Early $^{11}$C Flumazenil/H$_2$O Positron Emission Tomography Predicts Irreversible Ischemic Cortical Damage in Stroke Patients Receiving Acute Thrombolytic Therapy

Wolf-Dieter Heiss, MD; Lutz Kracht, MD; Martin Grond, MD; Jobst Rudolf, MD; Bernd Bauer, PhD; Klaus Wienhard, PhD; Gunter Pawlik, MD

Background and Purpose—Central benzodiazepine receptor ligands, such as $^{11}$C flumazenil (FMZ), are markers of neuronal integrity and therefore might be useful in the differentiation of functionally and morphologically damaged tissue early in ischemic stroke. We sought to assess the value of a benzodiazepine receptor ligand for the early identification of irreversible ischemic damage to cortical areas that cannot benefit from reperfusion.

Methods—Eleven patients (7 male, 4 female, aged 52 to 75 years) with acute, hemispheric ischemic stroke were treated with alteplase (recombinant tissue plasminogen activator; 0.9 mg/kg according to National Institute of Neurological Disorders and Stroke protocol) within 3 hours of onset of symptoms. At the beginning of thrombolysis, cortical cerebral blood flow ($^{15}$O H$_2$O) and FMZ binding were assessed by positron emission tomography (PET). Those early PET findings were related to the change in neurological deficit (National Institutes of Health Stroke Scale) and to the extent of cortical damage on MRI or CT 3 weeks after the stroke.

Results—Hypoperfusion was observed in all cases, and in 8 patients the values were below critical thresholds estimated at 12 mL/100 g per minute, comprising 1 to 174 cm$^3$ of cortical tissue. Substantial reperfusion was seen in most of these regions 24 hours after thrombolysis. In 4 cases, distinct areas of decreased FMZ binding were detected. Those patients suffered permanent lesions in cortical areas corresponding to their FMZ defects (112 versus 146, 3 versus 3, 2 versus 1, and 128 versus 136 cm$^3$). In the other patients no morphological defects were detected on MRI or CT, although blood flow was critically decreased in areas ranging in size up to 78 cm$^3$ before thrombolysis.

Conclusions—These findings suggest that imaging of benzodiazepine receptors by FMZ PET distinguishes between irreversibly damaged and viable penumbra tissue early after acute stroke. (Stroke. 2000;31:366-369.)

Key Words: flumazenil ligands penumbra stroke, acute stroke, ischemic thrombolytic therapy tomography, emission computed

Therapeutic strategies in acute ischemic stroke are targeted at rescuing from infarction ischemic but potentially viable tissue, known as the “ischemic penumbra.” This is vital because treatment can only be effective as long as tissue has not become necrotic. Of all the treatment efforts tested in controlled clinical trials, only thrombolytic therapy was shown to be effective, but only when initiated shortly after onset of clinical signs of cerebral ischemia. Markers of irreversible tissue damage or indicators of neuronal integrity would be helpful for the selection of patients who might benefit from reperfusion induced by thrombolysis or from other therapeutic approaches, such as neuroprotection. γ-Aminobutyric acid receptors are abundant in the cortex and sensitive to ischemic damage; therefore, specific radioligands to their subunits, the central benzodiazepine receptors, could be used as markers of preserved morphological integrity before initiation of therapy. Since previous studies have demonstrated that irreversibly damaged cortex can be reliably detected by reduced binding of the labeled benzodiazepine receptor ligand $^{11}$C flumazenil (FMZ) in experimental focal ischemia as well as in patients with acute ischemic stroke several hours after onset of symptoms, the value of this marker of neuronal integrity was investigated for the very early identification of ischemic tissue that had suffered irreversible damage.

