Adenosine

A Sensitive Indicator of Cerebral Ischemia during Carotid Endarterectomy

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Background: For the human brain, there are no data available concerning the significance of adenosine and its metabolites as biochemical indicators of cerebral ischemia. Since adenosine may counteract key pathogenetic mechanisms during cerebral ischemia, its sensitivity and specificity as a marker of cerebral ischemia were investigated in relation to hypoxanthine and lactate.

Methods: Arterial and jugular venous concentration changes of adenosine, hypoxanthine, and lactate were studied in 41 patients undergoing carotid endarterectomy. Cerebral tissue oxygenation was monitored continuously by somatosensory-evoked potentials. A carotid artery shunt (n = 6) was placed only after complete loss of somatosensory-evoked potentials.

Results: Before carotid artery clamping jugular venous concentrations of adenosine, hypoxanthine, and lactate in subsequently shunted patients were 229 ± 88 nM, 1105 ± 116 nM, and 0.85 ± 0.52 mM, respectively (mean ± SD). In patients who required shunting, carotid artery clamping induced a significant increase in jugular venous adenosine (389 ± 114 nM) and jugular venous hypoxanthine (1444 ± 168 nM). In contrast, the increase in jugular venous lactate (0.91 ± 0.48 mM) did not reach statistical significance. Focal cerebral ischemia was indicated by jugular venous adenosine with a sensitivity and specificity of 0.83 and 0.71, respectively.

Conclusions: Carotid artery clamping induced significant increases in jugular venous adenosine and hypoxanthine in patients with inadequate collateral blood flow. In addition, focal cerebral ischemia was reflected by changes in adenosine concentrations. (Key words: ATP degradation products; brain ischemia; human.)

In animal experiments adenosine has been shown to be a sensitive indicator of cerebral ischemia and a modulator of cerebral perfusion. In addition, because of its actions on membrane ion conductances and intracellular metabolic signaling in neurons and glial cells, adenosine exerts neuroprotective effects attenuating the toxic release of excitatory amino acids and excessive intracellular accumulation of free ionic calcium. Furthermore, adenosine can effectively inhibit the free oxygen radical production of human polymorphonuclear leukocytes, which is an important protective function during reperfusion, because activated neutrophils may be causally involved in reperfusion injury. In the human brain, however, no data are available that characterize the role of adenosine and its metabolites during ischemia and reperfusion in patients.

The present study investigated the sensitivity and specificity of purine compounds to indicate cerebral ischemia in patients undergoing carotid endarterectomy. Focal cerebral ischemia was verified by monitoring somatosensory-evoked potentials (SSEPs), and a carotid artery shunt was placed to restore cerebral perfusion when total loss of SEP amplitude occurred.

Materials and Methods

Patients

After institutional approval, informed consent was obtained from 41 patients (age 67 ± 9 yr) undergoing carotid endarterectomy. In all but one patient elective surgery was performed because of high grade obstruction of carotid arteries. One patient with acute signs of cerebral ischemia underwent emergency surgery. Except for six patients without prior neurologic symptoms,
Table 1. Characteristics of Unshunted (n = 35) and Shunted Patients (n = 6)

<table>
<thead>
<tr>
<th>Age (mean ± SD) (yr)</th>
<th>Unshunted Patients</th>
<th>Shunted Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>31</td>
<td>4</td>
</tr>
<tr>
<td>Male</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Neurologic classification [number (%)]</td>
<td>Unshunted Patients</td>
<td>Shunted Patients</td>
</tr>
<tr>
<td>Asymptomatic lesions</td>
<td>5 (14)</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Transient ischemic attack</td>
<td>23 (66)</td>
<td>4 (67)</td>
</tr>
<tr>
<td>Minor stroke</td>
<td>7 (20)</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Angiographic aspects [number (%)]</td>
<td>Unshunted Patients</td>
<td>Shunted Patients</td>
</tr>
<tr>
<td>Ipsilateral carotid artery</td>
<td>Unshunted Patients</td>
<td>Shunted Patients</td>
</tr>
<tr>
<td>Stenosis 70-90%</td>
<td>33 (94)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>Stenosis &gt;91%</td>
<td>2 (6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Contra lateral carotid artery</td>
<td>Unshunted Patients</td>
<td>Shunted Patients</td>
</tr>
<tr>
<td>Stenosis &lt;70%</td>
<td>26 (74)</td>
<td>3 (50)</td>
</tr>
<tr>
<td>Stenosis 70-99%</td>
<td>3 (9)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Occlusion</td>
<td>6 (17)</td>
<td>3 (50)</td>
</tr>
<tr>
<td>Disturbance of ipsilateral middle cerebral artery blood flow</td>
<td>8 (23)</td>
<td>2 (33)</td>
</tr>
</tbody>
</table>

