Superoxide Dismutase Activity in Serum of Patients With Acute Cerebral Ischemic Injury

Correlation With Clinical Course and Infarct Size

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Abstract

Background and Purpose Superoxide dismutase (SOD) is one of the major free radical scavenging systems that might play a role in both degenerative and acute diseases of the central nervous system.

Methods We measured SOD activity in the serum of 41 patients with acute ischemic stroke with a chemiluminometric assay based on the generation of oxygen free radicals by xanthine and xanthine oxidase.

Results SOD activity was significantly lower in patients with ischemic stroke than in age-matched
control patients with nonvascular, neurological illnesses (n=24; P<.034, Wilcoxon rank test). The activity was inversely correlated with the size of infarction on CT (P=.01, Spearman correlation) and the severity of neurological deficits (P<.001, Spearman correlation). The decreased SOD activity recovered within 5 days after stroke to values found in serum of control patients.

**Conclusions** Our data suggest that the SOD activity in serum is reduced in stroke patients, and replacement of antioxidative activity could be beneficial in the acute treatment of cerebral ischemia.

**Key Words:** central nervous system • free radicals • stroke, acute • superoxide dismutase

### Introduction

Reactive oxygen metabolites have long been implicated in the development of brain lesions in reperfusion after cerebral ischemia. However, only recent advances in methodology have allowed investigators to measure reactive oxygen metabolites directly, showing that superoxide anions are being released during reperfusion after cerebral ischemia. A variety of enzymatic systems have the capacity to generate reactive oxygen metabolites. In addition to the release from mitochondria, the NADPH-dependent oxidase system on the surface of granulocytes and activated macrophages and the xanthine oxidase in endothelial cells are main sources of reactive oxygen metabolites.

These constantly produced superoxide radicals are scavenged by a number of antioxidant enzymes, including superoxide dismutase (SOD), glutathione reductase, and catalase. Additionally, chemical antioxidants such as glutathione, ascorbic acid, and vitamin E are also likely to be involved in the detoxification of free radicals. During reperfusion after ischemia, perturbation of the antioxidative defense mechanisms is a result of the overproduction of oxygen radicals, inactivation of detoxification systems, and consumption of antioxidants.

Investigations in animal models of cerebral ischemia suggest a particular role of SOD in the reperfusion injury. However, the reports of the effect of cerebral ischemia on SOD expression and activity in vivo are contradictory. A small decrease in SOD activity was observed 7 days after middle cerebral artery occlusion in a rat model of focal ischemia. This was thought to be the consequence of ongoing additional damage to the peri-infarct tissue. Also, in gerbil focal ischemia/reperfusion and global ischemia models, SOD activity in the cerebral tissues decreased by approximately 20% when assayed by the chemiluminescence method. Other investigators found an increased immunoreactivity against mitochondrial Mn-SOD and cytosolic CuZn-SOD after transient forebrain ischemia in neurons of the gerbil hippocampus and increased SOD activity in the rat after global ischemia. Matsumiya and collaborators found increased mRNA levels but decreased protein concentrations of CuZn-SOD in the CA1 region of the hippocampus after transient forebrain ischemia in the cat, which was thought to reflect a less functional antioxidant system in the vulnerable CA1 neurons. These contradictory results might be caused by the different SOD isoforms, methods, and animal models used to investigate the impact of cerebral ischemia on SOD levels and activity.
In the present study we measured the SOD activity in the serum of patients with stroke and correlated the findings with both functional outcome and infarct size.

Subjects and Methods

The antioxidative activity in serum was measured sequentially in 41 patients with acute ischemic stroke who were admitted to the Department of Neurology within 24 hours after onset of symptoms (13 men and 28 women, aged 28 to 91 years [mean, 62.8 years]). Twenty-four age-matched patients who were being treated at the same time in the neurological department for nonvascular diseases (eg, intervertebral disk protrusion, polyneuropathies, or muscular diseases) served as controls (10 men and 14 women, aged 35 to 81 years [mean, 60.3 years]).

