Brief Report

Excitotoxicity and Metabolic Changes in Association With Infarct Progression

Johannes Woitzik, MD; Alexandra Pinczolits, MSc; Nils Hecht, MD; Nora Sandow, MD; Michael Scheel, MD; Christoph Drenckhahn, MD; Jens P. Dreier, MD; Peter Vajkoczy, MD

Background and Purpose—We investigated to what extent excitotoxicity and metabolic changes in the peri-infarct region of patients with malignant hemispheric stroke are associated with delayed infarct progression.

Methods—In 18 patients with malignant hemispheric stroke, 2 microdialysis probes were implanted within the peri-infarct tissue at a distance of 5 and 15 mm to the infarct. Precise probe placement was achieved by intraoperative laser speckle imaging. Glutamate, glucose, pyruvate, and lactate levels were monitored for 5 days after surgery. Delayed infarct progression was determined from serial MRI on the day after surgery and after the monitoring period.

Results—Initial stroke volume ranged from 122 to 479 cm³ with a median of 295 cm³. Nine of 18 patients (50%) had delayed infarct progression (median, 44 cm³; range, 19–93 cm³). In these patients, glucose and individual pyruvate levels were significantly lower when compared with patients without infarct progression, whereas glutamate and the lactate–pyruvate ratio were significantly elevated in patients with infarct progression early after surgery (12–36 hours) at the 15-mm microdialysis probe location. Lactate was elevated but without difference between groups.

Conclusions—Excitotoxic or metabolic impairment was associated with delayed infarct progression and could serve as a treatment target. (Stroke. 2014;45:00-00.)

Key Words: glutamic acid ▪ metabolism

Infarct progression is one of the most serious in-hospital complications after stroke. Among several involved factors, excitotoxicity and metabolic compromise are discussed as major contributors to infarct progression.1,2 In patients with large space-occupying infarcts, decompressive hemicraniectomy is often performed to prevent herniation. This surgical exposure of the brain allows probe implantation to monitor tissue at risk for secondary brain damage. Although a number of investigations have focused on biochemical changes within the peri-infarct tissue,3,4 precise probe positioning may have been hampered because probe placement was not performed under direct visual confirmation of the infarct border.

Here, we investigated biochemical alterations in the peri-infarct tissue of patients with malignant hemispheric stroke in relation to delayed infarct progression. Precise probe implantation was achieved through visual confirmation of the infarct border by intraoperative laser speckle imaging.

Methods

Methods are available in the online-only Data Supplement.

Patient Management

Forty-seven patients with subtotal or total middle cerebral artery infarction with or without additional infarction of the ipsilateral anterior- or posterior-cerebral artery territory and surgical indication for decompressive hemicraniectomy were screened for eligibility of study participation between May 19, 2009, and April 30, 2011. A total of 18 patients were included after informed consent was obtained. After bone removal and durotomy, the infarct border was localized by cerebral blood flow measurement using a laser speckle imager (MoorFLPi; Moor Instruments Ltd, Axminster, United Kingdom). A sharp regional drop in cortical perfusion ≤20% of normal was used as a threshold for ischemic tissue (Figure IA in the online-only Data Supplement) and judged as valid after independent confirmation of a macroscopically visible change in cortical tissue appearance at the suspected infarct border. Next, 2 microdialysis catheters (100 kDa; CMA, Stockholm, Sweden) were implanted 5 and 15 mm from the infarct in the peri-infarct tissue. Because of the fact that we were only able to identify the infarct border at the brain surface, microdialysis probes were implanted directly subpially to the cortex.

Glutamate, glucose, pyruvate, and lactate levels were monitored for 5 days after surgery.

Infarct Volume and Outcome Analysis

MRI or computed tomography was performed after surgery and at the end of the monitoring period on days 5 and 6.

