Permanent Cortical Damage Detected by Flumazenil Positron Emission Tomography in Acute Stroke

Wolf-Dieter Heiss, MD; Martin Grond, MD; Alexander Thiel, MD; Mehran Ghaemi, MD; Jan Sobesky, MD; Jobst Rudolf, MD; Bernd Bauer, PhD; Klaus Wienhard, PhD

Background and Purpose—Therapy of acute ischemic stroke can only be effective as long as neurons are viable and tissue is not infarcted. Since γ-aminobutyric acid receptors are abundant in the cortex and sensitive to ischemic damage, specific radioligands to their subunits, the central benzodiazepine receptors (BZR), may be useful as indicators of neuronal integrity and as markers of irreversible damage. To test this hypothesis we studied the binding of the BZR ligand \[^{11}\text{C}\text{]flumazenil (FMZ)}\] early after ischemic stroke in comparison to the extent of final infarcts and hypometabolic cortical areas.

Methods—In 10 patients cerebral blood flow, cerebral metabolic rate for oxygen (CMRO₂), oxygen extraction fraction (OEF), and FMZ binding were studied by positron emission tomography 3.5 to 16 hours after onset of their first hemispheric stroke. Early changes in flow, oxygen metabolism, and FMZ binding were compared with permanent disturbances in glucose metabolism, and the size of the final infarcts was determined on MRI or CT 12 to 22 days after the stroke.

Results—In all patients except one cerebral blood flow was disturbed, with marked decreases in eight and a hyperperfusion in one patient corresponding to the location of neurological deficits. In these areas CMRO₂ was also reduced but to a variable degree, inducing highly variable OEF. Areas with markedly decreased CMRO₂ (<60 μmol/100 g per minute) corresponded to regions with decreased FMZ binding (<4.0 times the mean value in the white matter). In all patients the final cortical infarcts were visible on the early FMZ images. Infarcts could be discriminated from noninfarcted cortex by decreased FMZ binding despite a wide range of OEF. In finally hypometabolic cortex FMZ binding was initially decreased or normal, with OEF covering a wide range; this suggested neuronal loss and/or deactivation as the cause of metabolic disturbance. Additionally, a highly significant correlation was found between FMZ distribution within the first 2 minutes after injection and regional cerebral blood flow.

Conclusions—These results demonstrate that permanently and irreversibly damaged cortex can be detected by reduced FMZ binding early after stroke. Since FMZ distribution additionally images regional cerebral perfusion, BZR radioligands have a potential as clinically useful tracers in patients with acute ischemic stroke. The evidence of tissue damage furnished by these tracers might be of relevance for the selection of individual therapeutic strategies. (Stroke. 1998;29:454-461.)

Key Words: flumazenil ■ receptors, benzodiazepine ■ stroke, ischemic ■ tomography, emission computed
and transcranial Doppler sonography, electroencephalography, and, if necessary, recording of visual and somatosensory evoked potentials were performed and CT scan was repeated to render a complete picture of the patient’s condition. Fully informed consent for the study was obtained from the patient and from the next of kin.

Exclusion Criteria
Excluded from the study were patients whose state was complicated by other medical conditions, including hypertension with systolic pressure >200 mm Hg or diastolic pressure >120 mm Hg, diabetes mellitus with blood glucose >200 mg/100 mL on admission, severe liver disease, severe congestive heart failure, or severe arrhythmias. CT excluded hemorrhagic or nonischemic lesions as well as subarachnoid hemorrhage. Comatose patients or those suffering from other neurological disorders including a previous cerebrovascular accident were excluded, as were patients treated with anticoagulants and those with hemorrhagic tendency or recent surgery.

Radiological Investigations
The first set of PET studies followed immediately after the initial clinical assessment (including CT) and was started within 3.5 to 16 hours of symptom onset. The second set of PET studies was performed 12 to 22 days later, when the size and location of the final infarct were also determined on T1-weighted MRI scans that were obtained on a 1.0-T Magnetom Impact (Siemens Medical Systems) as 64 transaxial, 2.5-mm-thick slices acquired simultaneously with the use of a three-dimensional fast low-angle shot sequence or on CT scan (Somatom, Siemens Med Systems) as 50 transaxial slices of 3-mm thickness. PET studies were performed in a resting state with the use of an ECAT EXACT HR scanner (Siemens/CTI) in two- or three-dimensional data acquisition mode providing 47 contiguous 3-mm slices of 5-mm full width at half maximum in-plane reconstructed resolution. The first PET examination (3.5 to 16 hours after symptom onset) consisted of a total of three studies: CBF was measured according to the 15O]H2O intravenous bolus method with 60 mCi (2.2 GBq). Ten minutes later, 50 mCi (1.85 GBq) 15O gas was inhaled by the subject in a deep single breath followed by a breath holding of approximately 10 to 15 seconds. For both studies arterial blood activity was measured with a commercially available automated blood sampling system. From the multiple brain activity frames accumulated after H2 15O injection and 15O inhalation and the time–activity curves of the blood, the computer (SUN SPARC, Sun Microsystems Inc) after decay correction calculated regional values of CBF, CMRO2, and OEF pixel by pixel with the use of the operational equation of Mintun et al. Details of these procedures have been described previously.

