Topography and Temporal Evolution of Hypoxic Viable Tissue Identified by $^{18}$F-Fluoromisonidazole Positron Emission Tomography in Humans After Ischemic Stroke

R. Markus, MBChB, FRACP; D.C. Reutens, MD, FRACP; S. Kazui, MD; S. Read, PhD, FRACP; P. Wright, MBBS; B.R. Chambers, MD, FRACP; J.I. Sachinidis, PhD; H.J. Tochon-Danguy, PhD; G.A. Donnan, MD, FRACP

Background and Purpose—We sought to characterize the spatial and temporal evolution of human cerebral infarction. Using a novel method of quantitatively mapping the distribution of hypoxic viable tissue identified by $^{18}$F-fluoromisonidazole ($^{18}$F-FMISO) PET relative to the final infarct, we determined its evolution and spatial topography in human stroke.

Methods—Patients with acute middle cerebral artery territory stroke were imaged with $^{18}$F-FMISO PET (n = 110; 1-6 hours, 4; 6 to 16 hours, 4; 16 to 24 hours, 5; 24 to 48 hours, 6). The hypoxic volume (HV) comprised voxels with significant ($P<0.05; >1\text{ mL}$) uptake on statistical parametric mapping compared with 15 age-matched controls. Central, peripheral, and external zones of the corresponding infarct on the anatomically coregistered delayed CT were defined according to voxel distance from the infarct center and subdivided into 24 regions by coronal, sagittal, and axial planes. Maps (“penumbragrams”) displaying the percentage of HV in each region were generated for each time epoch.

Results—Higher HV was observed in the central region of the infarct in patients studied within 6 hours of onset (analysis of covariance [ANCOVA]; $P<0.05$) compared with those studied later, in whom the HV was mainly in the periphery or external to the infarct. HV was maximal in the superior, mesial, and posterior regions of the infarct (ANCOVA; $P<0.05$).

Conclusions—These observations suggest that infarct expansion occurs at the expense of hypoxic tissue from the center to the periphery of the ischemic region in humans, similar to that seen in experimental animal models. These findings have important pathophysiological and therapeutic implications. (Stroke. 2003;34:2646-2652.)

Key Words: fluoromisonidazole ▪ hypoxia ▪ penumbra ▪ stroke ▪ tomography, emission computed

Cerebral infarction after ischemic stroke involves a complex series of pathophysiological changes that evolve in time and space. Hypoperfused, hypoxic, but initially viable tissue is progressively transformed to infarction as a result of a time-dependent cascade of functional and metabolic changes induced by ischemia. Investigators using serial multitracer PET studies in middle cerebral artery (MCA) occlusion stroke models in cats and baboons have shown that transformation of the potentially reversible region to irreversible injury is a dynamic process both temporally and spatially, with infarct expansion occurring from the center to the periphery of the ischemic region, reaching a maximum volume 24 hours or later after occlusion. Therapeutic strategies to limit infarct size and improve functional outcome after acute stroke are aimed at rescuing this potentially reversible ischemic region. In humans, infarct expansion at the expense of potentially viable tissue has been documented even 24 hours after stroke onset. However, the temporal evolution and spatial topography of this potentially salvageable region have not been systematically characterized in humans. This is of importance because the impact of tissue salvage on clinical outcome may depend on the location of this region as well as its volume.

Here, we characterize the geographical distribution and evolution of tissue with increased uptake of $^{18}$F-fluoromisonidazole ($^{18}$F-FMISO), a 2-nitroimidazole derivative that is selectively trapped in hypoxic viable cells in patients with acute stroke. The heterogeneous topography of infarcts in humans precludes simple comparison of the spatial extent of hypoxic tissue between stroke patients. We have implemented an objective and reproducible technique of mapping the 3-dimensional spatial extent of hypoxic tissue...
Subjects and Methods

Subjects

Patients were recruited from the acute stroke unit at the Austin & Repatriation Medical Center (Melbourne, Australia). The Human Research Ethics Committee of the hospital approved the study protocol. Written informed consent was obtained from the subjects or their next of kin. Inclusion criteria for the study were (1) symptoms and signs of an acute hemispheric stroke, (2) 18 F-FMISO uptake, identified with a previously validated method of statistical inference and with normal brain CT scans.

