Probability of Cortical Infarction Predicted by Flumazenil Binding and Diffusion-Weighted Imaging Signal Intensity

A Comparative Positron Emission Tomography/Magnetic Resonance Imaging Study in Early Ischemic Stroke

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Background and Purpose—The differentiation of reversible from irreversible ischemic damage is essential for identifying patients with acute ischemic deficits who may benefit from therapeutic interventions. Diffusion-weighted imaging (DWI) has become the method of choice to detect ischemic lesions. Positron emission tomography (PET) of the central benzodiazepine receptor ligand 11C flumazenil (FMZ) has been shown to be a reliable marker of neuronal integrity. These 2 imaging parameters were compared with respect to the probability to predict cortical infarction in early ischemic stroke.

Methods—In 12 patients with acute stroke, results from DWI (median, 6.5 hours after symptom onset) and FMZ–PET (interval, 85 minutes between DWI and PET) were compared with infarct extension 24 to 48 hours after onset of stroke on T2-weighted magnetic resonance imaging (T2-MRI). Probability curves predictive of eventual infarction were computed using respective DWI, FMZ, and apparent diffusion coefficient (ADC) values for voxels of interest (VOI) later classified as representing infarcted or noninfarcted tissue.

Results—Ninety-five percent limits predictive of cortical infarction were determined for relative FMZ binding (<3.2), DWI signal intensity (<1.18), and ADC values (<0.83). Cortical regions with values beyond these 95% limits did not necessarily overlap with nor were fully congruous with final cortical infarct volumes. The respective median volumes for these regions were FMZ median 10.9, range 0 to 99.7 cm³; DWI median 15.2, range 0 to 116.0 cm³; ADC median 12.4, range 0 to 112.7 cm³; and final infarct median 14.9, range 0 to 114.7 cm³. Overall, 83.5% of the final infarct, on average, was predicted by decreased FMZ binding, 84.7% by increased DWI signal intensity, and 70.9% by a decreased ADC value. The portions of the final infarct not predicted in the early investigation (false-negatives) were 4.8 cm³ (median) for FMZ, 3.7 cm³ for DWI, and 6.0 cm³ for ADC. The false-positive volumes not included in the final infarct were 0 cm³ (median) for FMZ, 5.1 cm³ for DWI, and 3.6 cm³ for ADC.

Conclusions—These results indicate that FMZ–PET and DWI are comparable in the prediction of probability of ischemic cortical infarction, but FMZ–PET carries a lower probability of false-positive prediction. The final infarcts include tissue not identified by these imaging modalities; at the time of the study, these tissue compartments are viable and could benefit from treatment. The discrepancy in predictive probability could be related to the fundamental difference of the measured variables: benzodiazepine receptor activity is a reliable marker of neuronal integrity in the cortex, and movement of water molecules in the extracellular space might be a more variable indicator of tissue damage. (Stroke. 2004;35:1892-1898.)

Key Words: stroke, ischemic ■ infarcts ■ magnetic resonance imaging, diffusion-weighted ■ tomography, emission computed ■ flumazenil

The detection of irreversibly damaged tissue in early stages of acute ischemic stroke has important therapeutic implications, because invasive strategies, eg, thrombolysis, cannot benefit and may even cause deleterious hemorrhages when the morphological integrity of the tissue is destroyed. Diffusion-weighted magnetic resonance imaging (DWI) has become a widely accepted modality for the assessment of an ischemic lesion and, especially in the combination with perfusion-weighted imaging (PWI), can be applied for the selection of patients amenable for acute therapy if a mismatch between these procedures suggests viable penumbral tissue. However, it was shown repeatedly that an area with increased
DWI signal does not necessarily turn into infarction but may finally normalize with spontaneous or treatment-induced recovery of neurological deficits, limiting the prediction of irreversible tissue damage. Such measurements assign irreversible tissue damage by the value of metabolic rate for oxygen decreased below a defined threshold. Quantitative measurements of cerebral blood flow (CBF) and cerebral metabolic rate of oxygen (CMRO₂) require arterial blood sampling, which is prohibited with invasive therapeutic intervention, eg, thrombolysis, and thereby further complicates the complex logistics of PET studies. As a simpler noninvasive but semiquantitative approach, the central benzodiazepine receptor ligand [¹¹C]-flumazenil (FMZ) can be applied as a marker of neuronal integrity. In several experimental and clinical studies, this tracer has been validated as a reliable predictor of irreversible cortical damage. The aim of this study was to define the prediction value of the DWI lesion and the volume with significantly decreased FMZ binding in acute cortical ischemia for the size of the final infarct determined by magnetic resonance imaging (MRI) in a new sample of patients with acute ischemic stroke.