Subjects and Methods

Eleven patients (7 male, 4 female, aged 52 to 75) with acute, hemispheric ischemic stroke were treated with 0.9 mg/kg recombinant tissue plasminogen activator (alteplase, Actilyse) according to the National Institute of Neurological Disorders and Stroke protocol within 3 hours of onset of symptoms, after their informed consent had been obtained. Five to 10 minutes before the beginning of recombinant tissue plasminogen activator infusion (95 to 180 minutes after onset of symptoms), cerebral blood flow (CBF) was measured by positron emission tomography (PET) (ECAT EXACT HR, CTI/Siemens) after intravenous bolus injection of $^{15}$O-labeled.
Areas of CBF and FMZ Binding Decreased Below Respective Thresholds, Final Infarct Size, and Change in National Institutes of Health Stroke Scale Score in Individual Patients

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age, y</th>
<th>NIHSS</th>
<th>CBF 3h</th>
<th>FMZ 3h</th>
<th>CT/MRI Infarct</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 h</td>
<td>3 mo</td>
<td>Volume, cm³</td>
<td>Mean, %</td>
<td>Volume, cm³</td>
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<tr>
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<td>8</td>
<td>0</td>
<td>78.0</td>
<td>37</td>
</tr>
<tr>
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<td>5</td>
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<tr>
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<tr>
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<td>21</td>
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<tr>
<td>Maximum</td>
<td>75</td>
<td>16</td>
<td>18</td>
<td>173.8</td>
<td>44</td>
</tr>
</tbody>
</table>

NIHSS indicates National Institutes of Health Stroke Scale.
*Relative to noninfarcted hemisphere.
†Cortical regions with FMZ binding decreased below 4 times the mean value of white matter.

**Results**

The Table shows the areas of CBF and FMZ binding decreased below the respective thresholds, final infarct size, and the change of National Institutes of Health Stroke Scale score in the individual patients. Eight of the patients exhibited cortical tissue of different size perfused below the critical threshold. All had additional areas perfused in the moderately hypoperfused range. Only 4 patients, however, exhibited significant defects in FMZ binding within their severely hypoperfused regions, and corresponding infarcts were detected on final CT/MRI. In the other cases, the hypoperfusion could be reversed by thrombolysis to values above the 70% threshold 24 hours after the stroke, and no cortical defects on morphological images were found. Therefore, severe decreases in early FMZ binding significantly (P<0.005 by Fisher’s exact test) predicted irreversible cortical damage. As reflected in their National Institutes of Health Stroke Scale changes, all but 1 infarct patient improved clinically. The defects in FMZ binding were not related to the size of the critically hypoperfused area: in small ischemic areas of 2 patients, irreversible damage was indicated by early loss of FMZ binding (Figure 1). In patient 10, even a small penumbral area was associated with decreased FMZ binding, predicting a small cortical infarct. The discrepancies in these small volumes are likely due to differences in the partial

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1. Water (60 mCi=2.2 GBq), and FMZ was injected intravenously (20 mCi=240 MBq) 5 minutes later to assess benzodiazepine receptor binding 30 to 60 minutes after tracer administration. CBF was measured again 24 hours after the stroke. The early PET findings were related to the individual change in neurological deficit and were compared with the extent of morphological damage on MRI and CT obtained 3 weeks after the stroke. With the use of an interactive program, all PET images were individually coregistered with the respective MRI or CT volume along the anterior-posterior commissural line. Subsequently, the cerebral hemispheres and, if present, the cortical part of the infarct were segmented from the MRI or CT volumes by means of an interactive data language (Research System Inc) and C-based image analysis system operating at a spatial resolution of 1 mm. The cortical rim was defined by thresholding the FMZ images at 3 times white matter activity and mirroring the noninfarcted hemisphere to the side of the infarction along a plane in the interhemispheric fissure defined on the morphological images.9

Thus, the outer border of the cortex was defined by the contour from MRI or CT, while the inner border of the cortex was defined by the FMZ (and in the area of the infarction by the mirrored FMZ).