Initial noncontrast cranial CT to exclude cerebral hemorrhage and a second scan 7 to 10 days after stroke onset to define the infarct volume (IV).

Image Analysis

Image analysis was performed with in-house software implemented for MATLAB (Mathworks Inc). Postprocessing time for PET images averaged 20 minutes per patient. All image data sets were transformed into standard coordinate space with the Automated Image Registration software package (AIR 3.0). The FMISO PET and CT templates comprised the average of 15 healthy subjects previously registered to standard stereotaxic coordinate space. 10

The 18 F-FMISO images were normalized by the mean activity in the contralateral hemisphere and filtered with a gaussian kernel of 6-mm full-width half-maximum to remove high-frequency noise. Statistical parametric maps of 18 F-FMISO tracer uptake were generated for the voxel-wise comparison of each patient with the group of control subjects. The hypoxic volume (HV) was defined as the region of significantly increased (P<0.05) 18 F-FMISO uptake, identified with a previously validated method of statistical inference based on the theory of gaussian random fields. 11 The infarct volume (IV) was defined as voxels comprising the hypodense lesion on the late CT scan outlined manually by an experienced observer blinded to the results of the 18 F-FMISO PET.

Penumbragram Construction

The central and peripheral regions of the IV were defined relative to the center of gravity (COG) of the final infarct. The coordinates of the COG in each of the p dimensions (x, y, and z) are given by the following:

\[ COG_p = \frac{\sum c_i n_{i,p}}{\sum n_{i,p}} \]

where \( C_p \) refers to the \( p \)th coordinate of dimension \( p \) and \( n_{i,p} \) refers to the number of voxels within the infarct with that coordinate. The Euclidean distance between each voxel in the IV and the COG was then calculated. Voxel forming the center and periphery of the IV were operationally defined as those with distances less than and greater than the median distance, respectively. Horizontal, coronal, and sagittal planes passing through the COG were used to further subdivide the IV, yielding 16 regions within the IV and 8 regions external to the final infarct. The penumbragram (Figure 1) maps the

Figure 1. Schematic representation of the construction of a penumbragram displaying 3-dimensional distribution of HV relative to predefined zones of the infarct and surrounding regions. Euclidean distance from the COG was used to operationally define central (yellow), peripheral (red), and external (blue) zones of the IV. In the penumbragram, superior and inferior halves of the IV defined by the horizontal plane through the COG are shown separately, with the inner, middle, and outer circles representing the central, peripheral, and external zones of the IV. Further subdivisions in the anterior/posterior and mesial/lateral planes relative to the COG give 12 regions in each half. Displaying the percentage of voxels with significant 18 F-FMISO uptake that anatomically correspond to each of these 24 regions generates a map demonstrating 3-dimensional distribution of hypoxic tissue relative to the infarct. This permits quantitative analysis of the distribution of hypoxic tissue between patients or groups of patients.
distribution of HV corresponding to each of these regions and permits quantitative statistical analysis between patients or groups of patients.

Data and Statistical Analyses
Statistical analysis was performed by use of SPSS, version 7.5.

Temporal Evolution of Hypoxic Viable Tissue in Acute Ischemic MCA Territory Stroke
A composite penumbragram was generated for each patient subgroup (<6, 6 to 16, 16 to 24, and >24 hours), reflecting the average distribution of the HV at each time interval. To examine the temporal evolution of HV, the distribution of HV within the central region, peripheral region, and regions external to the final infarct was examined by multivariate analysis of covariance (ANCOVA) with regional volume (mL) as the dependent variable, region and time interval as main effects, and total HV (mL) for each patient as a covariate. A Bonferroni correction was applied to correct for repeated measurements.