Patients and Methods

Twelve patients (5 men, 7 women, aged 37 to 74 years, mean age 62.4 years) with acute hemispheric stroke were included in this study. In all of them, imaging with DWI-MRI was performed 2.7 to 19 hours after symptom onset (median 6.5 hours). PET scanning followed MRI within 20 to 130 minutes (median, 85 minutes). Between MRI and PET, none of the patients received benzodiazepines or showed significant clinical changes (ie, National Institutes of Health Stroke Scale [NIHSS] changes ≥4 points). Initial assessment included general medical standardized neurological examinations including the NIHSS and computed tomography (CT) scanning. The patients who arrived beyond the 3-hour time window received standard medical therapy (n=9). Three patients arrived within 3 hours after symptom onset and received intravenous thrombolysis according to our previously reported protocol. Fully informed consent to the study was obtained from the patients and from their next of kin.

MRI was performed on a Philips Intera 1.5-T whole-body Scanner (Philips) with single-shot spin-echo-planar-imaging (EPI) sequences providing 20 slices with a slice thickness of 6 mm. For DWI, 2 b values (b=0 and b=1000 s/mm²) were used. The gradients were applied to each of the x-, y-, and z-directions and the calculated isotropic images were used for further analysis of the DWI intensity maps. Further parameters of DWI acquisition included an echo time of 96 ms, a repetition time of 3560 ms, a matrix size of 128×128, and a field of view of 230×230 mm. The maps of the apparent diffusion coefficient (ADC) were calculated according to the Stejskal–Tanner equation: ADC = -ln(S₁/S₀) / δboom, where S₁ is signal intensity, DWI intensity and ADC values were expressed as the ratio relative to the contralateral homotopic regions. Size and location of the final infarct was determined 24 to 48 hours later on the T2-weighted MRI. Two patients showed no infarct on the follow-up MRI.

PET studies were performed in a resting state on an ECAT EXACT HR scanner (Siemens CTI) in 3-dimensional data acquisition mode providing 47 contiguous 3-mm slices at 5-mm full-width-half-maximum in-plane reconstructed resolution. In all patients, 20 mCi (740 MBq) [¹¹C] flumazenil (FMZ) was injected intravenously and the distribution and accumulation of this tracer was followed-up for 60 minutes by serial scanning. Benzodiazepine receptor (BZR) density was estimated from the distribution of FMZ 30 to 60 minutes after bolus injection. Because quantification of receptor density is not generally feasible, ratios of cortical FMZ binding in the affected hemisphere relative to contralateral oval center (white matter above lateral ventricles) activity were used for further analysis, as described previously.

The early PET and DWI findings were compared with outcome. T2-MRI obtained at 24 to 48 hours. Using an interactive program, all PET images were individually coregistered with the respective MRI volumes along the anterior–posterior commissural line. Because of the difference in spatial resolution, PET images were rescaled to a slice thickness of 6 mm to enable a voxel-based analysis. Because FMZ binds primarily to cerebral cortex, only cortical areas were used in the comparative analysis.