the indication for carotid endarterectomy was symptomatic high grade carotid stenosis ≥ 70%. Preoperatively, the clinical status of the patients was determined according to their history of neurologic symptoms and angiography of the cerebral vasculature (table 1).

**Antesthesia**

After premedication with midazolam (3.75-7.5 mg), anesthesia was induced with 1-3 mg midazolam, 2-5 μg/kg fentanyl, 0.15-0.3 mg/kg etomidate, and 0.5 mg/kg atracurium. Following intubation, anesthesia was maintained with nitrous oxide in oxygen (N₂O:O₂ = 50:50) and 0.2–0.6% isoflurane. Atracurium and fentanyl were subsequently administered as necessary. All patients were mechanically ventilated to maintain normocapnia with PaCO₂ of 38–41 mmHg. In each patient ECG data and end-tidal CO₂ and arterial blood pressure changes using a 20-gauge radial artery catheter (Abbott, Wiesbaden, Germany) were continuously recorded. A thorough neurologic examination was performed immediately after the patient regained consciousness, 1 h later, and then daily until the patient was discharged.

**Intraoperative Monitoring Techniques**

After induction of general anesthesia, SSEPs were recorded following contralateral median nerve stimulation (Nicolet “Spirit”). Subdermal needle electrodes were placed over the second cervical vertebra and the areas of the ipsilateral somatosensory cortex (C3' and C4'), respectively. The reference electrode was placed on the forehead (Fpz). For one SSEP recording 256 stimulations were averaged. Baseline SSEP tracings (n = 5) were recorded immediately preoperatively for which the mean value was set to 100%. It is important to note that the indication for shunt placement was exclusively dictated by the changes in SSEP when a complete loss of the N20/P25 SSEP amplitude was registered during carotid cross-clamping. According to this criterion intraoperative shunting of the carotid artery was performed in 6 of 41 patients. Intraoperatively, a jugular venous catheter was introduced under direct fluoroscopic guidance into the ipsilateral jugular bulb by the surgeon to determine the concentrations of lactate, adenosine, and hypoxanthine in jugular venous blood. The correct catheter position in the jugular bulb during the surgical procedure was again verified by routine intraoperative complementation-angiography at the end of the operation. After endarterectomy and reperfusion of the carotid artery, angiography was performed by injection of contrast medium in the common carotid artery and fluoroscopy in an anteroposterior and lateral projection. Before carotid cross-clamping, all patients received heparin (5,000 U) and hydroxyethyl starch.

Parameters were determined before carotid cross-clamping (C₁), at 10 min of carotid artery occlusion, immediately before reperfusion, at short-term intervals during reperfusion (30 s, 60 s, 90 s, 120 s, 150 s, and 180 s), and 15 min after reperfusion (C₂), respectively. The dead volume of the jugular venous catheter (1 ml) was discarded before blood samples were taken.

In patients without shunt placement, removal of the carotid artery clamp after endarterectomy was taken as start of the reperfusion period. In patients with shunt placement, however, opening of the shunt after its insertion was defined as the beginning of reperfusion.