With exception of patient numbers 6 and 13, where an MRI scan was not possible for logistic reasons, matched diffusion-weighted imaging and fluid attenuated inversion recovery sequences from the MRI performed postoperatively and on days 5 and 6 were used for determination of the infarct volume and brain swelling with iPlan Cranial software. In patient numbers 6 and 13, infarct volume and

Received December 17, 2013; final revision received January 20, 2014; accepted January 23, 2014.
From the Department of Neurosurgery (J.W., A.P., N.H., N.S., P.V.), Center for Stroke Research Berlin (J.W., A.P., N.H., N.S., C.D., J.P.D., P.V.), Departments of Neuroradiology (M.S.) and Neurology (C.D., J.P.D.), Charité-Universitätsmedizin Berlin, Berlin, Germany.
The online-only Data Supplement is available with this article at http://stroke.ahajournals.org/lookup/suppl/doi:10.1161/STROKEAHA.113.004475/-/DC1.
Correspondence to Johannes Woitzik, MD, Department of Neurosurgery, Charité-Universitätsmedizin Berlin, Hindenburgdamm 30, 12200 Berlin, Germany. E-mail johannes.woitzik@charite.de
© 2014 American Heart Association, Inc.
Stroke is available at http://stroke.ahajournals.org
DOI: 10.1161/STROKEAHA.113.004475
swelling were assessed from serial computed tomographic scans. For volumetric analysis, the infarct volume was corrected for hemispheric swelling. The initial neurological deficit was assessed using the Glasgow Coma Scale and Modified National Institutes of Health Stroke Scale. Outcome was evaluated at 6 months using the modified Rankin Scale.

**Results**

The initial stroke volume ranged from 122 to 479 cm$^3$ with a median volume of 295 cm$^3$. Nine of 18 patients (50%) had delayed infarct progression (median 44 cm$^3$; range, 19–93 cm$^3$; Figure 1B in the online-only Data Supplement). A mild perfusion mismatch of 15, 20, and 15 cm$^3$ was found at the early MRI time-point in patient numbers 11, 15, and 17, respectively. All other patients with available MRI data did not show a perfusion mismatch. Demographic, clinical, and postoperative monitoring data are listed in Table I in the online-only Data Supplement.

**Glutamate**

The mean glutamate concentrations were not significantly different (5 mm: 49±41 versus 52±68 μmol/L in patients with and without infarct progression; 15 mm: 64±62 versus 21±20 μmol/L in patients with and without infarct progression). However, early after surgery (12–36 hours), glutamate levels at the 15-mm distance were significantly elevated in patients where infarct progression occurred (Figure).

**Metabolic Changes**

Glucose levels did not differ 5 mm from the infarct (1.8±0.9 versus 1.6±0.5 mmol/L in patients with and without infarct progression), whereas significantly lower glucose levels were found at the 15-mm distance in patients with infarct progression (1.2±0.4 versus 1.9±0.4 mmol/L in patients with and without infarct progression; P<0.05; Figure).

Lactate was significantly elevated when compared with normal range. However, lactate did not differ between patient groups (5 mm: 6.8±3.8 versus 6.7±2.3 mmol/L in patients with and without infarct progression; 15 mm: 5.9±1.2 versus 5.9±2.6 mmol/L in patients with and without infarct progression; Figure).

The mean pyruvate levels did not differ (5 mm: 130±40 versus 168±65 μmol/L in patients with and without infarct progression; 15 mm: 113±63 versus 164±46 μmol/L in patients with and without infarct progression). However, in patients with infarct progression, individual pyruvate levels were significantly lower (5 mm: 108 and 120 hours after surgery; 15 mm: 12 and 108 hours after surgery; Figure).

Similarly, the mean lactate–pyruvate ratio did not differ (5 mm: 63±23 versus 53±33 in patients with and without infarct progression; 15 mm: 106±97 versus 47±31 in patients with and without infarct progression), but individual ratios during the early observation period (15 mm: 12–36 hours) were significantly higher in patients who showed delayed infarct progression (Figure).

**Discussion**

Lesion expansion is closely linked to a border zone of malperfusion, the penumbra, surrounding the already infarcted tissue. However, at delayed time points, the typical hemodynamic penumbra rarely exists, and lesion expansion may predominantly rely on other mechanisms, such as excitotoxicity, metabolic changes, lactacidosis, apoptosis, inflammation, and spreading depolarizations. Against this background, we exclusively studied delayed periods of infarct maturation (beyond 24 hours after stroke onset) where no significant hemodynamic perfusion–diffusion mismatch was measurable in our patients. Interestingly, half of our patients with malignant hemispheric stroke nevertheless had significant delayed infarct progression, which was associated with altered levels of glutamate, glucose, and pyruvate in the immediate peri-infarct region.
To investigate whether there is a gradient from the infarct border to the periphery, we implanted 2 microdialysis probes at different distances to the infarct border. Interestingly, all measured biochemical markers were significantly altered at both distances. When distinguishing between patients with and without infarct progression, however, differences in the levels of glutamate, glucose, and the lactate–pyruvate ratio were predominantly found further away (15 mm) from the infarct. Therefore, our data suggest that altered biochemical changes can be detected in a rather widespread area surrounding the infarct. The association between these alterations and delayed infarct progression might be restricted to a rather circumscribed area.

