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 [11C]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.

Key Words: flumazenil receptors, benzodiazepine stroke, ischemic tomography, emission computed

Inhibitory GABAergic synapses are present in high concentration on all cortical neurons, and therefore the distribution of GABA receptors can be used as an indicator of neuronal integrity. Radioligands of central BZR—a subunit of the postsynaptic GABAergic complex—have been successfully applied to detect neuronal loss in various brain disorders affecting predominantly cortical cells, including focal epilepsy (review in References 5 and 6) and Alzheimer’s disease. In subacute to chronic states after acute cerebral ischemia, these tracers—FMZ for PET and [123I]iomazenil for SPECT—delineate the extension of cortical infarcts and also indicate incomplete infarction of reperfused cortex appearing structurally intact on CT or MRI. Recent data from experimental focal ischemia demonstrate that reduced BZR binding identifies irreversibly damaged tissue as early as 2 to 3 hours after transient occlusion of the middle cerebral artery. In this article we report the first application of the central BZR ligand FMZ to patients with acute ischemic stroke. The findings are compared with acute changes in rCBF and in rCMRO₂, with persistent alterations of rCMRglc, and with morphological damage assessed on MRI or CT 2 to 3 weeks after the stroke.

Subjects and Methods

Patient Selection

Inclusion Criteria

Ten patients (8 male, 2 female) aged 52 to 76 years (mean, 62 years) with their first acute hemispheric ischemic stroke were included in this study. The diagnosis was made clinically and based on focal neurological deficits of acute onset that persisted throughout the study. Initial assessment included general and neurological examination, ECG, chest radiography, routine electrolyte biochemistry and hematology determinations, and CT scan. During the following days, neck
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 performed 12 to 22 days later, when the size and location of the final infarct were also determined on T1-weighted MRI or 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.

**Selected Abbreviations and Acronyms**

- BZR = benzodiazepine receptors
- FMZ = [11C]flumazenil
- GABA = γ-aminobutyric acid
- OEF = oxygen extraction fraction
- PET = positron emission tomography
- (r)CBF = (regional) cerebral blood flow
- (r)CMRO2 = (regional) cerebral metabolic rate for oxygen
- (r)CMRglc = (regional) cerebral metabolic rate for glucose
- ROC = receive-operator characteristic
- ROI = region of interest
- SPECT = single-photon emission computed tomography
- VOI = volume of interest

In which blood samples were available, a two-compartment, two-parameter model could be applied to estimate regional receptor distribution. The ratios of distribution volume between cortical regions and white matter regions compared well with corresponding values of activity distribution between 30 and 60 minutes. Since a quantification of receptor density was not generally feasible, relative values of FMZ binding in comparison to averaged white matter activity were used for further analysis.

The second PET session 12 to 22 days after the stroke (with one exception) included measurement of rCMRglc after intravenous injection of 10 mCi (370 MBq) [15O]2-fluoro-2-deoxy-D-glucose following the previously described procedure and using activity-adjusted rate constants.

**Data Analysis**

With the use of an interactive program, all PET images were individually coregistered to the MRI or CT volume along the anterior-posterior commissural line. Subsequently, the cerebral hemispheres and the infarct comprising both gray and white matter were segmented from the MRI or CT volumes by means of an IDL (Interactive Data Language Research System Inc) and C-based image analysis system operating at a spatial resolution of 1 mm3. The cortical rim was defined by thresholding the FMZ images at three 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 CT or MRI images. Thus, the outer border of the cortex was defined by the contour from MRI or CT, whereas 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 only be reliably assessed in the cortex, only cortical areas were used for the comparative analysis of early changes in flow, oxygen metabolism, and FMZ binding and permanent morphological and metabolic defects. This analysis was based on the following criteria defined on all pertinent images of the individual patients: regions with critically disturbed perfusion below a threshold of 12 mL/100 g per minute25,26; areas with CMRglc, depressed below the critical value of 60 μmol/100 g per minute25,26; and cortical areas with FMZ binding decreased below 4.0 times the mean value in the white matter. This threshold was chosen since it was 2 SD below the mean value of normal cortex (5.9 ± 0.97); additionally, the respective decrease of more than 30% below the contralateral cortex could clearly be discriminated on the images. These abnormalities assessed on the early PET images were related to the area of finally infarcted cortex defined on late MRI or CT and to the regions with permanently depressed glucose metabolism (rCMRglc < 25 μmol/100 g per minute) on the late PET study.

**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 CMRglc, 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 = axb) 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, CMRglc, and FMZ binding decreased below the respective thresholds given with the size of final infarct and areas of permanently depressed
Compromised Cortical Regions in Individual Patients

<table>
<thead>
<tr>
<th>Pt</th>
<th>Age/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></td>
<td></td>
<td></td>
<td></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 CMR gluc (<25 μmol/100 g per minute).