After completion of the 15O studies, 20 mCi (740 MBq) FMZ was injected intravenously, and the distribution and accumulation of this tracer were followed for 60 minutes by serial scanning. The initial tracer distribution reached within 2 minutes after injection served as an indicator of the perfusion pattern in comparison to the flow values determined by H2 15O. BZR density was estimated from the distribution of FMZ 30 to 60 minutes after the bolus injection. In a few cases

### Statistical Methods
First, for analysis of the linear relationship between volumes with FMZ binding decreased below a predefined threshold and final infarct volume in MRI, a regression analysis was calculated. The significance threshold was set to P=0.01.

Second, the set of spherical VOI was tested for linear relationships between FMZ binding and CMRO2, OEF, and CBF with the use of Pearson correlation coefficients. A significance threshold of P=0.01 was used for the analysis.

To assess sensitivity and specificity of FMZ binding for predicting finally infarcted brain tissue on MRI after 2 weeks, an ROC analysis was performed for all measured physiological parameters within the VOI set. Sensitivity and specificity were analyzed at certain predefined physiological thresholds for all parameters. Finally, a nonlinear curve-fit was computed with the use of a power function (y=a×x^b) to describe the relationship between CBF and FMZ distribution. All computations were performed with SAS Version 6.11 for Unix (Statistical Analytical System, SAS Institute).

### Results
The Table shows the areas of CBF, CMRO2, and FMZ binding decreased below the respective thresholds given with the size of final infarcts and areas of permanently depressed...
Compromised Cortical Regions in Individual Patients

<table>
<thead>
<tr>
<th>Pt</th>
<th>Age, y/Sex</th>
<th>Location of Infarct</th>
<th>Decreased FMZ Binding</th>
<th>Hypoperfusion</th>
<th>Reduced CMRO₂</th>
<th>Hypometabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Infarct Volume, cm³</td>
<td>Value Relative</td>
<td>Value, (mL/100 g/min)</td>
<td>Value, (μmol/100 g/min)</td>
</tr>
<tr>
<td>1</td>
<td>57/M</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>54/F</td>
<td>L frontotemporal</td>
<td>Silent infarction</td>
<td>4.81</td>
<td>2.98</td>
<td>18.01*</td>
</tr>
<tr>
<td>3</td>
<td>63/M</td>
<td>L paracentral gyrus</td>
<td>0.91</td>
<td>0.37</td>
<td>2.69</td>
<td>1.45</td>
</tr>
<tr>
<td>4</td>
<td>64/M</td>
<td>L frontoparietal</td>
<td>5.24</td>
<td>3.56</td>
<td>2.91</td>
<td>1.32</td>
</tr>
<tr>
<td>5</td>
<td>52/M</td>
<td>R MCA territory</td>
<td>44.11</td>
<td>45.29</td>
<td>2.04</td>
<td>63.67</td>
</tr>
<tr>
<td>6</td>
<td>76/M</td>
<td>L frontotemporal</td>
<td>3.30</td>
<td>0.13</td>
<td>3.60</td>
<td>0.67</td>
</tr>
<tr>
<td>7</td>
<td>65/M</td>
<td>L posterior insula</td>
<td>18.88</td>
<td>16.57</td>
<td>2.45</td>
<td>22.51</td>
</tr>
<tr>
<td>8</td>
<td>60/F</td>
<td>R posterior insula</td>
<td>8.03</td>
<td>9.30</td>
<td>2.88</td>
<td>18.78</td>
</tr>
<tr>
<td>9</td>
<td>55/M</td>
<td>L anterior insula</td>
<td>29.32</td>
<td>25.46</td>
<td>2.81</td>
<td>33.09</td>
</tr>
<tr>
<td>10</td>
<td>73/M</td>
<td>R posterior insula</td>
<td>32.22</td>
<td>16.35</td>
<td>2.83</td>
<td>107.07</td>
</tr>
</tbody>
</table>

Pt indicates patient; L, left; R, right; and MCA, middle cerebral artery. Location and volume of final infarction are given in comparison to volume and value of decreased FMZ binding (below relative value of 4.0), of hypoperfusion (<12 mL/100 g per minute), of reduced CMRO₂ (<60 μmol/100 g per minute), and of reduced CMRglc (<25 μmol/100 g per minute).

