Spermidine: A Predictor for Neurological Outcome and Infarct Size in Focal Cerebral Ischemia?

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Background and Purpose—Polyamines are mainly restricted to the intracellular space. During focal cerebral ischemia, polyamines are released from the intracellular compartment. Experimental studies have implicated a marked elevation in brain tissue and blood. The aim of our study was to investigate whether the elevation of polyamines in the blood of patients with focal cerebral ischemia correlates with the clinical outcome and the infarct volume.

Methods—Polyamines were measured in 16 patients with focal cerebral ischemia and in 8 healthy control subjects. Blood samples for polyamine measurement were taken at admission and at fixed time points for the next 28 days. Polyamines were analyzed in red blood cells by a high-pressure liquid chromatography system. Clinical findings were recorded with the NIH Stroke Scale score. Volume of infarction was analyzed from cranial CT at admission and on days 4 to 6 after ischemia.

Results—A significant increase of the spermidine level in the peripheral blood could be observed in all patients with focal cerebral ischemia as compared with control subjects ($P<0.01$), starting with the admission. Spermidine values correlated positively with the clinical outcome at several time points in the first 48 hours ($r=0.90$ to 0.40; $P<0.01$) and with the infarct volume in cranial CT on days 4 to 6 ($r=0.91$; $P<0.01$).

Conclusions—As hypothesized from experimental data, polyamine levels in blood increase in patients after focal cerebral ischemia. The results indicate that the peripheral spermidine level is closely associated with the clinical outcome as well as with the infarction volume. Therefore, polyamines may be used as a novel predictor for the prognosis of patients with focal cerebral ischemia. (Stroke. 2001;32:43-46.)

Key Words: biological markers cerebral ischemia, focal outcome polyamines

The endogenous polyamines putrescine, spermidine, and spermine are low-molecular-weight aliphatic amines that are found in high concentrations in the brain. Polyamine metabolism is regulated by the activity of the first key enzyme ornithine decarboxylase (ODC). Polyamines are predominantly found intracellularly, whereas only smaller amounts could be observed in the extracellular space or in the peripheral blood. Animal studies have shown that polyamines play an important role in the ischemic cascade. Polyamines activate N-methyl-D-aspartate (NMDA) receptors, followed by a calcium influx. Other possible mechanisms are calcium-related events at the cell membrane and release of neurotransmitters from nerve endings. However, polyamines have not been determined in clinical studies of cerebral ischemia, and their clinical significance is thus unknown.

Because polyamines are released from the intracellular compartment during focal cerebral ischemia, the aim of our study was to analyze their value as a marker of neuronal tissue destruction in the peripheral blood and a potential predictor for clinical outcome in the acute stage of stroke.

Subjects and Methods

Patients
The polyamine level was determined in 16 patients (6 women and 10 men; mean age ± SD, 70.4 ± 8.3 years) with a first-ever acute focal cerebral ischemia (<6 hours) of the middle cerebral artery (MCA) as determined by clinical signs and cranial CT. Adults of any age were included, and informed consent was obtained according to the Helsinki Declaration of Ethical Requirements. CT as well as blood samples were part of the routine workup.

Exclusion criteria were (1) brain stem or lacunar stroke, (2) a transient neurological deficit with rapid recovery during workup, (3) a previous ischemia in the same territory, and (4) primary or secondary cerebral hemorrhage with preexisting disability.

All patients underwent a complete cerebrovascular workup, including extracranial and transcranial Doppler ultrasound, electrocardiography, echocardiography, and serum investigations for exclusion of coagulation disorders.
An age-matched healthy group (68.8±15.3, n=8) with no previous neurological disorder or severe general disease served as control subjects. The level of polyamines was determined at the same time points as in the patient group.

