Effects of Hypothermia on Excitatory Amino Acids and Metabolism in Stroke Patients
A Microdialysis Study

Christian Berger, MD; Wolf-Rüdiger Schäbitz, MD; Dimitrios Georgiadis, MD; Thorsten Steiner, MD; Alfred Aschoff, MD; Stefan Schwab, MD

Background and Purpose—The objective of this study was to assess the effect of therapeutic moderate hypothermia on excitatory amino acids and metabolism by applying cerebral microdialysis in patients suffering from space-occupying middle cerebral artery infarction.

Methods—This was an open, prospective, observational study of 12 patients undergoing moderate hypothermia (33°C) as rescue therapy for large, life-threatening middle cerebral artery infarction. Microdialysis probes were placed concomitantly with intracranial pressure (ICP) measuring devices in the frontal lobe of the infarcted and/or noninfarcted hemisphere. Using the CMA 600 Microdialysis Autoanalyzer, we analyzed glutamate, glycerol, pyruvate, and lactate.

Results—According to follow-up cranial CT scans, 3 different compartments of microdialysis measurements could be defined. First, noninfarcted brain tissue had stable dialysate concentrations but a significant effect of hypothermia on glutamate (2.6 versus 3.6 μmol/L), lactate (1.8 versus 3 mmol/L), and pyruvate (50 versus 95.8 μmol/L). Second, measurements from peri-infarct tissue had a significant effect of hypothermia on glutamate (4.8 versus 12.6 μmol/L), glycerol (58 versus 82 μmol/L), lactate (0.7 versus 1.3 mmol/L), and pyruvate (13.3 versus 36.8 μmol/L). Third, dialysate concentrations obtained from irreversibly damaged tissue were excessive for glutamate (453 μmol/L), glycerol (1187 μmol/L), lactate (12 μmol/L), and pyruvate (4 μmol/L). In this extreme compartment, no effect of hypothermia was observed.

Conclusions—Cerebral microdialysis is a safe and feasible bedside method for neurochemical monitoring indicating normal brain tissue, potentially salvageable brain tissue, and irreversibly damaged areas in stroke. We could demonstrate that hypothermia decreases glutamate, glycerol, lactate, and pyruvate in the “tissue at risk” area of the infarct but not within the infarct core. Thus, future treatment strategies for life-threatening stroke should be guided by close neurochemical monitoring. (Stroke. 2002;33:519-524.)

Key Words: cerebral metabolism ■ excitatory amino acids ■ hypothermia ■ microdialysis ■ stroke

Large, space-occupying hemispheric strokes carry a high mortality risk of up to 80% because of fatal brain edema formation. Their treatment is a major challenge for modern neurointensive care units and requires close monitoring of vital parameters and intracranial pressure (ICP) to ensure sufficient perfusion pressure. Besides the use of osmotic agents to reduce brain edema and mortality, more invasive techniques such as craniotomy and moderate hypothermia have been applied recently. Experimental studies in rats demonstrated a beneficial effect of mild hypothermia on the release of excitatory amino acids such as glutamate, dopamine, and aspartate. Their suppression and an overall decrease in cerebral metabolism could be responsible for a possible neuroprotective effect. So far, this has not been shown in patients, because monitoring these changes is not part of a routine monitoring program in intensive care units.

Cerebral microdialysis is currently the only technique that allows continuous monitoring of a variety of substances, including excitatory amino acids and metabolites from the extracellular fluid (ECF). Most microdialysis studies have been applied in neurosurgical patients undergoing frontal lobe resection, after severe head injury or subarachnoid hemorrhage, during temporal lobe resection during aneurysm surgery, and recently in hemispheric stroke. The safety and feasibility of this method were demonstrated in several microdialysis studies.

We applied cerebral microdialysis in patients suffering from large, space-occupying middle cerebral artery (MCA) infarction undergoing moderate hypothermia. The objectives of this study were to assess the effect of therapeutic hypothermia on the concentrations of glutamate, glycerol, lactate, and pyruvate in the ECF of the infarcted and noninfarcted
brain hemisphere, to test the hypothesis that glutamate is decreased under hypothermia, and to evaluate the predictive value of neurochemical monitoring with respect to potentially salvageable brain tissue and its role as a potential therapeutic guide in large, life-threatening MCA infarction.