**Analysis of Lactate and Purine Compounds**

For lactate determination 2 ml of blood were collected in precooled syringes containing fluoride/EDTA (4.0 mm) and immediately stored on ice. Lactate was measured using enzyme methods.²

Purine compounds were measured as previously described.¹⁰ In brief, blood samples (1 ml) were collected in precooled dipyrindamole solution (1 ml, 5 × 10⁻⁵ M, 4°C) to prevent nucleoside uptake by erythrocytes.¹¹ After immediate centrifugation at 4°C, plasma supernatant (1 ml) was deproteinated with perchloric acid (70%, 0.1 ml). Following neutralization (KH₂PO₄) and centrif-
igation, nucleosides were determined by high-performance liquid chromatography. One hundred microliters were automatically injected onto a C-18 column (NovaPak C18, 3.9 mm × 150 mm; Waters, Eschborn, Germany). A linear gradient that started with 100% KH₂PO₄ (0.001 M, pH 4.0) and increased to 60% of 60/40 methanol/water (vol/vol) in 15 min at a flow rate of 1.0 ml/min. This was followed by a reversal of the gradient to initial conditions over the next 3 min. Absorbance of the column eluate was monitored at 254 nm (adenosine, hypoxanthine) using a photodiode array detector (Waters). Purine compounds were quantified with a computer assisted program (Millenium; Waters).

In in vitro experiments blood samples were aspirated through the same catheter type as inserted into the jugular bulb and known amounts of adenosine were added to the human blood. Under these conditions the recovery of adenosine was 81 ± 4% (n = 5).

**Statistical Analysis**

Results are expressed as mean ± SD. All data were tested for normal distribution by the Kolmogorov-Smirnov test. Differences within or between groups were analyzed by analysis of variance followed by a post hoc Scheffé test. To avoid an enormous number of multiple comparisons, statistical analysis was performed only for the following time points: C1, before reperfusion, the peak value during reperfusion, and C2. To calculate sensitivity and specificity for the maximal increase in jugular venous metabolite concentration (x-fold of control) to predict cerebral ischemia, the best cut-off point was determined by inspection of the data to provide the best separation of patients with (shunted) or without (unshunted) cerebral ischemia. Statistical significance is at the P < 0.05 level.

**Results**

As can be seen in table 1, shunt placement became necessary in six patients without adequate collateral blood flow. In the remaining patients SSEPs showed only minor changes during carotid artery occlusion (table 2). SSEP amplitude was totally abolished in all shunted patients within 16 ± 4 min after carotid artery occlusion. The recovery of SSEP amplitude was obtained within 3 min (R₃) of reperfusion. No differences were found in perioperative hemodynamic parameters, PaCO₂, or hemoglobin concentrations between the groups throughout the observation period (table 2). It is important to note that

<table>
<thead>
<tr>
<th>Table 2: Somatosensory Evoked Potentials, Parameters of Hemodynamic Data, and Blood Gas Analysis</th>
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<tbody>
<tr>
<td>Unshunted Patients (n = 30)</td>
</tr>
<tr>
<td>Occlusion</td>
</tr>
<tr>
<td>10 min</td>
</tr>
<tr>
<td>C1</td>
</tr>
<tr>
<td>SSEP (mV)</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
</tr>
<tr>
<td>HR (min⁻¹)</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
</tr>
<tr>
<td>pH</td>
</tr>
</tbody>
</table>

C1 = before carotid cross-clamping; C2 = 6 min in unshunted or 16 ± 4 min in shunted patients; before reperfusion; R₃ = 3 min after reperfusion; SSEP = values given as percent of the individual N20/25 amplitude on somatosensory evoked potentials after induction of anesthesia; MAP = mean arterial pressure; HR = heart rate; PaCO₂ = arterial carbon dioxide tension; PaO₂ = arterial oxygen tension.