**Excitotoxicity**

In all patients, extracellular glutamate levels were initially above the ischemia threshold and with ongoing time, glutamate levels steadily decreased but reached normal ranges only in patients without infarct progression (Figure). In patients with infarct progression, glutamate was significantly higher at a distance of 15 mm during the early observation period. This may reflect progressive tissue damage or could also be part of the underlying pathological mechanism for infarct progression itself.

**Extracellular Metabolic Biochemistry**

Glucose and pyruvate were significantly lower in patients with delayed infarct progression (Figure) and naturally, this indicates a more intact metabolism in patients without infarct progression. However, recent studies have also suggested neuroprotection through pyruvate, which needs to be addressed in future studies. The widespread increase in lactate was in line with previous experimental findings. The significantly higher lactate–pyruvate ratio during the early monitoring period in patients with infarct progression (Figure) underlines the higher metabolic compromise in this group.

**Conclusions**

Delayed infarct progression in patients with malignant hemispheric stroke is associated with a disarrangement of biochemical markers within the peri-infarct region. Whether the observed excitotoxic or metabolic impairment rather reflects progressive tissue damage or possibly represents a suitable target for novel treatment strategies should be a main focus of future studies.

**Acknowledgments**

We thank S. Seidlitz, J. Kopetzki, C. Altendorf, and N. Gase for research assistance and G. Bohnier and J. Fiebach for help with imaging processing.

**Sources of Funding**

The study was funded by the Deutsche Forschungsgemeinschaft (DFG-WO 1704/1–1, DFG DR 323/5-1; Bundesministerium für Bildung und Forschung (Center for Stroke Research Berlin, 01 EO 0801) and Kompetenznetz Schlaganfall.

**Disclosures**

None.

**References**

Excitotoxicity and Metabolic Changes in Association With Infarct Progression
Johannes Woitzik, Alexandra Pinczolits, Nils Hecht, Nora Sandow, Michael Scheel, Christoph Drenckhahn, Jens P. Dreier and Peter Vajkoczy

Stroke. published online March 18, 2014;
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/early/2014/03/18/STROKEAHA.113.004475

Data Supplement (unedited) at:
http://stroke.ahajournals.org/content/suppl/2014/03/18/STROKEAHA.113.004475.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/
ONLINE SUPPLEMENT

Excitotoxicity and metabolic changes in association with infarct progression

Johannes Woitzik, MD\textsuperscript{1,2}; Alexandra Pinczolits, MSc\textsuperscript{1,2}; Nils Hecht, MD\textsuperscript{1,2}; Nora Sandow, MD\textsuperscript{1,2}; Michael Scheel, MD\textsuperscript{2,3}; Christoph Drenckhahn, MD\textsuperscript{2,4}; Jens P. Dreier, MD\textsuperscript{2,4}; Peter Vajkoczy, MD\textsuperscript{1,2}

\textsuperscript{1}Department of Neurosurgery, \textsuperscript{2}Center for Stroke Research Berlin, \textsuperscript{3}Department of Neuroradiology, \textsuperscript{4}Department of Neurology, Charité - Universitätsmedizin Berlin, Berlin, Germany

Supplemental Methods

\textit{Patient management}

The study was approved by the local research and ethics committee of the Charité-Universitätsmedizin Berlin. Informed consent was obtained from the patient or legal representative.