**Patients and Methods**

This open, prospective, observational study requiring written consent from the patient’s next of kin was approved by the local Ethics Committee (approval number 34/97). We included patients suffering from a clinically severe stroke syndrome with deteriorating level of consciousness, hypotension on initial cranial computed tomography (CCT) scan exceeding two thirds of the MCA territory, and a perfusion deficit of the whole MCA territory on perfusion-weighted MRI. Besides antiedema therapy with mannitol, patients were treated with moderate hypothermia (33°C) as rescue therapy as described previously. Briefly, a cooling blanket (Polar Bair, Augustine Medical) with cool ventilator air fanning the patient’s body surface was used for external cooling. Once the body core temperature reached 33°C, it was kept between 33°C and 34°C for 48 to 72 hours. During the next 24 hours, the patient was passively rewarmed to a normal temperature if ICP could be kept below critical levels of 20 mm Hg. All patients were sedated with midazolam and fentanyl, received neuromuscular blockade with continuous infusion of atracurium (0.3 to 0.6 mg/kg IV), and remained mechanically ventilated during all neurosurgical procedures and throughout the hypothermia phase until rewarming was accomplished.

A 10-mm, flexible microdialysis probe (M, 20-kDa cutoff) with an external diameter of 0.62 mm (CMA/70 custom probe, CMA/Microdialysis) and an ICP measuring device (Codman) were inserted through the same burr hole into the frontal lobe of the infarcted or noninfarcted hemisphere or both. The probe position within the infarcted hemisphere was aimed at the penumbral zone as defined by the area of a perfusion-diffusion mismatch on MRI. The microdialysis probes were perfused at 0.3 mL/min with a sterile isotonic solution containing Na+ 147 mmol/L, K+ 4.0 mmol/L, Ca++ 2.3 mmol/L, and Cl− 156 mmol/L, and the dialysates were sampled in microvials. Each microvial was to be replaced after 60 to 120 minutes. We measured the concentrations of glutamate, glycerol, pyruvate, and lactate online using the CMA 600 Microdialysis Analyzer. The CMA 600 Microdialysis Analyzer is a self-calibrating autoanalyzer designed for microdialysis samples measuring glutamate, glycerol, pyruvate, and lactate as the rate of formation of a colored substance (quinoneimine or quinonedimine) in a filter photometer at 546 nm. All reagents required for analysis were obtained from CMA Microdialysis.

Physiological variables such as blood gas analysis and blood pressure and clinical events were documented regularly and added to the database of the mainframe computer, which also was used for analysis of the microdialysis results.

Dialysate concentrations and ICP obtained from probes placed in the noninfarcted hemisphere were categorized into those measured during hypothermia (<34°C) and those measured after successful rewarming (normothermia; ≥36.5°C) and were compared as pooled time-averaged data by use of the nonparametric Mann-Whitney U test. To assess only values for noninfarcted tissue, data from patients with secondary ischemia were excluded from further analysis. Statistical analysis for microdialysis results and ICP obtained from the peri-infarct region of the infarcted hemisphere was performed similarly. Data obtained from probes placed in unsalvageable infarcted tissue as assessed on follow-up CCT scan were excluded from further analysis. In patients with bilateral probe placement, paired comparisons between the infarcted and noninfarcted hemisphere were performed by use of the Wilcoxon signed-rank test. Regression analysis was performed to assess the correlation between the various substances analyzed by microdialysis. Analyses were performed with StatView statistical software. A value of P < 0.01 was considered statistically significant.

**Results**

A total of 12 patients were included in this study. Their characteristics are given in the Table. Overall, we performed 4206 single analyses of dialysate concentrations with a mean of 292 analyses per patient over a measuring period of 68 ± 12 hours per patient. Microvials were regularly replaced every 60 to 120 minutes.

One patient (patient 6) died of malignant brain edema with subsequent contralateral and transtentorial herniation during the hypothermia period. Three other patients died at least 1 week after rewarming: 1 died of multiorgan failure and 2 died of cardiac failure. No complications of microdialysis and probe insertion such as hematoma or infusion edema were observed on follow-up CCT scans or clinically at discharge. In a postmortem examination of the patient who died of

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age, y</th>
<th>Infarcted Hemisphere</th>
<th>Measured Hemisphere</th>
<th>Start of Hypothermia After Stroke Onset, h</th>
<th>Duration of Hypothermia, h</th>
<th>Outcome</th>
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herniation, the probe position within the brain tissue could not be detected.

Microdialysis Results Obtained From the ECF of Noninfarcted Brain Tissue

Microdialysis results and ICP values obtained from the noninfarcted hemisphere of 8 patients under hypothermic (<34.0°C) and normothermic (>36.5°C) conditions are depicted in Figure 1A. In these patients, no obvious hypodensity surrounding the probe was detected on follow-up CCT scans, whereas patient 6 developed secondary ischemia and therefore was excluded from further analysis. During normothermia, the mean of glutamate concentrations significantly increased from 2.6 to 3.6 μmol/L, lactate increased significantly from 1.8 to 3.0 mmol/L, and pyruvate increased from 50.5 to 95.8 μmol/L. Glycerol concentrations were significantly higher during hypothermia (62.2 versus 22.4 μmol/L). The ratio of lactate to pyruvate (L/P) differed nonsignificantly between the hypothermic and normothermic periods (42.9 versus 37.6), as did the ICP (13.3 versus 14.5 mm Hg).