*P < 0.05 versus C1, *P < 0.05 versus C2.
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Table 3. Arterial and Jugular Venous Concentrations of Lactate, Adenosine, and Hypoxanthine

<table>
<thead>
<tr>
<th></th>
<th>Unshunted Patients (n = 35)</th>
<th>Shunted Patients (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C1 33 ± 6 min</td>
<td>Peak</td>
</tr>
<tr>
<td>Lactate (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>0.71 ± 0.27</td>
<td>0.69 ± 0.20</td>
</tr>
<tr>
<td>jv</td>
<td>0.73 ± 0.24</td>
<td>0.72 ± 0.25</td>
</tr>
<tr>
<td>Adenosine (nM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>263 ± 67</td>
<td>279 ± 73</td>
</tr>
<tr>
<td>jv</td>
<td>266 ± 86</td>
<td>318 ± 66</td>
</tr>
<tr>
<td>Hypoxanthine (nM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>956 ± 137</td>
<td>946 ± 146</td>
</tr>
<tr>
<td>jv</td>
<td>1,051 ± 141</td>
<td>1,214 ± 178</td>
</tr>
</tbody>
</table>

Values are mean ± SD.
C1 = before carotid cross-clamping; 33 ± 6 min in unshunted or 16 ± 4 min in shunted patients = before reperfusion; C2 = 15 min after reperfusion; a = arterial; jv = jugular venous.
*P < 0.05 versus C1.
†P ≤ 0.05 shunted versus unshunted patients.

that the mean occlusion time in unshunted patients was 33 ± 6 min and significantly longer than in the shunted group (16 ± 4 min). In the latter group (n = 6) shunt opening was taken as the start of reperfusion.

**Brain Metabolites**

At baseline, arterial and jugular venous lactate concentrations were significantly higher in the shunted than in unshunted patients (table 3). While the arterial and jugular venous concentrations remained constant in the unshunted group during the observation period, the jugular venous lactate concentration in the shunted group was significantly increased during reperfusion.

No significant differences between shunted and unshunted patients in arterial or jugular venous concentrations of adenosine and hypoxanthine were observed at baseline. In shunted patients a significant increase in jugular venous concentrations of both metabolites were found already at the end of the clamping period and during reperfusion.

The changes in jugular venous — arterial metabolite concentration differences are shown in figure 1. In shunted patients, clamping of the carotid artery induced significant increases in jugular venous — arterial concentration differences of lactate, adenosine, and hypoxanthine. In contrast to lactate, which was still elevated 15 min after reperfusion, adenosine and hypoxanthine showed a rapid washout from brain tissue, reaching control values within the observation period.

In figure 2 the relationship between the increase in jugular venous — arterial metabolite concentration differences during cross-clamping of the carotid artery in shunted patients and the time of focal cerebral ischemia is displayed. Particularly at 16 ± 4 min, the jugular venous — arterial concentration difference of adenosine is significantly higher than that of lactate or hypoxanthine.

The receiver operating characteristic (ROC) curves for jugular venous adenosine, hypoxanthine, and lactate indicating cerebral ischemia are shown in figure 3. Based on a 1.3-fold increase in jugular venous adenosine as the best cut-off point, the sensitivity and specificity of adenosine for the prediction of cerebral ischemia were 0.83 and 0.71, respectively (table 4).

**Discussion**

During normoxia the concentration of tissue ATP is maintained by the balance of ATP degradation and ATP synthesis. During cerebral ischemia, however, ATP synthesis is reduced despite steady use of ATP, leading to enhanced degradation of AMP to adenosine by activated 5'-nucleotidase. Berne et al.12 demonstrated with animal experiments that adenosine is intracellularly formed during brain ischemia and released *via* cellular diffusion13 and nucleoside transporters14 to enhance the extracellular adenosine concentration.1 In addition, extracellular adenosine can also be formed during ischemic activation of N-methyl-D-aspartate (NMDA) receptors, when 5'-AMP is released and converted to adenosine by activation of ecto-5'-nucleotidase.15 Although the role of adenosine during brain ischemia has been characterized in animal experiments, its significance has not been studied in patients when cerebral blood flow (CBF) is disturbed by ipsilateral carotid artery occlusion. Thus, the present study is the first attempt to characterize the interrelation between cerebral tissue oxygenation and brain energy metabolism in humans.