Exclusion criteria of the present study were as follows: Age below 18 years, pregnancy, MRI incompatible medical device implants, coagulation abnormalities, pre-morbidity or reduced life expectancy. In four patients, no surgery was performed due to co-morbidities and poor prognosis. One patient/legal representative did not give informed consent. In 24 patients, study inclusion was not possible due to simultaneous monitoring of other patients, equipment failure or unavailability of the study team. For surgical decompression, anesthesia was performed with propofol and remifentanil. After surgery, patients were transferred to the intensive care unit. Intracranial pressure was monitored via a pressure sensor (Raumedic, Münchberg, Germany) and maintained below 20 mmHg. Blood gases, electrolytes and glucose were controlled every 4 hours.
**Laser speckle contrast imaging**

The laser speckle imager (MoorFLPI, Moor Instruments Ltd., Axminster, UK) was positioned perpendicular over the infarcted hemisphere at a distance of 300 mm. Blood flow within the imaging field (18 x 24 cm) was visualized as a 2-dimensional color-coded map of perfusion and recorded with purpose designed data acquisition software (MoorFLPI measurement software, Version 3.0, Moor Instruments, Axminster, UK) at 25 Hz using a temporal filter of 100 frames per image resulting in a scan rate of 0.25 Hz to allow optimal spatial resolution (760 x 568 pixel, exposure time 8.4 ms).\(^1\)\(^,\)\(^2\) Identical focal lens settings and exposure times were maintained during all measurements. Illumination of the surgical field by light sources other than the laser was avoided and an adjustable polarizing filter was used to eliminate specular reflection from shiny tissue.

**Image-guided navigation**

In selected cases, the infarct border was additionally identified by image-guided navigation: Preoperative MRI with magnetization prepared rapid gradient echo (MPRAGE), diffusion weighted imaging (DWI) and fluid attenuation inversion recovery (FLAIR) sequences was performed and the cortical area of the infarct (DWI, FLAIR) was outlined in the MPRAGE sequence with iPlan\(^\text{®}\) Cranial planning software (BrainLAB, Feldkirchen, Germany). During surgery, the infarct border was identified with a BrainLAB Curve\(^\text{™}\) image-guidance system (BrainLAB, Feldkirchen, Germany) before performing the durotomy in order to avoid misalignment through inadvertent brain shift. Next, the dura was opened above the suspected infarct border and the border was transposed to the underlying cortex by outlining it with a silk ligature (Supplementary Figure IA).

**Microdialysis**

The catheters were perfused at a constant rate of 0.3 \(\mu\)l/min with artificial cerebrospinal fluid (NaCl 147 mmol/L, KCl 2.7 mmol/L, CaCl\(_2\) 1.2 mmol/L and MgCl\(_2\) 0.85 mmol/L; CMA, Stockholm, Sweden). Vial changes were performed every hour and samples were immediately analyzed at bedside for extracellular concentration of glutamate, glucose, lactate and pyruvate with the CMA 600 Microdialysis Analyzer (CMA, Stockholm, Sweden).
**MRI and infarct volume analysis**

All MRI scans were performed in an axial manner with 1 or 6 mm slice thickness. For assessment of a perfusion-diffusion mismatch, perfusion-weighted imaging (PWI) was performed with a serial T2* weighted single-shot gradient echo-planar imaging (EPI) sequence. Gadolinium-contrast agent (0.1mmol/kg bw; Magnevist®; Schering) was injected as a bolus rate of 4-6 mL/second.

**Statistical analysis**

Microdialysis data are recorded as 12-hour mean values for each patient for the first 5.5 days post-surgery. Data are presented as mean ± standard deviation. Baseline characteristics were compared with student’s t-test. To compare groups with and without infarct progression, a two-way analysis of variance (ANOVA) on repeated measures with subsequent Bonferroni post hoc test for multiple comparisons was used. P<0.05 was considered statistically significant.
Supplementary Table I:

Summary of demographic, clinical and postoperative monitoring data (yrs=years; m=male; f=female; GCS=Glasgow Coma Scale; mNIHSS=Modified National Institutes of Health Stroke Scale; MCA=middle cerebral artery; ACA=anterior cerebral artery; PCA=posterior cerebral artery; R=remifentanil; M=midazolam; P=propofol; mRS=modified Rankin Scale). There was no baseline difference concerning age, initial stroke size or swelling (age: 59±10 years and 58±17 years; initial stroke volume: 304±90 cm³ and 231±90 cm³; swelling: 21±10% and 16±3% in patients with and without infarct progression, respectively).