The predictive values of initial changes (1) in FMZ binding, (2) in the intensity of DWI changes, and (3) in ADC on final outcome were calculated as described previously. In brief, cortical areas were categorized in each individual patient as infarction or normal according to their appearance on follow-up MRI. To avoid large variances inherent in pixel data, contiguous spherical volumes of interest (VOIs) of 6 mm diameter were fitted into the cortical rim to cover the infarct and more than twice the volume of surrounding noninfarcted cortex (Figure I, available online at http://stroke.ahajournals.org). Depending on the individual infarct size, each patient contributed 16 to 59 volumes of interest (median 30) representing 30% infarct and 70% noninfarcted tissue. This method yielded a total of 456 VOIs, which were used for the following pooled analysis. For all values of FMZ binding, DWI intensity, and ADC across all patients’ VOIs, the threshold probability integrals of final infarction or noninfarction were iteratively computed. The positive prediction curve was obtained by gradually moving the FMZ, DWI, and ADC test threshold from the lowest to the highest observed values and by calculating the positive prediction rate at each step, ie, the proportion of infarcted cortical areas of all VOIs exhibiting values at or below the respective threshold. From the curves, the positive 95% prediction limits of FMZ binding, DWI intensity, and ADC were obtained as the values on the abscissa at the prediction rate of 0.95. The hereby defined thresholds were used for further analysis using an interactive data language (Research Systems Inc) and a C-based image analysis system operating at a spatial resolution of 1 mm². An individual cortical atlas was generated thresholding the early T1-weighted MRI that showed no morphological alteration at this early time point. Cortical tissue compartments were defined voxel-by-voxel using the positive FMZ, DWI, and ADC limits to estimate their relative size with respect to morphological outcome (online Figure). The descriptive statistics of variables exhibiting a non-normal distribution are given as median and range. The differences among the false-positive volumes predicting infarction were analyzed by the nonparametric test for paired samples (Wilcoxon test).

Results

The patients included in this study had hemispheric ischemic strokes of different severity, leading to infarcts involving the cortex within the territories of the middle cerebral artery to a variable degree (n=9) and the posterior cerebral artery (n=1). The clinical deficit ranged from mild (and transient) paresis of an arm to severe contralateral sensorimotor deficits. Age, sex, time elapsed from onset of symptoms to DWI, time between DWI and PET, and the size of the cortical infarct are listed in Table 1. On later MRI, it was shown that an infarct did not develop in 2 patients, 1 of them showed a small lesion on early DWI. In 2 patients, small lesions were detected on the final MRI, in both of them early DWI abnormalities were slightly larger. Three patients (patients 7, 8, 9) received...
intravenous rt-PA within the 3-hour window before the imaging studies, but this treatment was only effective in 1 (patient 8), who experienced a change in the NIHSS from 8 to 2 in the 24 hours after the study. In the 2 other patients NIHSS was unaffected (score 15 to 16 in patient 7, and 14 to 14 in patient 9).

FMZ Binding and Morphological Outcome

The distribution of pooled regional FMZ binding values with respect to “infarcted” and “noninfarcted” VOIs on late MRI scans (Figure 1A) exhibited a distinct positive skew in eventually infarcted volumes of interest and a more symmetrical distribution in eventually noninfarcted volumes of interest. There was considerable overlap of these distributions. The relationship between cortical FMZ binding and the tissue condition found on late MRI scans can be calculated in predictive curves. Using conventional probability levels of 0.95, this prediction curve yielded the weighted mean positive 95% prediction limit of early cortical FMZ binding for infarcted tissue (Figure 1B). FMZ binding values the mean binding in the contralateral white matter identified tissue that turned into infarction.

MRI-Derived Variables and Morphological Outcome

Histograms and positive prediction curves relating early relative DWI signal intensity and ADC values to the appearance of the cortex on late MRI scans were derived as has been described for FMZ binding. The DWI signal relative to the contralateral homotopic area exhibits a rather small and symmetric distribution for the eventually noninfarcted volumes of interest and a rather broad distribution for the eventually infarcted volumes of interest (Figure 1C). Again, there was some overlap between these distributions. The calculated positive predictive curve is rather steep and yields a well-defined 95% prediction limit for cortical infarction at 1.18 (i.e., 118% compared with the unaffected contralateral area) (Figure 1D). The ADC maps consisted of inhomogeneous areas with more variable values limiting the volumetric analysis for prediction of tissue outcome. Therefore, the histograms of values for infarcted and noninfarcted tissue demonstrated a broad overlap (Figure 1E) and the predictive curve was less steep than for DWI (Figure 1F). The 95% probability threshold for final infarction was calculated at 0.83 (i.e., 83% of the contralateral value).