*Hyperperfusion.
defined and equally spaced within the cortical rim. A total of 332 spheres placed in that way were subsequently labeled according to their location in finally infarcted, hypometabolic, and normal tissue. When these ROIs were used, the three categories clustered with respect to FMZ binding (Fig 5). Infarcted tissue peaked at a value of 2.5 times the mean binding within the white matter, with some overlap reaching into the normal range (4.0). Hypometabolic tissue showed a broad distribution reaching into the normal values. FMZ binding was significantly correlated to CMRO2 (Fig 6a), which also separated infarcted from normal tissue with only a small overlap. The relationship to rCBF was looser (r=.56), especially because of regions with pathological hyperperfusion, and separation among various tissue compartments was less clear on the basis of rCBF values. The uncoupling between flow and oxygen metabolism in pathologically perfused tissue became evident when OEF was related to final tissue outcome: A clustering of tissue categories for low or high values was not observed (Fig 5b), and OEF only showed a weak correlation with final tissue outcome (Fig 7b), the calculated ROC curve was only slightly better than random chance, and a discriminating point could not be defined.

In all the patients FMZ distribution within the first 2 minutes after bolus injection showed the perfusion pattern to be in excellent agreement with the flow maps obtained after H215O injection (Figs 1 to 3). The usefulness of FMZ as a tracer of perfusion was further tested by comparing the regional FMZ uptake within the first 2 minutes to the absolute flow values. FMZ uptake was determined as percentage of the mean of the contralateral hemisphere and related pixel by pixel to rCBF in milliliters per 100 g per minute. The correlation analysis of cortical pixels within the infarct and in the noninfarcted ipsilateral hemisphere demonstrated the significant correspondence (R2=.88) between these procedures (Fig 8). The nonlinear regression line shown could serve as a calibration curve for estimating flow from FMZ distribution without necessitating additional H215O injection and arterial blood sampling.
Discussion

Irreversible tissue damage is characterized by a coupled reduction of CBF and CMRO$_2$ below certain thresholds.\textsuperscript{23,24} It is in accordance with these previous findings that CMRO$_2$ and CBF reduced below these thresholds at the early stage were also predictive of final infarction in our study. However, the broad clinical application of this examination is limited by the complex logistics involved in PET studies, by the necessity of arterial blood sampling, and by the short half-life of the tracers. Therefore, widely applicable technologies are still needed for the early detection of irreversibly damaged ischemic tissue.

Early signs of infarction on CT\textsuperscript{26} and changes in diffusion-weighted MRI\textsuperscript{27} indicate gross irreversible tissue destruction, but neuronal loss in silent infarction may remain unrecognized, and the time course of the development of morphological changes may delay conclusive findings. Whereas neuronal damage in basal ganglia was indicated on early CT in the majority of our patients, cortical damage was only indicated in three patients, even though nine patients ultimately experienced cortical infarction or considerable neuronal loss.

One of the earliest indicators of irreversible neuronal damage might be dysfunction of the GABA receptors,\textsuperscript{28} which are more sensitive to ischemia than glutamate receptors.\textsuperscript{29,30} Ligands to central BZR, which can also be labeled for single photon detection, were shown to be early indicators of irreversible damage in experimental focal ischemia\textsuperscript{12} and reliable markers of neuronal loss in gross and silent infarction.\textsuperscript{9,11} Our results demonstrate for the first time the usefulness of FMZ to visualize permanent infarcts early after the onset of cerebral ischemia. FMZ resembles CMRO$_2$ in its ability to detect early damaged neurons (Fig 8), but the quantitative determination of oxygen consumption is burdened by the necessity of multitracer application, arterial blood sampling, and active cooperation of the patient during bolus inhalation; additionally, the spatial resolution for oxygen tracers is impaired by unfavorable counting statistics and the high energy of the emitted positrons. As demonstrated in our examples, the images for FMZ binding have superior quality because of the high amount of accumulated counts and the favorable properties of the tracer.

