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. (Stroke. 1998;29:454-461.)

Key Words: flumazenil receptors, benzodiazepine stroke, ischemic tomography, emission computed
Selected Abbreviations and Acronyms

- BZR = benzodiazepine receptors
- FMZ = \(^{11}C\)flumazenil
- GABA = \(\gamma\)-aminobutyric acid
- OEF = oxygen extraction fraction
- PET = positron emission tomography
- (r)CBF = (regional) cerebral blood flow
- (r)CMRO\(_2\) = (regional) cerebral metabolic rate for oxygen
- (r)CMRglyc = (regional) cerebral metabolic rate for glucose
- ROC = receive-operator characteristic
- ROI = region of interest
- SPECT = single-photon emission computed tomography
- VOI = volume of interest

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 arhythmias. 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 30 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 using the \(^{15}O\)H\(_2\)O intravenous bolus method with 60 mCi (2.2 GBq). Ten minutes later, 50 mCi (1.85 GBq) \(^{15}O\) 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 H\(_2\)O injection and \(^{15}O\)O inhalation and the time-activity curves of the blood, the computer (SUN SPARC, Sun Microsystems Inc) after decay correction calculated regional values of CBF, CMRO\(_2\), 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 \(^15\)O 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 H\(_2\)\(^{18}\)O. BZR density was estimated from the distribution of FMZ 30 to 60 minutes after the bolus injection. In a few cases 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 rCMR\(_2\) after intravenous injection of 10 mCi (370 MBq) \[^{15}F\]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 the 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 mm\(^3\). 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 minute\(^{-1}\), areas with CMRO\(_2\) depressed below the critical value of 60 \(\mu\)mol/100 g per minute\(^{-1}\), and cortical regions 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 (rCMRglyc < 25 \(\mu\)mol/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 CMRO\(_2\), 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 each predefined physiological thresholds for all parameters. Finally, a nonlinear curve-fit was computed with the use of a power function \(y = ax^b\) 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, CMRO\(_2\), and FMZ binding decreased below the respective thresholds given with the size of final areas and areas of permanently depressed
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 CMRO$_2$ 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$_{glc}$ 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 region with severely depressed CMRO$_2$ was also found. On late MRI an infarction could not be delineated, but late PET studies demonstrated significantly decreased rCMR$_{glc}$ and FMZ binding in a rather large area, suggesting considerable neuronal loss (“silent infarction”). 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$_{glc}$ permanently reduced below 25 μmol/100 g per minute), or normal (ipsilateral cortex appearing normal on MRI or CT and with rCMR$_{glc}$ 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

### 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 CMRO$_2$</th>
<th>Hypometabolism</th>
</tr>
</thead>
<tbody>
<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>18.01$^*$</td>
<td>4.76</td>
</tr>
<tr>
<td>3</td>
<td>63/M</td>
<td>L paracentral gyrus</td>
<td>0.91</td>
<td>1.45</td>
<td>0.47</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>64/M</td>
<td>L frontoparietal</td>
<td>5.24</td>
<td>1.32</td>
<td>30.53</td>
<td>5.71</td>
</tr>
<tr>
<td>5</td>
<td>52/M</td>
<td>R MCA territory</td>
<td>44.11</td>
<td>63.67</td>
<td>72.26</td>
<td>68.68</td>
</tr>
<tr>
<td>6</td>
<td>76/M</td>
<td>L frontotemporal</td>
<td>3.30</td>
<td>0.67</td>
<td>14.80</td>
<td>11.43</td>
</tr>
<tr>
<td>7</td>
<td>65/M</td>
<td>L posterior insula</td>
<td>18.88</td>
<td>22.51</td>
<td>45.63</td>
<td>24.58</td>
</tr>
<tr>
<td>8</td>
<td>60 /F</td>
<td>R posterior insula</td>
<td>8.03</td>
<td>18.78</td>
<td>7.79</td>
<td>36.62</td>
</tr>
<tr>
<td>9</td>
<td>55/M</td>
<td>L anterior insula</td>
<td>29.32</td>
<td>33.09</td>
<td>37.21</td>
<td>26.75</td>
</tr>
<tr>
<td>10</td>
<td>73/M</td>
<td>R posterior insula</td>
<td>32.22</td>
<td>107.07</td>
<td>42.73</td>
<td>26.28</td>
</tr>
</tbody>
</table>

$^*$Hyperperfusion.

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 CMRO$_2$ (<60 μmol/100 g per minute), and of reduced CMR$_{glc}$ (<25 μmol/100 g per minute).
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 to FMZ binding in the analyzed region (Fig 6b). Whether or not tissue with increased OEF turned into necrosis was significantly dependent on CMRO₂. Finally, infarcted tissue showed mean initial CMRO₂ of 60.5 μmol/100 g per minute compared with salvaged tissue with 94.5 μmol/100 g per minute (P = .0001). This means that regions with normal, increased, or decreased OEF in the acute stage could finally be infarcted, hypometabolic, or normal.

The set of regional data was additionally used for an analysis of the sensitivity and the specificity of the different variables for the discrimination between finally infarcted and noninfarcted tissue. ROC curves for the separation of infarcted from noninfarcted regions based on the different variables are shown in Fig 7. FMZ binding showed a high reliability for the prediction of final tissue status with a sensitivity of 70.0% and a specificity of 95.2% at a threshold of four times; if the peak of the curve at 4.85 is used, a specificity of 84% and a sensitivity of 85% are obtained. This ROC curve is nearly identical to that from CMRO₂, in which case a sensitivity of 82.5% and a specificity of 83.4% are reached with a threshold of 60 μmol/100 g per minute. The values for rCBF are lower: a sensitivity of 77.5% and a specificity of 86.2% are obtained with the threshold set at 12 mL/100 g per minute. OEF within a region had no value for prediction of final 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.
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 “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 emolliion, according to Reference 25) or deactivation by impaired afferent pathways (“diascisis”)\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 rCMR \(_{\text{glc}}\) together with reduced FMZ binding indicates neuronal loss in incomplete cerebral infarction, whereas discordant rCMR \(_{\text{glc}}\) reduction not paralleled by decreased FMZ binding suggests deactivation. These two conditions can be deduced from our data: Concordant reduction of rCMR \(_{\text{glc}}\) and FMZ binding is an indicator of neuronal loss in incomplete infarction (Fig 3), while discordant rCMR \(_{\text{glc}}\) 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 (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, which was shown to be reversed by intravenous recombinant tissue plasminogen activator followed by clinical improvement. However, as indicated by the high correlation between FMZ distribution within the first 2 minutes after injection and rCBF measured by \(^{15}\text{O}\), 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. 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,\textsuperscript{5,18,51,52} 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 might be reached for the determination of BZR distribution. However, this decision is usually based on the clinical situation and CT findings.\textsuperscript{53} 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 neuronal 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.\textsuperscript{54}

References


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

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

Stroke. 1998;29:454-461
doi: 10.1161/01.STR.29.2.454

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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