Comparison of Changes in FMZ Binding and in DWI/ADC Values for Prediction of Tissue Outcome

To further investigate the differences in the predictive value of FMZ binding and DWI/ADC values the mean positive 95% predictive limits were used to form various subcompartments for the tissue outcome in a voxel-based analysis. As can be seen on Table 2, the sizes of the final infarcts were in most cases different from volumes of tissue predicted by either imaging parameter as infarcted. Despite a close correlation between FMZ and DWI predicted findings, the volumes do not completely overlap. This indicates that there are differences between these imaging parameters that can be analyzed by differentiating subcompartments according to their FMZ binding or DWI appearance (Table 3). Overall, 83.5% of the final infarct (14.9 cm³ median) was predicted by a decreased FMZ binding, 84.7% by an increased DWI signal, and 70.9% by a reduced ADC value. In a median volume of 8.0 cm³ of the final infarct, both FMZ and DWI were beyond the threshold; in 1.7 cm³ and 3.4 cm³ only FMZ and only DWI, respectively, was at or outside the critical value, and only a small part of the final infarct was not predicted by FMZ or DWI value beyond the critical limit (median 1.1, mean 2.0 cm³). The false-positive and false-negative rates, however, showed more significant differences: only a small part (median 0, mean 0.9 cm³) of the finally noninfarcted tissue had initially decreased FMZ binding.
whereas 5.1 cm$^3$ of finally normal tissue showed an increased DWI signal (25.9% of the total volume of DWI increase) and 3.6 cm$^3$ showed a decreased ADC value (22.3% of the volume). These differences were significant ($P<0.01$, Wilcoxon test). The volumes of portions of infarct not predicted by decreased FMZ (4.8 cm$^3$ = 16.5% of final infarct) and by changed DWI signal (3.7 cm$^3$ = 15.3% of final infarct) were comparable, whereas the ADC value was again less reliable (6.0 cm$^3$ = 29.1% of final infarct). Figure 2 illustrates the high predictive value for early infarct detection on FMZ and DWI. In contrast, Figure 3 shows markedly increased DWI signal in a large area where FMZ binding was not affected; on final MRI, no lesion was detected. In most cases, however, the differences with respect to areas with reduced FMZ binding or changed DWI signals were at the borderline of the ischemic territory and usually turned into infarction.

**Discussion**

It was the objective of this comparative study to define probability thresholds for the early prediction of subsequent cortical infarction by various PET-based and MRI-based imaging parameters: The relative values of FMZ uptake $<3.2$, DWI $>1.18$, and ADC $<0.83$ predicted infarction at follow-up MRI with a 95% probability. These cutoff values may serve to compare the respective imaging modality with respect to probability of final infarction. This study was not intended to prove the clinical applicability of any imaging method or its impact on therapeutic decisions. However, this
comparative approach allows combination of the advantages of each imaging technique to yield complementary insight into the dynamics of the ischemic damage. Although our results are derived exclusively from cortical data, they represent pathophysiological mechanisms relevant for ischemic brain damage.