Figure 1. Microdialysis results from the noninfarcted hemisphere (A) and peri-infarct region of the infarcted hemisphere (B). Bars represent mean ± SEM. Concentrations are given in μmol/L for glutamate, glycerol, and lactate and in mmol/L for pyruvate. Glutamate and lactate results are multiplied by 10 for better discrimination on the bar plot. ICP is given in mm Hg. Dark bars indicate time-averaged values obtained during hypothermia (<34°C); light bars, time-averaged values obtained after rewarming to normothermic conditions (>36.5°C). * P < 0.01 for comparison between hypothermic and normothermic phases.

During normothermia, the probe position within the brain tissue could not be detected.

Microdialysis Results Obtained From the ECF of Peri-Infarct Brain Tissue

At the penumbral stage were similar to those obtained from the infarct core. In the remaining 5 patients, paired comparisons demonstrated significant differences (peri-infarct versus noninfarcted tissue) for glutamate (5.8 versus 2.4 μmol/L), glycerol (56 versus 31 μmol/L), the L/P ratio (83 versus 43), and pyruvate (12.3 versus 38.1 μmol/L) during hypothermia, whereas ICP and lactate concentrations were not significantly different. After rewarming, significant differences were still observed for glutamate (11.3 versus 3.6 μmol/L), glycerol (87 versus 22.6 μmol/L), the L/P ratio (75 versus 38), and pyruvate (42.3 versus 97.1 μmol/L) but again not for ICP and lactate.

Microdialysis Results From the ECF of the Infarct Core

Analyses obtained from probes within the infarct core in 2 patients as seen on CCT scan differed from concentrations measured in the penumbral region with a tendency toward more extreme values: glutamate concentrations reached 453 μmol/L; glycerol, 1187 μmol/L; lactate, 12 mmol/L; and the L/P ratio, 582. Pyruvate decreased to a minimum of 4 μmol/L. These changes were unrelated to ICP values but changed drastically over time so that statistical comparison was not appropriate.

Performing regression analyses between various substances, we observed a clear correlation between lactate and pyruvate ($R^2 = 0.89$, P < 0.001) measured in the ECF of noninfarcted hemispheres both during hypothermia and after rewarming. This correlation was abolished in the ECF of the infarct core (Figure 2). Instead, we obtained a positive correlation between glutamate and glycerol ($R^2 = 0.89$, P < 0.001) and between glycerol and lactate ($R^2 = 0.76$, P < 0.001) in infarcted tissue, whereas this could not be observed in nonischemic tissue (Figure 3).

Discussion

This is the first report on cerebral microdialysis in a series of stroke patients treated with moderate hypothermia that describes concentration values for glutamate, glycerol, lactate, and pyruvate from the ECF of the infarcted and noninfarcted hemispheres under hypothermic (<34.0°C) and normothermic (>36.5°C) conditions. Measurements in the peri-infarct...
region led to significantly higher values for glutamate, glycerol, and the L/P ratio than in the noninfarcted hemisphere, whereas pyruvate remained in a significantly lower concentration range. Microdialysis performed in the core of the infarct region revealed extremely high concentrations of glutamate and glycerol and the L/P ratio. Similar results were obtained from measurements in the primarily noninfarcted hemisphere in 1 patient who developed secondary brain ischemia as a result of the herniating effect of the infarcted hemisphere. In all cases, ICP levels did not correlate with the results of microdialysis.

Baseline values were obtained from 8 patients without signs of secondary ischemia of the noninfarcted hemisphere on follow-up CCT scans. Concentrations for lactate, pyruvate, and glycerol and the L/P ratio under normothermic conditions are similar to those given for anesthetized patients undergoing neurosurgery in the posterior fossa. Measurements of glutamate concentrations in normal ECF are rare and vary, depending on the technique used for microdialysis. Reinstrup et al observed an average concentration of 7 µmol/L in awake patients and 17 µmol/L in anesthetized patients. In this study, we obtained an average concentration of 3.5 µmol/L under normothermic conditions, whereas Hillered et al reported normal concentrations of 1 to 2 µmol/L. In a study measuring glutamate during neurovascular operations, glutamate concentrations ranging from 8 to 25 µmol/L were obtained. Because volatile anesthetics are known to attenuate the release of glutamate and to increase its uptake into nerve cells, variations in glutamate concentrations between different studies might be explained by the use of different sedatives. Differences might also arise from the use of different perfusion velocities (0.3 versus 1.0 µL/min) and different membrane lengths of microdialysis catheters.