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In patients without shunt placement no significant changes in SSEP were observed during carotid artery clamping (33 ± 6 min). Obviously, these patients exhibited sufficient collateral blood flow to the occlusion-dependent brain area, because no major changes in Δlactate and only slight increases in Δadenosine were obtained. These results are confirmed by the study of Haljamae et al., who did not find increased jugular venous lactate concentrations at the end of the clamping period in patients without signs of cerebral ischemia.

According to the adenosine hypothesis, any decrease in cerebral tissue oxygenation should be accompanied by increases in the adenosine production. Moreover, Matsumoto et al. demonstrated enhanced extracellular purine concentrations in the presence of a reduction in CBF. In particular, focal brain ischemia in cats was induced by occlusion of the middle cerebral artery and the threshold of cerebral blood flow for an increase in the extracellular concentrations of adenosine, inosine, and hypoxanthine was determined to be at a CBF just below 25 ml·100 g⁻¹·min⁻¹. When CBF was lowered to between 25-20 ml·100 g⁻¹·min⁻¹, adenosine was already markedly increased (5- to 15-fold), and adenosine was even more enhanced (6- to 76-fold) at a CBF below 20 ml·100 g⁻¹·min⁻¹. In addition, using nuclear magnetic resonance spectroscopy, Naritomi et al. demonstrated that ATP and phosphocreatine were significantly depleted at threshold values of CBF of 12-14 ml·100 g⁻¹·min⁻¹ and 18-23 ml·100 g⁻¹·min⁻¹, respectively. The results of the present study in the shunted group demonstrate that a complete loss of adequate collateral blood flow; shunted patients are those with inadequate collateral blood flow. Cₘ = before carotid cross-clamping; O₀ = at 10 min of carotid artery occlusion; Oₙ = before reperfusion, which occurred at 33 ± 6 min of carotid artery occlusion in unshunted and 16 ± 4 min in shunted patients; Rₚ = 3 min after reperfusion; Cₚ = 15 min after reperfusion; Δ = jugular venous – arterial concentration differences of the respective parameters. Values are given as mean ± SD. Statistical analysis was performed only for the following time points: Cₘ, 33 ± 6 min in unshunted or 16 ± 4 min in shunted patients, the peak value during reperfusion, and C₂. #P ≤ 0.05 shunted versus unshunted; *P ≤ 0.05 versus C₁.
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Fig. 2. Relationship between changes in jugular venous – arterial metabolite (Δ metabolite) concentration (x-fold of control) during carotid artery cross-clamping and the time of focal cerebral ischemia in patients with inadequate collateral blood flow (shunted patients). Values are given as mean ± SD; #P < 0.05 versus hypoxanthine; *P < 0.05 versus lactate.

Xanthine, and lactate in the ipsilateral jugular venous outflow. Furthermore, when brain ischemia was determined by a complete loss of SSEP the sensitivity and specificity of adenosine to indicate cerebral ischemia were rather high (0.84 and 0.71, respectively). The increase in cerebral metabolites reflects a considerable impairment in the energy metabolism of the human brain. Particularly, adenosine was enhanced in proportion to the time of focal cerebral ischemia (fig. 2). In addition, both adenosine and hypoxanthine showed a characteristically transient and rapid washout from brain tissue, which is inversely proportional to the changes in cerebral tissue oxygenation. The slightly later occurrence of the reperfusion peak in the case of hypoxanthine is most likely explained by the time-related enzymatic degradation sequence of adenosine to hypoxanthine via inosine.