Comparative studies of different complex imaging modalities, however, usually have deficiencies related to the difficulty in performing several demanding investigations in individual patients within a short time window. The fact that PET requiring more complex logistics, after MRI, caused a delay of 85 minutes, which may have contributed to some of the observed differences because it actually shifts the PET study closer to the time when the infarct has reached its final extension. However, despite this delay, the cortical portions of the final infarct predicted correctly by PET and DWI were comparable (83.5% versus 84.7%), and only the rate of false-positive predictions was higher with DWI. The reversibility of some lesions observed in DWI early after stroke has been described in various clinical conditions including transient ischemic attacks,6,21 small ischemic infarcts,22,23 and use of thrombolytic therapy.7,24 These observations led to a correction of the original concept that the DWI lesion represents the irreversible infarct in early stroke. However, it was shown in animal experiments that reversal of early DWI abnormalities does not necessarily reflect potential tissue salvage,25 and re-expansion of changes on DWI has been observed probably as a consequence of reperfusion injury.26 In our small sample of patients with stroke, 25.9% of the total volume with a DWI signal >1.18 did not evolve into infarction. This finding represents the most important difference between the DWI and FMZ–PET studies in early stroke; in most patients, FMZ binding reduced below the limit predicted infarction, and only in a few cases very small tissue portions (median 0, mean 0.9 cm³) with reduced FMZ binding were not included in the final infarcts.

Differences in the tissue volumes identified as abnormal by the particular imaging modality might be caused by technical reasons, eg, the differences in spatial resolution. Slice thickness for PET and size of VOIs were chosen with respect to DWI, and the accordingly used size of regions of interest made a new calculation of FMZ thresholds necessary. This methodical adjustment explains the small difference of this new group of patients to the probability threshold for FMZ binding.

| TABLE 2. Summary Statistics of Final Infarct, FMZ, DWI, and ADC Volumes (Values in cm³) |
|-----------------|-----------------|---------------|-----------------|---------------|---------------|---------------|
| Patient ID     | Infarct Total Correct Positive | Total Correct Positive | Total Correct Positive | Total Correct Positive | Total Correct Positive |
| 1              | 11.6 7.2 2.7 | 13.6 7.1 6.6 | 112.3 98.9 105.3 | 97.9          |
| 2              | 0.0 0.0 0.0 | 0.0 0.0 0.0 | 0.0 0.0 0.0 | 0.0          |
| 3              | 111.0 90.4 90.4 | 112.3 98.9 105.3 | 105.3 97.9 |
| 4              | 34.0 22.6 22.6 | 32.8 27.7 35.5 | 29.2          |
| 5              | 57.4 53.8 50.3 | 61.2 51.4 47.6 | 43.6          |
| 6              | 1.2 0.6 0.6 | 5.0 0.9 2.0 | 0.7          |
| 7              | 17.5 8.6 7.0 | 16.4 12.6 14.5 | 11.4          |
| 8              | 114.7 99.7 99.7 | 116.0 102.8 112.7 | 101.6        |
| 9              | 0.0 0.0 0.0 | 5.1 0.0 6.2 | 0.0          |
| 10             | 16.6 15.6 14.2 | 21.5 14.6 15.9 | 10.5          |
| 11             | 13.1 13.1 13.1 | 13.9 10.3 10.3 | 8.0           |
| 12             | 1.3 1.3 1.3 | 3.8 1.3 2.3 | 1.1          |
| Median         | 14.9 10.9 10.1 | 15.2 11.5 12.4 | 9.3           |
| Min            | 0.0 0.0 0.0 | 0.0 0.0 0.0 | 0.0          |
| Max            | 114.7 99.7 99.7 | 116.0 102.8 112.7 | 101.6        |
| Mean           | 31.5 26.1 25.2 | 33.5 27.3 29.9 | 25.6          |
| SD             | 38.38 35.55 35.66 | 41.15 37.36 39.59 | 37.05        |

| TABLE 3. Absolute and Relative Size (Median Values) of Cortical Subcompartments Identified by a Threshold-Based Volumetric Analysis of FMZ, DWI, and ADC Imaging |
|-----------------|-----------------|---------------|---------------|---------------|---------------|---------------|
| FMZ <3.2        | FMZ >3.2        | DWI >1.18     | DWI <1.18     | ADC <0.83     | ADC >0.83     |
| Infarcted        | 10.1 cm³        | 4.8 cm³       | 11.5 cm³      | 3.7 cm³       | 9.3 cm³       | 6 cm³         |
| (median volume 14.9 cm³) | 83.5% | 16.5% | 84.7% | 15.3% | 70.9% | 29.1% |
| Noninfarcted     | 0 cm³           | normal cortex | 5.1 cm³      | normal cortex | 3.6 cm³      | normal cortex |
|                  | 0%              | 25.9%         | 22.3%         |               |               |