Spatial Topography of Hypoxic Viable Tissue in Acute Ischemic MCA Territory Stroke
A composite penumbragram summarizing the distribution of HV in all patients was used to study geographical distribution of hypoxic tissue. Differences in distribution of HV in the superior/inferior, mesial/lateral, and anterior/posterior regions relative to the center of the infarct were examined by ANCOVA with regional volume (mL) as the dependent variable, region as the main effect, and HV (mL) for each patient as a covariate. A Bonferroni correction was applied to correct for repeated measurements.

Results
Nineteen patients (10 men; mean±SD age, 76.7±12.5 years; Table 1) with acute MCA territory strokes were included in this study. Fifteen subjects (8 men; mean±SD age, 67.4±10.7 years) with no history of stroke or transient ischemic attack and with normal brain CT scans formed the control group.

The mean IV for the whole group was 141.8 mL, and mean hypoxic tissue volume was 48.5 mL. A substantial proportion of the final infarct was initially hypoxic and potentially viable (mean±SD, 32.2±32.7%; Table 1). Four subjects were studied with ¹⁸F-FMISO PET within 6 hours (mean±SD, 4.8±0.9 hours), 4 within 6 to 16 hours (mean±SD, 9.8±3.3 hours), 5 within 16 to 24 hours (mean±SD, 20.4±2.9 hours), and 6 within 24 to 48 hours (mean±SD, 39.7±7.0 hours) of stroke onset. Representative coregistered PET and CT image slices from each patient are shown in Figure 2.

Temporal Evolution of Hypoxic Viable Tissue in Acute Ischemic Stroke
The temporal evolution of hypoxic tissue is summarized in Figure 3. ANCOVA showed that there was a significant interaction between the interval from stroke onset to PET scanning and regional distribution of hypoxic tissue (P<0.05). Significantly higher HV was observed in the
central region of the infarct in the group of patients studied within 6 hours compared with those studied later. In the latter group, significantly higher HV was seen in the peripheral and external regions (P<0.005).

Spatial Topography of Hypoxic Viable Tissue in Acute Ischemic Stroke

The spatial topography of HV for patients with MCA territory strokes is summarized in Figure 4. The distribution of HV in the superior/inferior, mesial/lateral, and anterior/posterior regions relative to the center of the infarct for each patient is shown in Table 2. ANCOVA with a Bonferroni correction showed that HV was significantly higher in the superior, mesial, and posterior regions (P<0.05) in the group of patients with MCA territory strokes.

Discussion

The principal finding of this study was that significantly higher volumes of hypoxic viable tissue were observed in the region corresponding to the center of the infarct in patients studied within 6 hours of stroke onset, whereas in those studied at later times, hypoxic tissue was present mostly in the periphery or external to the infarct. The temporal change in location of hypoxic tissue is consistent with infarct expansion at the expense of hypoxic tissue beginning at the center of the ischemic region and extending to its periphery.

Figure 2. Representative image slices from each patient showing the region of hypoxic tissue identified by acute-stage 18F-FMISO PET superimposed on the final infarct defined on late CT. Infarct boundary is outlined in yellow. Hypoxic tissue that was viable at the time of acute PET but subsequently infarcted is shown in red; areas that survived are shown in green. Tissue without 18F-FMISO uptake within the final infarct is presumed to have infarcted by the time of the acute PET study. Representative image slice for each patient was chosen to show the maximal extent of hypoxic tissue and therefore does not correspond to center of the final infarct.