Since FMZ binding can be reliably assessed only in the cortex, only cortical areas were used for the comparative analysis of early changes in flow, FMZ binding, and permanent morphological defects. This analysis was based on the following criteria defined on all pertinent images of the individual patients: regions with critically disturbed cortical perfusion were operationally set to 50% [15 O]H₂O uptake relative to the mean of the contralateral hemisphere. This perfusion level was chosen because, in a previous quantitative CBF PET study in ischemic stroke, it had been shown to correspond to a gray matter flow of <12 mL/100 g per minute,10 which represents the widely accepted viability threshold since flow below this threshold commonly leads to infarction within a few hours. Moreover, a moderately hypoperfused zone comparable to the "penumbral zone" defined by Hakim et al was identified, with [15 O]H₂O uptake between 50% and 70% of the contralateral mean, representing 12 to 18 mL/100 g per minute. Likewise, cortical regions with FMZ binding <4.0 times the mean value of white matter were identified. This threshold was chosen because it was 2 SD below the mean value of normal cortex (5.9±0.97); a decrease of >30% below the contralateral cortex could also clearly be distinguished on the images. The abnormalities found on the early PET images were then related to the area of finally infarcted cortex as defined by late MRI or CT.
volume effects of the applied imaging procedures. In contrast, fairly large hypoperfused regions could also benefit from reperfusion and did not become infarcted, as long as reperfusion began before decreased FMZ binding indicated irreversible damage (Figure 2). The largest and most severely hypoperfused cortical area (patient 6; 174 cm³), however, included a rather large region of decreased FMZ binding (112 cm³). This suggested widespread irreversible neuronal damage at this early stage (Figure 2), with subsequent infarct growth as indicated by late CT.

Discussion

Penumbral tissue can be demonstrated by functional imaging procedures visualizing conditions such as the mismatch between blood flow and energy metabolism ("misery perfusion," expressed as increased oxygen extraction fraction in multitracer PET studies⁴³) or between changes in perfusion and water diffusion (perfusion disturbance without irreversible damage, assessed by subtraction of diffusion-weighted from perfusion-weighted MR images⁴⁷). Special tracers, such
as the imidazole derivative [18F]fluoromisonidazole, selectively identify hypoxic peri-infarct tissue that may represent the penumbra surrounding an infarct.18 Several studies have shown that this critically hypoperfused tissue can be salvaged by reperfusion therapy.19–21 The early detection of irreversibly damaged tissue within a critically perfused territory is more difficult, since CT does not disclose the full extent of irreversible damage during the first hours after a stroke.22–24 Likewise, the results of diffusion-weighted MRI might be misleading for various reasons.25,26 Tracers, however, that bind only to intact neurons, such as the central benzodiazepine receptor ligand FMZ, can be used for this purpose, namely, to distinguish potentially viable cortex from tissue that cannot be salvaged by any treatment. While the areas of severe early ischemia and decreased FMZ binding showed considerable overlap in this study, it was only the FMZ result that predicted morphological outcome. The CBF changes, by contrast, were too nonspecific. In all cases with normal FMZ binding, critical hypoperfusion, even in large areas, could be reversed by thrombolysis, as indicated by the repeated CBF measurement 24 hours after the attack. The similarity in clinical improvement between patients who developed cortical infarcts and those who did not may be explained by differences in topographical involvement and in the degree of subcortical damage.

As a tracer of neuronal integrity, FMZ clearly has some limitations, the most important being that benzodiazepine receptors are abundant only in cerebral cortex and that receptor binding can only be assessed in a steady state after tracer injection. However, the early phase of tracer distribution can be used to estimate blood flow,7 thus minimizing those limitations to some extent because additional tracer injections can be avoided. Studies of benzodiazepine receptors need not involve the complex logistics required by11C injections can be avoided. Studies of benzodiazepine receptors in humans have shown that predicted morphological outcome. The CBF changes, by contrast, were too nonspecific. In all cases with normal FMZ binding, critical hypoperfusion, even in large areas, could be reversed by thrombolysis, as indicated by the repeated CBF measurement 24 hours after the attack. The similarity in clinical improvement between patients who developed cortical infarcts and those who did not may be explained by differences in topographical involvement and in the degree of subcortical damage.

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