An uncoupled decrease of rCBF with oxygen consumption preserved at a higher level was coined “misery perfusion”\textsuperscript{31} and used as an indicator of viable tissue. The fate of this tissue within the ischemic penumbra\textsuperscript{32} indicated by increased OEF, however, is undefined, with some tissue compartments recovering and others turning into necrosis in the further course.\textsuperscript{33,34} In several cases in our study, regions with increased OEF were found in finally infarcted as well as hypometabolic or normal areas, and in the regions outside the infarcts with permanently depressed glucose metabolism neuronal loss indicative of silent infarction (a focal incomplete ischemic tissue necrosis not leading to emollision, according to Reference 25) or deactivation by impaired afferent pathways (“diaschisis”)\textsuperscript{35} can be assumed. In our study there was a significant difference in rCMRO$_2$ between misery perfused regions eventually turning into infarcted or hypometabolic tissue and those regions finally outside the compromised areas; this difference was observed as a trend previously.\textsuperscript{34} As in previous studies,\textsuperscript{17,34,36,37} an increased OEF therefore was not predictive of the further course and cannot be used for discrimination between permanently damaged and potentially salvageable tissue. For that purpose a marker of neuronal integrity is needed to detect irreversibly damaged neurons early after onset of cerebral ischemia.

Our results demonstrate that FMZ can be used for early detection of irreversible damage in areas of coupled decrease of
flow and metabolism as well as in areas with increased OEF; as soon as FMZ binding is reduced, at least a proportion of neurons is irreversibly damaged irrespective of some continuing metabolic activity of the remaining tissue. Loss of neurons was previously demonstrated in the surrounding of gross infarcts and was related to permanently reduced blood flow.38

In the permanent state, reduced rCMRglc together with reduced FMZ binding indicates neuronal loss in incomplete cerebral infarction,25,39 whereas discordant rCMRglc reduction not paralleled by decreased FMZ binding40 suggests deactivation. These two conditions can be deduced from our data: Concordant reduction of rCMRglc and FMZ binding is an indicator of neuronal loss in incomplete infarction (Fig 3), while discordant rCMRglc decrease with normal FMZ binding suggests deactivation in the surrounding of infarcts or in cortex above white matter lesions (Fig 1). However, the part of the final infarct that is caused by delayed neuronal death and progressive ischemic damage41,42 or due to additional disturbances of flow in case of progressive arterial thrombosis43 cannot be detected by early BZR studies. These tissue compartments were indicated in some of our patients by those ROIs within infarcted tissue clustering at normal FMZ values (Fig 5).

For the decision on the potential of therapeutic strategies—reperfusion, neuroprotection, or rehabilitation—the study of the intactness of GABAergic receptors by BZR ligands might yield useful information in addition to the detection of the impairment in blood supply by SPECT or PET,44–48 which was shown to be reversed by intravenous recombinant tissue plasminogen activator followed by clinical improvement.49 However, as indicated by the high correlation between FMZ distribution within the first 2 minutes after injection and rCBF measured by H215O, regional perfusion can also be assessed semiquantitatively by FMZ. As a consequence, only one tracer—and one study—is necessary for the determination of regional perfusion and tissue damage.

The advantages of BZR radioligands as tracers for perfusion and markers of neuronal integrity in ischemia are confronted with certain limitations; the most important disadvantage is the low density of BZR in basal ganglia, white matter, and brain stem.50,51 Therefore, neuronal damage in these structures cannot be assessed reliably by FMZ. The quantitative determina-
tion of BZR density requires repeated injections of tracer with different specific activity, which is impractical in the clinical setting. For fast decision making about acute therapeutic intervention, eg, the initiation of thrombolytic therapy, the complete study might take too much time since a steady state must be reached for the determination of BZR distribution. However, this decision is usually based on the clinical situation and CT findings. In these instances the study of BZR receptors would be of scientific value to demonstrate which portion of the critically hypoperfused tissue is irreversibly damaged within the time window appropriate for initiation of thrombolytic therapy. This time window could be extended beyond the Food and Drug Administration–approved period of 3 hours if normal FMZ binding indicates largely preserved neuronal integrity. With FMZ as a reliable marker for early assessment of irreversible damage, the effect of neuroprotective compounds preventing delayed neuronal loss and ischemic damage by moderate but prolonged biochemical and perfusional disturbances could be evaluated. The BZR ligands (FMZ for PET or iomazenil for SPECT) therefore have a potential as clinically useful tracers in patients with acute ischemic stroke in whom areas with neuronal loss or permanent infarction can be detected early. The result of a BZR study might be relevant for the selection of patients for individual therapeutic interventions targeted to mechanisms with different time windows.

References

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