Figure 3. Penumbragrams characterizing the temporal evolution of the hypoxic tissue in patients after acute ischemic stroke. Composite penumbragram for each time epoch (<6, 6 to 16, 16 to 24, and 24 to 48 hours) after stroke onset are shown. Number in each region refers to percentage of total HV. Higher volume of hypoxic tissue is observed in the central region in patients studied within 6 hours of onset (P<0.05; ANCOVA) vs those studied at later time points, when it occurs mainly in the periphery or external to the infarct. Superior, mesial, and posterior predominance of hypoxic tissue distribution is seen at all time points.
Analysis of the spatial topography of the hypoxic tissue in MCA territory strokes revealed a superior, mesial, and posterior predominance, perhaps reflecting the pattern of collateral flow.

Our observation that the hypoxic potentially viable region temporally decreases from the center to the periphery of the infarct in human stroke is concordant with findings from sequential multitracer PET studies in animals. In baboons after MCA occlusion, hypometabolic tissue progressively expanded laterally and posteriorly from the central ischemic region over 24 hours. Reperfusion within 6 hours restricted the infarct to the deep MCA territory. In cats after MCA occlusion, infarct expansion progressed from the center to the periphery of the region with increased oxygen extraction fraction (OEF) over 24 hours. In rats studied by MRI, restricted diffusion was observed 1 hour after MCA occlusion only at the center of the perfusion deficit but at 24 hours encompassed the entire region, which at 1 week showed changes in infarction on histology. These qualitative descriptions are compatible with the concept of dynamic infarct expansion occurring at the expense of the ischemic penumbra.

The spatial and temporal evolution of the ischemic penumbra has been assumed to be similar in humans and animals, although this assumption has not been previously tested. In humans, investigators using multitracer PET have identified hypoperfused tissue with preserved energy metabolism, compatible with penumbra in the acute stages after stroke. This tissue was observed predominantly over the cortical mantle and was present on early but not follow-up PET scans. Late CT showed a corresponding area of infarction. Survival of this tissue was associated with a better neurological

### Table 2. Distribution of Hypoxic Tissue Volume in MCA Territory Stroke

<table>
<thead>
<tr>
<th>Patient</th>
<th>Superior, mL</th>
<th>Inferior, mL</th>
<th>Mesial, mL</th>
<th>Lateral, mL</th>
<th>Anterior, mL</th>
<th>Posterior, mL</th>
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<td>21</td>
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<td>16.3</td>
<td>7.0</td>
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<td>21.8</td>
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</tbody>
</table>

Mean±SD 28.8±29.1 19.8±20.4 29.7±31.1 18.8±19.3 19.4±22.1 29.2±28.9
outcome. Investigators using MRI have described several different patterns of mismatch between perfusion and diffusion abnormalities, reflecting the heterogeneous topography of human stroke. However, this technique has yet to be used to obtain a quantitative evaluation of the temporal evolution of the penumbra.

In this study, analysis of the spatial topography in patients with MCA territory ischemic stroke revealed a predominance of hypoxic viable tissue in the superior, mesial, and posterior aspects of the final infarct. Similar to our findings in humans, in baboons after MCA occlusion, increased OEF was observed on the superior and posterior aspects of the final infarct. Although we describe its use in individuals with single well-defined final infarcts, the algorithm can be applied to each infarcted region in patients with multiple infarcts. Infarct volume measurement on CT using manual tracing is reproducible with low intraobserver and interobserver variability. Infarct volumes were measured on CT scans performed 7 to 10 days after stroke onset because patients had died by the 3-month follow up. Although we cannot exclude that fogging effect may have obscured infarct hypodensity, the infarct contour outlined on the CT done on day 7 to 10 was similar to that on a CT performed later in the subset that survived, suggesting that the effect was minimal. CT scans at 7 to 10 days after stroke onset have been used to measure the IV in clinical trials of acute stroke therapy.