*Hyperperfusion.

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glucose metabolism for the individual patients. No abnormalities were observed in only 1 patient, and this patient recovered without persisting neurological defects and without a lesion on CT or MRI. On initial CT, early signs of infarction could be detected in 8 patients. In 5 of those patients only subcortical hypodensity was found, in 1 patient cortical hypodensity covered less than one third of the MCA territory, and in 2 patients it covered more than one third. In 8 patients marked cortical flow decreases of variable extension were present on the early scans, with CMRO₂ changes to a variable degree leading to increased OEF in several regions. Within the areas of compromised blood supply, regions with FMZ binding decreased below the defined threshold (4.0 times the mean value in the white matter) were found that corresponded to the location of the infarcts defined on final CT or MRI. This was obvious for large territorial infarcts of the middle cerebral artery (Fig 1), but small cortical lesions were also detected (Fig 2). In 4 patients the area of permanently depressed rCMR gluc extended beyond the finally infarcted cortex. In 1 patient a marked focal hyperperfusion was found in the location corresponding to the neurological deficits. Within this area FMZ binding was reduced in a smaller region to 2.98, and a small area with severely depressed CMRO₂ was also found. On late MRI an infarction could not be delineated, but late PET studies demonstrated significantly decreased rCMR gluc and FMZ binding in a rather large area, suggesting considerable neuronal loss (“silent infarction”) 23. This patient suffered from permanent moderate aphasia (Fig 3). Overall, there was a significant correlation of the volume of initially reduced FMZ binding and the volume of final infarction (Fig 4).

For the analysis of the predictive value of initial changes in flow, oxygen metabolism, and FMZ binding on the final outcome, cortical areas were categorized as infarcted (on late MRI or CT), hypometabolic (cortex outside the infarcts with rCMR gluc permanently reduced below 25 μmol/100 g per minute), or normal (ipsilateral cortex appearing normal on MRI or CT and with rCMR gluc in the normal range). To avoid high variability of values as a result of pixel size and pixel distribution in regions suffering from partial volume effects, small regions of interest (spheres with 3-mm diameter) were

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**Figure 1.** Coregistered transaxial PET images at the caudate/ventricular level of CBF, early FMZ distribution (Dis) and steady state FMZ binding (Bdg), and OEF at 12 hours and CMR gluc and MRI at 2 weeks after moderate left hemiparesis and hemihypesthesia of acute onset in a 52-year-old male patient. The large territorial defect is visible in all PET modalities with different extensions. The contour delineates the cortical infarct as determined on late MRI. FMZ binding precisely predicts the extension of the final infarct, whereas CBF and FMZ distribution (as a marker of perfusion) delineate a considerably larger volume of disturbed perfusion. In the cortical region outside the infarct with initially disturbed perfusion, OEF is increased, indicating preserved CMRO₂ at 12 hours after ictus. The permanently decreased CMR gluc in this region could be caused by neuronal loss and/or diaschisis.
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 = 0.56), 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 to FMZ binding in the analyzed region (Fig 6b). 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 = 0.88) between these procedures (Fig 8). The non-linear 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\textsubscript{2} below certain thresholds.\textsuperscript{23,24} It is in accordance with these previous findings that CMRO\textsubscript{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\textsubscript{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 “miserly 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\textsubscript{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. In the permanent state, reduced rCMRglc together with reduced FMZ binding indicates neuronal loss in incomplete cerebral infarction, whereas discordant rCMRglc decrease not paralleled by decreased FMZ binding 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, 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 damage or due to additional disturbances of flow in case of progressive arterial thrombosis 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.

Figure 6. Cortical regions of interest labeled according to final outcome for early FMZ binding (relative to mean white matter values) versus CMRO2 (a) and early FMZ binding versus OEF (b). A significant correlation exists between FMZ binding and CMRO2, with a clustering of finally infarcted ROIs at low values. FMZ binding and OEF values are not significantly correlated; high OEF values are found in all categories of tissue outcome.

Figure 7. ROC curves for the prediction of infarcted versus noninfarcted cortical regions. a, ROC curve for flumazenil binding indicates good separation between infarcted and noninfarcted ROIs, with a sensitivity of 70.0% and a specificity of 95.2% for the FMZ binding value 4.0 times the mean white matter value, which was used as threshold. b, ROC curve for OEF is not different from random chance; early OEF has no predictive value for separation of finally infarcted and noninfarcted cortical regions.
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 in whom areas with neuronal loss or permanent 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 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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