In the case of infarcted tissue on the follow-up scan (upper row), values are given as the percentage of infarct size. In the case of noninfarcted tissue on the follow-up scan (lower row), values are given as the percentage of lesion volume on FMZ, DWI, or ADC imaging.
binding (3.4× the value in white matter) observed in our previous study.19 ADC values might be additionally affected by inhomogeneity introduced by cerebrospinal fluid;27 this could lead to lower cutoff values for prediction of infarction.28 A further problem in the comparison of PET and MRI data is the accuracy of coregistration, which in some instances is impaired by distortion of MR images. This well-known phenomenon could account, at least in part, for the lack of absolute congruency of volumes with reduced FMZ and ADC and the rather large portions where either FMZ binding or DWI value predicted final infarction.

Differences in the findings with different imaging modalities in acute ischemia are also caused by the different basic mechanisms investigated and therefore can yield complementary insight into pathophysiological processes. PET measurements are based on clearly defined biochemical concepts and use: (1) regional blood flow and oxygen consumption for the definition of thresholds, penumbra, and damaged tissue;29–31 (2) the binding to benzodiazepine/GABA receptors of nerve cells as a marker of neuronal integrity;14 and (3) uptake of 18F-misonidazol for identification of hypoxic tissue.32 DWI changes seem to reflect much more complex pathophysiological alterations, including redistribution of water from the extracellular to the intracellular space as a consequence of reduced adenosine-triphosphate activity,33 with consequent swelling of cells and shrinkage of the intracellular space. For the methods applied in this study, it must be kept in mind that DWI also images lesions within the white matter, which escape detection by FMZ–PET because BZRs are mainly localized on cortical neurons.

The differences in underlying pathophysiological processes explain discrepancies in the imaging findings in early stroke. In a few instances, DWI changes were reversible in cortical areas with normal FMZ binding indicating (transient) swelling of cells before BZRs are affected. However, decreased FMZ binding predicted infarction in some areas before DWI was visibly affected pointing to the high sensitivity of FMZ as a marker of neuronal integrity. An important difference concerns false-positive predictions. The volume with FMZ binding reduced below the limit, which did not develop into infarction, was significantly smaller (median 0% of total volume with reduced FMZ binding) than that for the changed DWI signal (25.9%) or the increased ADC value (22.3%). That indicates that tissue with decreased FMZ binding usually did not recover. In contrast, tissue with changed DWI signal had some potential for survival. The presented data from a limited patient sample indicate a high specificity of decreased FMZ binding for reliable prediction of infarction. Overall, decreased FMZ binding predicted final infarction in a slightly smaller volume (83.5% of the final infarct) than decreased DWI signal (84.7%), but the respective volumes do not completely overlap. Because the volume of decreased FMZ binding and of changed DWI signal, or both, did not completely correspond to the final infarct, a further extension of infarction must be postulated between DWI/PET studies and final MRI. These portions of the final infarct in which FMZ above (16.5% of final infarct) or DWI signal below the threshold (15.3% of final infarct) did not predict damage might reflect tissue compartments amenable for treatment.

In conclusion, our results indicate that combined MRI/PET imaging in patients with acute stroke reveal complementary information on the dynamics of pathophysiological events in ischemic brain tissue with (1) comparable prediction values between FMZ and DWI for the final extent of infarction and (2) a lower false-positive prediction value for noninfarcted tissue by FMZ. Further studies are required to define the value of these imaging data for the identification of each patient’s individual therapeutic window for successful and safe initiation of reperfusion or neuroprotective therapy.
References
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