<table>
<thead>
<tr>
<th>No.</th>
<th>Age (yrs), sex</th>
<th>Initial GCS</th>
<th>Initial mNIHSS</th>
<th>Location of infarction</th>
<th>Time from stroke to operation (h)</th>
<th>Postoperative sedation time (h) / used drugs</th>
<th>Initial stroke volume (cm³)</th>
<th>Swelling (%)</th>
<th>Delayed infarct progression (cm³)</th>
<th>mRS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>62, m</td>
<td>14</td>
<td>20</td>
<td>left MCA</td>
<td>15</td>
<td>39 / R, M</td>
<td>283</td>
<td>24</td>
<td>58</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>65, m</td>
<td>15</td>
<td>18</td>
<td>right MCA</td>
<td>14</td>
<td>8 / R, M</td>
<td>306</td>
<td>18</td>
<td>43</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>59, m</td>
<td>15</td>
<td>13</td>
<td>right MCA</td>
<td>22</td>
<td>-</td>
<td>203</td>
<td>7</td>
<td>44</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>43, f</td>
<td>14</td>
<td>21</td>
<td>right MCA</td>
<td>20</td>
<td>-</td>
<td>159</td>
<td>21</td>
<td>49</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>72, f</td>
<td>15</td>
<td>16</td>
<td>left MCA/ACA</td>
<td>29</td>
<td>8 / R, P</td>
<td>311</td>
<td>21</td>
<td>19</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>60, f</td>
<td>3</td>
<td>-</td>
<td>left MCA/ACA</td>
<td>52</td>
<td>101 / R, M</td>
<td>337</td>
<td>11</td>
<td>67</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>49, m</td>
<td>13</td>
<td>22</td>
<td>right MCA</td>
<td>13</td>
<td>76 / R, P</td>
<td>316</td>
<td>20</td>
<td>36</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>52, m</td>
<td>11</td>
<td>20</td>
<td>left MCA/ACA</td>
<td>31</td>
<td>163 / R, M</td>
<td>342</td>
<td>21</td>
<td>93</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>70, m</td>
<td>15</td>
<td>20</td>
<td>right MCA/ACA</td>
<td>32</td>
<td>78 / R, P</td>
<td>479</td>
<td>42</td>
<td>39</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>69, f</td>
<td>3</td>
<td>-</td>
<td>left MCA</td>
<td>27</td>
<td>60 / R, M</td>
<td>163</td>
<td>10</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>11</td>
<td>74, f</td>
<td>3</td>
<td>-</td>
<td>right MCA/ACA</td>
<td>32</td>
<td>-</td>
<td>381</td>
<td>17</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>12</td>
<td>54, m</td>
<td>3</td>
<td>-</td>
<td>left MCA</td>
<td>38</td>
<td>82 / R, P</td>
<td>262</td>
<td>18</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>13</td>
<td>51, m</td>
<td>14</td>
<td>12</td>
<td>left MCA</td>
<td>24</td>
<td>-</td>
<td>145</td>
<td>12</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>14</td>
<td>29, m</td>
<td>15</td>
<td>20</td>
<td>left MCA</td>
<td>54</td>
<td>50 / R, M</td>
<td>122</td>
<td>20</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>44, m</td>
<td>14</td>
<td>11</td>
<td>right MCA</td>
<td>12</td>
<td>24 / R, P</td>
<td>222</td>
<td>17</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>16</td>
<td>82, f</td>
<td>11</td>
<td>17</td>
<td>right MCA</td>
<td>23</td>
<td>24 / R, P</td>
<td>309</td>
<td>15</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>17</td>
<td>68, m</td>
<td>13</td>
<td>28</td>
<td>right MCA/ACA</td>
<td>28</td>
<td>91 / R, M</td>
<td>343</td>
<td>19</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>18</td>
<td>52, f</td>
<td>15</td>
<td>17</td>
<td>right MCA</td>
<td>34</td>
<td>99 / R, M</td>
<td>159</td>
<td>18</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>
Supplementary Figure I:

Intra- and postoperative imaging. A: Intraoperative photograph of the left frontal cortex. The green silk ligature (white arrows) was placed on the cortical surface to outline the infarct border, which was identified by image-guided navigation. In the left upper corner a color-coded laser speckle image of cortical blood flow from the area of the infarct border (dashed rectangle) is illustrated as an example of intraoperative infarct border visualization. The scale bar marks arbitrary perfusion units (CBF-Flux). A microdialysis probe was implanted to the cortical tissue with a distance of 5 mm (A) and 15 mm (B) to the infarct. B: MRI (FLAIR sequences) in a 49-year old male patient with a right-sided malignant hemispheric stroke (patient no. 7) after surgery (left panel) and after completion of the monitoring period on day 5 (right). The infarct progression is indicated with arrows.
Supplemental References
