was the only mineral found to be decreased in acute stroke patients in our study. Acute decrease in selenium levels in serum are associated with acute inflammatory processes [25]. Bor et al. [26] found decreased

selenium levels after acute myocardial infarction. Levels of our stroke risk patients were in the normal range, while levels were decreased after acute stoke. We can only speculate that this is due to acute inflammatory processes after stroke.

There are limitations of our study and the number of examined patients is small. Only 4 of the 11 patients with acute infarction developed severe stroke and large cerebral ischemic lesion; most of the patients only had minor or no permanent deficit. This might explain why we could only find small changes of the antioxidant system. However, we could show that there are differences of the antioxidant status between patients at stroke risk and patients with acute stroke. Our data support the hypothesis that in acute stroke, activation of the GSH system helps to overcome oxidative stress. It is possible that antiplatelet drugs might have influenced the antioxidant systems as well. However, the use of stroke prevention drugs was similar in both groups and all stroke patients were treated with either ASS, clopidogrel or anticoagulation after exclusion of intracerebral hemorrhage. Nevertheless, further studies are needed to find out whether antioxidant changes will have prognostic and therapeutic implications for stroke and stroke risk patients.

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