The study was approved by the local ethics committee. Patients with (1) concomitant cardiac, renal, hepatic, or cancerous disease; (2) recent head trauma; (3) recent history of transient ischemic attacks; or (4) CT and/or MRI results inconclusive for the location of the ischemic lesion were excluded from this study. All patients were evaluated by CT and/or MRI on day 1 and on day 4 after stroke.

Clinical examination was performed on admission and daily thereafter and was scored according to the 58-point Scandinavian Stroke Scale (SSS). On admission and 10 days after stroke, the neurological deficit was assessed by the SSS.

Blood samples were drawn between 8 AM and noon from indwelling venous catheters within the first 24 hours after stroke and again 72 hours, 5 days, and 10 days thereafter. Samples were immediately centrifuged (1500g, 10 minutes), and serum was stored at -80°C until assayed. Serum SOD activity was measured with a chemiluminometric method described in detail before. Briefly, xanthine oxidase (grade III from buttermilk, 420 mU/mL, Sigma), 0.1 mmol/L lucigenin (Sigma), and serum samples were diluted in 50 mmol/L potassium phosphate buffer, pH 10.0. Serum samples were diluted 1:10. At this concentration, the influence of natural reducing agents such as ascorbate or epinephrine is excluded in the assay, and the inhibition of chemiluminescence is caused by SOD. Mn-SOD (Sigma) dilutions or buffer was used as control. The superoxide-producing reaction was initiated by the automated dispensing of 60 µL of 1.45 mmol/L xanthine. All reagents were freshly prepared in 50 mmol/L potassium phosphate buffer, pH 10.0. The buffer and xanthine solutions were kept at room temperature, the other solutions on ice. Chemiluminescence was measured for 20 minutes in 1-minute cycles in BioLumat LB 9501. Mean blank values given by cuvettes without xanthine oxidase were subtracted from the peak counts per minute. Results are expressed as micrograms per milliliter SOD according to a standard curve with bovine Mn-SOD.

Statistical Analysis
For statistical analysis we used the Wilcoxon rank test and Spearman correlation, as appropriate. \( P < .05 \) was assigned as statistically significant. Data are given as mean±SEM.

Results
Of the 41 patients with stroke, 9 had large hemispheric infarcts, in 8 patients due to cardiac embolism occluding the internal carotid artery and in 1 due to dissection of the internal carotid artery with supraocclusional middle cerebral artery embolism. Three of these patients died from transtentorial herniation during the 10 days of investigation. Nine patients showed a medium-sized infarct on cranial CT. Five of these patients had an occlusion of the internal carotid artery; in 4 of them the middle cerebral artery was occluded. Twenty-three patients had small cerebral infarcts due to either MCA branch occlusion or lacunar infarcts. Initial SSS ranged from 2 to 56 (mean, 25.98±14.3). Ten days after stroke, the mean SSS was 29.88±18.0 (range, 6 to 58).

The chemiluminescence triggered by xanthine/xanthine oxidase was significantly less inhibited by serum of patients after acute stroke compared with age-matched control patients (1489.12±28.7 versus 1335.9±32.6, mean±SEM; \( P < .034 \), Wilcoxon rank test), indicating less SOD activity in their serum. SOD activity was decreased most profoundly within 24 hours after onset of neurological deficits and recovered to values not different from control subjects within 5 days after stroke (Fig 1). We found a significant correlation between the infarct volume on the cranial CT scan and the SOD activity in the serum obtained within 24 hours after stroke (\( P = .01 \), Spearman correlation). Patients with large infarcts (>150 cm\(^3\)) had a higher chemiluminescence and thus a lower SOD activity than those with small infarcts (\( P < .001 \), Wilcoxon rank test) (Fig 2). Accordingly, the degree of neurological deficit of stroke patients scored by SSS on admission correlated well with the SOD activity (\( r = .599, P < .001 \), Spearman correlation). In patients with low SSS scores on admission a higher chemiluminescence was observed (Fig 3).