**Neuroimaging**

All patients were scanned by cranial CT (GE ProSpeed SX Power, GE Medical Systems) at admission and on days 4 to 6 after ischemia. A set of 5-mm-thick, contiguous, axial CT images was obtained. The infarction volume was computed from the data set by the semiautomated infarct segmentation method. The volume of infarction was measured within each slice and calculated to give a total volume. The obtained volume was correlated to the entire MCA territory according to anatomic guidelines and defined as small infarctions (less than one third of the MCA territory), moderate infarctions (one third to two thirds of the MCA territory), and large infarctions (more than two thirds of the MCA territory).

**Polyamine Analysis**

Venous blood samples were collected without compression from all subjects at admission (within 6 hours after onset of symptoms), in the first 2 days every 6 hours, and on days 3, 5, 7, 14, and 28 after the onset of neurological symptoms. One patient died after 7 days and was excluded from further statistical analysis.

Cooled heparinized blood samples were immediately sedimented by centrifugation (5000 rpm, 2 minutes); plasma and leukocytes were carefully discarded. Erythrocytes were washed 3 times with isotonic NaCl, hemolyzed with distilled water, and extracted with HClO4. The extract was neutralized with KOH and frozen at −20°C.

The neutralized extracts were derivatized with o-phthalaldehyde, and polyamines were separated by means of a reversed-phase HPLC column (Partisil 10 ODS 3, CS-Chromatographie Service) and quantified by fluorescence detection.

**Statistical Analysis**

For the follow-up of the spermidine level, an unpaired Wilcoxon test with a Holm correction was used. For further evaluation, a multiple linear regression model was used. Statistical significance was set at P<0.01. Data are given as mean±SD.

**Results**

**Clinical Aspects**

In 6 patients, a cardioembolic source of stroke was likely because of atrial fibrillation, mitral valve operation with stop of anticoagulation therapy, mitral valve insufficiency, and global heart insufficiency. Three patients showed paradox embolism caused by a patent foramen ovale with a right-to-left shunt. The other patients had obstructions of the internal carotid artery; in 2 patients, the underlying cause of ischemia remained undetermined.

**Clinical Characteristics**

Risk factors were determined for smoking, alcohol, hypercholesterolemia, diabetes, hypertension, heart insufficiency, and coagulation disorders. No correlation was found between the polyamine level and either of these risk factors.

**NIH Stroke Score**

The mean of the NIH Stroke Score at admission was 14.4±5.6 (range, 6 to 23; median, 14.0). Three patients had a severe stroke (NIH score ≥22), 10 had a moderate stroke (NIH score 10 to 22), and 3 had a mild stroke (NIH score <10).

**Infarct Volume**

All patients showed embolic infarction on the follow-up CT scan on day 4 after ischemia. The mean infarct volume was 95.1±98.2 mm³. In 2 patients, no early signs of stroke could be detected in the initial CT scan. In the control CT on day 4, all patients revealed an ischemic area with small infarcts in 7 patients, moderate infarctions in 5 patients, and large infarctions in 4 patients.

**Spermidine Level in Red Blood Cells**

In the control group, mean blood level for spermidine was 5.9±1.7 nmol/mL erythrocytes (range, 2.3 to 10.7 nmol/mL erythrocytes). There were no significant alterations in the spermidine level throughout the entire observation period (Figure 1). The polyamines spermine and putrescine were not detectable in each blood sample taken but showed a similar tendency as the spermidine values (data not shown). During the entire observation period, the spermidine level was higher than the level in the control group, with a marked increase at day 14 followed by a drop on day 28 (Figure 1).

During the observation period, the spermidine level showed a nonsignificant generalized increase compared with...
control subjects (Figure 1). A significant elevation of spermidine level in red blood cells compared with control values after focal cerebral ischemia could be seen at admission ($P<0.0027$), further at 6 hours after ischemia ($P<0.0085$), and on days 7 and 14 ($P<0.0004$, $P<0.0005$, respectively), followed by a marked drop on day 28 (Figure 1).