Pyruvate and lactate represent important end products of the aerobic and anaerobic metabolism that derive mainly from the glycolytic chain degrading glucose via fructose-1,6-diphosphate, and glyceraldehyde-3-phosphate to pyruvate. They are contained in a redox equilibrium with NADH. This equilibrium is shifted to the side of pyruvate under aerobic conditions and to the side of lactate under anaerobic conditions. This balance is expressed by a linear correlation between lactate and pyruvate or numerically by the L/P ratio. We observed a positive correlation and a stable L/P ratio in noninfarcted brain tissue under both normothermic and hypothermic conditions, indicating an intact L/P redox equilibrium. This L/P ratio increased significantly in penumbral regions and even more so in the irreversibly damaged brain tissue. Here, the correlation between lactate and pyruvate was abolished.

Glutamate serves as a neurotransmitter and plays a crucial role as excitatory amino acid. Excessive concentrations have been measured in primary or secondary ischemic brain tissue. Mild hypothermia is assumed to exhibit a neuroprotective effect on the ischemic brain by attenuation of the

Figure 2. Positive correlation between lactate and pyruvate concentrations obtained from the noninfarcted hemisphere (P<0.001). This correlation was completely abolished in irreversibly damaged tissue of the infarcted hemisphere. Data are pooled for hypothermia and normothermia.

Figure 3. Positive correlation between glutamate and glycerol concentrations obtained from measurements in the infarct core (P<0.001) but not in the noninfarcted hemisphere (R²=0.003). Data are pooled for hypothermia and normothermia.
release of excitatory amino acids. However, these studies have been performed with hypothermia starting before the ischemic event. Boris-Moller and Wieloch even questioned the hypothesis that neuroprotection is achieved by abolishing glutamate increase. They found an attenuation of glutamate only in the striatum, whereas cortical glutamate levels, although lower at baseline, were not attenuated by hypothermia. In this study, 3 different glutamate levels reflect 3 different conditions of neuronal tissue. First, baseline concentrations of glutamate in noninfarcted tissue were significantly lower during hypothermia. Second, glutamate levels that were elevated 3- to 4-fold and decreased to baseline levels during hypothermia indicated peri-infarct region and salvageable brain tissue. Third, glutamate levels in infarcted and irreversibly damaged brain tissue were excessively high during hypothermia. Thus, hypothermia appears to exert a neuroprotective effect by glutamate attenuation in penumbral regions and also possibly within the infarct core, although we could not demonstrate this statistically.

In the infarct core, glutamate concentrations are independent of brain temperature. Its release into the ECF can reflect a vesicular release from the neurotransmitter pool as a result of depolarization, inhibition of the cellular reuptake mechanism, leakage from dying cells undergoing autolysis, or a disturbed blood-brain barrier. We observed a positive correlation between glutamate and glycerol in the infarct core region. Thus, we assume that most glutamate molecules were released by autolysis of neuronal cells, because glycerol is one of the end products of membrane phospholipid degeneration, reflecting the disruption of cellular membranes leading to neuronal autolysis.

In clinical practice, microdialysis is a useful monitoring technique to detect the development of secondary neuronal ischemia and to assess the potential reversibility of ischemic damage. Thus, it may be more sensitive than EEG and somatosensory evoked potentials monitoring. ICP monitoring in patients with large, space-occupying infarcts is important and superior in its time resolution, but monitoring of glutamate is an earlier predictor of irreversible brain damage. At least in penumbral brain tissue, early glutamate alterations are not correlated with ICP measurements. Other studies confirmed the value of cerebral microdialysis as a monitoring tool for the acutely injured brain to detect impending danger for still viable brain tissue. However, the method is invasive, and positioning the probes into penumbral tissue may be difficult even with advanced neuroradiological methods. Certainly, new treatment strategies for life-threatening hemispheric infarction such as hypothermia warrant close monitoring of all possible indicators of treatment success or failure. With further insight into cerebral metabolism and release of excitatory amino acids during ischemia, we might be able to guide therapy or develop new measures for the treatment of ischemia in the future.

In summary, cerebral microdialysis is a feasible bedside method for monitoring extracellular metabolic substances (lactate, pyruvate, glycerol) and neurotransmitters such as glutamate that indicate normal brain tissue, potentially salvageable brain tissue, and irreversibly damaged areas in stroke. We could demonstrate that hypothermia applied as a rescue therapy in large hemispheric stroke decreases glutamate, glycerol, lactate, and pyruvate in the “tissue at risk” area of the infarct but not within the infarct core. Thus, future treatment strategies for life-threatening stroke should be guided by close neurochemical monitoring.

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References


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