Misonidazole is a 2-nitroimidazole derivative that is selectively retained in hypoxic tissue after reduction by cellular reductases and binding to cellular components. This tracer is not retained in normoxic cells in which the molecule is immediately reoxidized and is not available for further reduction and trapping or in irreversibly injured cells in which the enzymes responsible for the initial reduction are compromised. Lythgoe et al studied the relationship between diffusion MRI and autoradiographic markers of hypoxic tissue (123I-iodoazomycin arabinoside, a 2-nitroimidazole derivative with binding characteristics in hypoxic tissue similar to FMISO) and blood flow (99mTc-hexamethylpropylene amine oxime) injected 2 hours after MCA occlusion in a rat model of stroke. The area of hypoxic tracer retention and diffusion MRI abnormality corresponded to regions in which blood flow was <34% of normal. The region with mild hypoperfusion (<66% but >34% of normal) that was not recruited to infarction had normal tracer uptake and diffusion, suggesting that hypoxic tracer uptake may discriminate between the ischemic penumbra, where tissue is at risk of infarction, and oligemia, where it is not at risk. Tracer uptake was reduced in the region with a diffusion abnormality and severe hypoperfusion (<7% of normal). The hypoxic region identified by 18F-FMISO PET in humans has not been directly compared with multitracer PET; this is an area of current study. In this study, a mean of 32% of the final infarct was initially hypoxic and viable. This is comparable to the observation that penumbral tissue defined by multitracer PET comprised 10% to 52% (mean, 32%) of the final IV. We observed significant amounts of hypoxic tissue in 6 of 28 patients (21%) studied 24 hours after stroke onset. Serial MRI studies in humans have shown substantial infarct enlargement occurring in up to 33% of patients 24 hours after stroke onset. Further studies are necessary to establish whether hypoxic tissue observed at delayed time points after stroke onset retains the capacity for reversibility or is already irreversibly damaged without a potential for recovery. The observation that even at these delayed time points a proportion of hypoxic tissue survived spontaneously without infarction (Figure 2) suggests that its fate may not be predetermined. These converging data from animals and humans suggest that after ischemic stroke the geographical distribution and temporal evolution of hypoxic tissue labeled with 18F-FMISO are likely to mirror the behavior of the ischemic tissue at risk of infarction.

Our observation that the spatial distribution of hypoxic tissue evolves over time, principally involving the central region of the infarct in the first 6 hours, has important clinical and pathophysiological implications. A number of neurobiological factors are likely to explain the spatial and temporal evolution of the penumbra. Susceptibility to ischemia may differ in different parts of the vascular territory according to predominant cell type, vascular architecture, and collateral circulation. In addition, different survival time profiles within compartments in the penumbra may reflect disparate pathophysiological mechanisms leading to infarction, as postulated by Heiss et al. For example, critically hypoperfused tissue within the central ischemic region may have a more rapid evolution to irreversible damage and a shorter therapeutic time window for tissue salvage than tissue bordering this zone, in which additional secondary and delayed cellular mechanisms may underlie progression to cell death.

Reperfusion by thrombolysis has been shown to improve clinical outcome when instituted within 3 and possibly up to 6 hours after stroke onset. In contrast, a variety of neuroprotectants given in isolation up to 24 hours after stroke onset have not shown benefit in humans despite their effectiveness in animal models. Further understanding of the time window during which therapeutic interventions are likely to be effective and the location of the tissue likely to benefit will improve our understanding of when and why acute stroke therapies succeed or fail. Our method of quantifying the spatial location of the penumbra is limited by the unlikely clinical applicability of 18F-FMISO PET, but it can easily be adapted to different functional imaging modalities in humans and animal models of stroke to gain further insight into the evolution and spatial location of the penumbra. Improved techniques to rapidly identify the pathophysiological state of tissue threatened by infarction would facilitate patient selection and choice of therapeutic strategy and may extend the therapeutic time window.
Conclusions
This study provides evidence that after ischemic stroke infarct expansion proceeds from the center to the periphery of the ischemic region in humans similar to previous reports from animal experiments. In MCA territory strokes, hypoxic viable tissue is maximal in the superior, mesial, and posterior regions relative to the infarct.

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References
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