**Correlation of Spermidine Level With Clinical Outcome and Infarct Volume**

For the determination of polyamines as a prognostic predictor, the spermidine levels were correlated with the clinical outcome (NIH Stroke Score on day 28) as well as with the infarct volume. At admission, there already was a significant correlation between the spermidine level and the NIH Stroke Score at day 28 ($r=0.90$; $P<0.01$) as well as with the infarct volume ($r=0.91$; $P<0.01$). Furthermore, there was a significant statistical correlation between several spermidine levels within the first 48 hours and clinical outcome (Figure 2). No significant correlation was achieved between the final NIH score and the spermidine level at 18 hours and days 7 to 14, respectively.

**Discussion**

Several markers of neuronal tissue destruction such as neuron-specific enolase, lactate, myelinbasic proteins, proteolipid protein, and S-100 protein have been investigated to provide quantitative information about the extension of infarction to have early information for clinical prognosis. Recent studies have shown that there is a continuous release of polyamines from cells into the extracellular space to regulate cellular concentration. Measurements of polyamine metabolism in brain slices after focal cerebral ischemia showed a marked increase in the ODC activity and an overshoot of the putrescine concentration, which is catalyzed by ODC. However, spermidine and spermine showed a significant reduction after recirculation in severely damaged areas, indicating a release not only into the extracellular space but a clearing into the peripheral blood. This hypothesis is supported by the finding that polyamine release plays an important role in alteration of the blood-brain barrier in focal cerebral ischemia and is important in the development of late vasogenic edema. Furthermore, it has been shown that spermidine can also be released from the brain cells by depolarization stimuli, as observed in the case of hypoxia, and stimulates NMDA receptor activity.

Polyamines modulate NMDA receptors for the opening of a channel permeable for sodium, calcium, and potassium ions and therefore play an important role in the ischemic cascade. The mechanisms responsible for the transport of polyamines across the cell wall in the early phase of ischemia are not fully understood. After release from brain tissue, polyamines are mainly transported in the blood by erythrocytes. In the presence of serum, the affinity of red blood cells for spermidine is 30-fold greater than that for other polyamines, which may explain the difficulties in the measurement of spermine and putrescine.

![Figure 2. Correlations of the NIH Stroke Score at day 28 and spermidine level at different time points (Pearson correlation coefficients).](http://stroke.ahajournals.org/)
The aim of our study was to investigate whether polyamine levels were elevated in human blood after focal cerebral ischemia and whether there is a correlation with the clinical outcome as well as with the volume of infarction after hemispheric stroke. Our results suggest that the measurement of spermidine is an early marker for these parameters. The results support the finding in experimental studies of focal cerebral ischemia, which showed a release of polyamines from the intracellular compartment after ischemia.23,32,33 All patients in our study showed an embolicogenic territorial infarction. However, many patients with an acute neurological deficit have only lacunar strokes in vulnerable areas such as the basal ganglia with severe microangiopathic alterations, before hemodynamic infarctions or an intracerebral hemorrhage. In these patients, the elevation of polyamines may not be associated with the clinical outcome or the volume of infarction.

The pathophysiological pathway of polyamines in focal cerebral ischemia is not yet fully understood. There may be an influence of the calcium buffering capacity of mitochondria by spermine; on the other hand, polyamine metabolism may sensitize NMDA receptors during the ischemic cascade.35–38

Our results indicate that the measurement of polyamines may be a useful marker for the prediction of the clinical course of focal cerebral ischemia. Therefore, polyamines reveal neuronal injury and represent an ideal marker for clinical outcome. Thus, polyamine measurement may be of help in studies of new neuroprotective agents to determine the group of patients who show a therapeutic benefit. Further studies are necessary for patients with acute focal neurological symptoms and other underlying causes, the early diagnosis of infarction, the differentiation between transitory ischemic events and manifest infarction, and the effectiveness of therapeutic interventions.

References
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