Adenosine, however, is not only a sensitive indicator of cerebral tissue oxygenation, but exhibits protective functions during brain ischemia. When gerbils were pretreated with the adenosine antagonist theophylline and then subjected to a short period of global forebrain ischemia, they showed moderately aggravated neurologic symptoms and significantly enhanced ischemic cell damage. The protective effect of adenosine during cerebral ischemia is mediated by a variety of functions of adenosine as a physiologic cell modulator. Endogenous adenosine can counteract the deleterious rise in free cytosolic Ca2+, which plays a key role in ischemic brain damage. In addition, the vasodila-

Fig. 3. Receiver operating characteristic (ROC) curves of cerebral ischemia for x-fold increases in jugular venous concentrations of adenosine, hypoxanthine, and lactate. Solid line = point by point ROC curve; Dotted line = best fit ROC curve.
arterial concentration difference of lactate was still elevated at the end of the study period. This finding could be explained by postischemic, reperfusion-dependent inhibition of pyruvate dehydrogenase, which may determine the degree to which glucose is metabolized aerobically versus anaerobically.27 As a consequence of anaerobic glycolysis during ischemia cells are acidified. In complete or near complete brain ischemia, tissue pH can fall to values as low as 6.5.28 Tissue acidosis may then promote edema formation by intracellular accumulation of Na⁺ and Cl⁻ as well as by inhibition of mitochondrial metabolism and H⁺ extrusion. Furthermore, acidosis interferes with calcium binding thereby contributing to a harmful rise in intracellular Ca²⁺.5 On the other hand, however, mild acidosis may also protect neurons from injury by blocking NMDA-mediated calcium influx.29 In addition, brain lactate is an obligatory aerobic energy substrate for functional recovery after hypoxia.30 In humans it is well accepted that enhanced production of lactate is a parameter of cerebral ischemia and that a jugular venous-arterial lactate difference of more than 0.3 mM is indicative of global brain ischemia in the clinical setting. If this condition persists, it will result in neuronal damage.31,32

One might argue that determination of jugular venous adenosine does not reflect actual concentration changes in the immediate vicinity of the ischemic brain area. Because the jugular bulb is a dilatation of the rostral internal jugular vein just below the jugular foramen, and only 2.7% (range, 0-6.6%) of the blood in the jugular bulb is derived from extracerebral structures,33 this fact indicates that the majority of the blood is collected from intracerebral tissue. However, blood from ischemic brain tissue is diluted with blood originating from the nonischemic regions of the brain. Furthermore, large variations in bilateral jugular venous lactate concentrations, although determined simultaneously, have been found after severe head injury.34 In patients with a predominant unilateral lesion, the highest sensitivity to detect cerebral ischemia was obtained by monitoring on that side. This supports our approach to determine metabolites in the ipsilateral jugular venous outflow, because focal cerebral ischemia during carotid endarterectomy occurs at the site of the operation. In addition to admixture of blood from nonischemic brain regions, factors including rapid incorporation and degradation of adenosine by erythrocytes and endothelial cells as well as enzymatic degradation by plasma adenosine deaminase to vasoinactive inosine can potentially alter the plasma concentrations of the vasoactive nucleoside.35 In the present study blood was collected in syringes containing ice-cold dipyridamole solution to prevent inactivation of adenosine by erythrocytes. In addition, samples were rapidly processed in the cold and plasma proteins were denaturated by the addition of perchloric acid. Taking all “dilutional” factors into account, measured changes in jugular venous adenosine actually underestimate by far the local nucleoside release at the site of its production. This explains why the percentage increases of jugular venous-arterial adenosine concentration differences are rather low in this clinical study in comparison with animal experimental studies measuring extracellular purine concentrations by microdialysis.1,36

In conclusion, jugular venous—arterial concentration differences of vasoactive adenosine in humans are temporarily increased during ischemia and reperfusion and are inversely proportional to changes in cerebral tissue oxygenation. In addition, adenosine indicates cerebral ischemia with a sensitivity and specificity of 0.83 and 0.71, respectively. The data presented suggest that adenosine plays a significant role during ischemia and reperfusion in the human brain. Moreover, because adenosine sensitively reflects disturbances in cerebral tissue oxygenation, this nucleoside is well suited to examine the action of drugs that are used clinically to improve cerebral perfusion.

References

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