**Figure 1.** Superoxide dismutase (SOD) activity in serum was measured as described in "Subjects and Methods." Serum from control patients contained significantly more SOD activity than that of stroke patients measured within 24 hours after onset of symptoms. Five days after stroke, SOD activity returned to control values. Data are given as mean±SEM. *\( P = .034 \), Wilcoxon rank test.

**Figure 2.** Patients with larger strokes had less free radical scavenging activity than those with smaller infarcts. Data are given as mean±SEM (*\( P < .005 \), **\( P < .001 \) vs control, Wilcoxon rank test).
We investigated the SOD activity in the serum of patients with acute cerebral ischemia and observed a significant decrease within 24 hours after stroke compared with age-matched control subjects. This seems to be in contrast to previous studies, which, however, used a different methodological approach. In previous investigations of the concentration of cytoplasmic CuZn-SOD in the serum of patients with stroke, either no significant difference was found or there was an increase in plasma and cerebrospinal fluid. The method we used is specific for SOD, excluding other antioxidants as a cause for reduced chemiluminescence created by the xanthine/xanthine oxidase system. However, it measures the total SOD activity and does not differentiate between the different SOD isoforms. In extracellular fluids, including serum, extracellular SOD, which is secreted by endothelial cells, is the major SOD isozyme, and the activity of SOD in serum closely parallels the concentration of extracellular SOD. Therefore, mitochondrial Mn-SOD and cytoplasmic CuZn-SOD might not be as relevant in serum, and a strong reduction in extracellular SOD activity could account for the reduction in the antioxidative capacity we found in stroke patients. Strand and Marklund reported an increased CuZn-SOD activity in the cerebrospinal fluid and found a good correlation with the size of the infarct on cranial CT scan and functional impairment. They suggested that CuZn-SOD activity in the cerebrospinal fluid is a good marker for the extent of brain damage, since this small molecule leaks easily from the injured brain tissue into the cerebrospinal fluid. However, no serum analysis was performed. In our study decreased SOD activity was already apparent within 24 hours after onset of symptoms and recovered subsequently over the next few days. Therefore, it seems likely that antioxidants are depleted as a consequence of an excessive production of oxygen free radicals very early after the ischemic insult. The generation of superoxide anion usually occurs at the time of reperfusion. Since it is unlikely that the occluded arteries recanalized at the same time in all patients investigated in this study, reactive oxygen metabolites from other sources including activated granulocytes in the penumbra of the ischemic injury may be the cause of the reduced SOD activity in serum.
infarct may add to the observed depletion of radical scavenging enzymes. The low SOD activity in
patients with large infarcts and poor outcome might reflect the increased amount of free oxygen
radicals released from a severe ischemic injury. Thus, increasing the antioxidative capacity in serum
within the first day after the onset of symptoms might be a therapeutic option to minimize the
oxidative injury caused by oxygen free radicals until the endogenous free radical scavenging systems
recover. In animal models of cerebral ischemia, polyethylene glycol–conjugated or liposome-
entrapped CuZn-SOD was used successfully to reduce brain injury by ischemia and reperfusion.
Infarct volume4 19 and posts ischemic blood-brain barrier permeability4 20 were reduced after
administration of SOD in piglets that had been subjected to focal cerebral ischemia. Further evidence
for the important role of SOD in the defense of free oxygen radical damage in reperfusion injury
came from transgenic animal experiments. Mice overexpressing SOD were highly resistant to
reperfusion injury and damage of the blood-brain barrier.5 21 22 The decrease in the infarct volume in
transgenic mice correlated well with an improved neurological outcome, whereas mice with a
knockout mutation for SOD had larger infarcts after focal ischemia.6 Supplementation of depleted
antioxidative capacity thus may also be beneficial in stroke patients.

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