Potential of Early \(^{18}\text{F}\)-2-Fluoro-2-Deoxy-D-Glucose Positron Emission Tomography for Identifying Hypoperfusion and Predicting Fate of Tissue in a Rat Embolic Stroke Model

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Background and Purpose—Experimental stroke models are essential to study in vivo pathophysiological processes of focal cerebral ischemia. In this study, an embolic stroke model in rats was applied (1) to characterize early development of regional cerebral blood flow and metabolism with positron emission tomography (PET) using \(^{15}\text{O}\)H\(_2\)O and \(^{18}\text{F}\)-2-fluoro-2-deoxy-D-glucose (FDG); and (2) to identify potential parameters for predicting tissue fate.

Methods—Remote occlusion of the middle cerebral artery was induced in 10 Wistar rats by injection of 4 TiO\(_2\) macrospheres. Sequential \(^{15}\text{O}\)H\(_2\)O-PET (baseline, 5, 30, 60 minutes after middle cerebral artery occlusion) and FDG-PET measurements (75 minutes after middle cerebral artery occlusion) were performed. \(^{15}\text{O}\)H\(_2\)O-PET data and FDG kinetic parameters were compared with MRIs and histology at 24 hours.

Results—Regional cerebral blood flow decreased substantially within 30 minutes after middle cerebral artery occlusion (41% to 58% of baseline regional cerebral blood flow; \(P<0.001\)) with no relevant changes between 30 and 60 minutes. At 60 minutes, regional cerebral blood flow correlated well with the unidirectional transport parameter \(K_l\) of FDG in all animals (\(r=0.86\pm0.09\); \(P<0.001\)). Tissue fate could be accurately predicted taking into account \(K_l\) and net influx rate constant \(K_i\) of FDG. The infarct volume predicted by FDG-PET (375.8±102.3 mm\(^3\)) correlated significantly with the infarct size determined by MRI after 24 hours (360.8±93.7 mm\(^3\); \(r=0.85\)).

Conclusions—Hypoperfused tissue can be identified by decreased \(K_i\) of FDG. Acute ischemic tissue can be well characterized using \(K_l\) and \(K_i\) allowing for discrimination between infarct core and early viable tissue. Because FDG-PET is widely spread, our findings can be easily translated into clinical application for early diagnoses of ischemia. (Stroke. 2012;43:193-198.)

Key Words: FDG-PET ■ macrosphere model ■ penumbra ■ PET ■ prediction ■ rCBF ■ stroke

A principal purpose of early imaging after stroke is to predict the fate of tissue and thus identify tissue compartments potentially accessible to therapeutic intervention. Imaging of experimental stroke models in the acute phase is essential to study pathophysiological processes related to cerebral ischemia in vivo and forms a basis for the development of novel therapies.

Positron emission tomography (PET) allows visualizing molecular and biochemical processes in the living brain. The standard tracer for measuring regional cerebral blood flow (rCBF) by PET is radiolabeled H\(_2\)O (\(^{15}\text{O}\)H\(_2\)O). However, \(^{15}\text{O}\)H\(_2\)O-PET has some disadvantages, like a short half-life (approximately 2 minutes) of \(^{15}\text{O}\)H\(_2\)O and a low signal-to-noise ratio. Cerebral glucose metabolism can be measured by PET using \(^{18}\text{F}\)-2-fluoro-2-deoxy-D-glucose (FDG), a tracer that is much more readily available than \(^{15}\text{O}\)H\(_2\)O. Like glucose, FDG is transported into brain tissue by glucose transporters and is phosphorylated by the enzyme hexokinase, but, in contrast to glucose, FDG is not further metabolized but trapped in metabolically active cells. Previous MRI studies in rats have suggested parameters like the mismatch of diffusion-weighted and perfusion-weighted imaging or apparent diffusion coefficient as predictors for final tissue outcome if acquired during the acute phase of stroke. However, a generally accepted method for the prediction of tissue fate from MRI data is still lacking. The purpose of this PET study in a rat embolic stroke model was (1) to characterize early alterations of rCBF using...
sequential [15O]H2O-PET; (2) to analyze FDG kinetics in acute ischemic tissue as well as (3) to identify potential parameters for the early prediction of tissue fate that might support the development of potential therapeutic interventions.

Methods

Animals and Surgery

All animal procedures were in accordance with the German Laws for Animal Protection and were approved by the local animal care committee and local governmental authorities. Male Wistar rats (n = 13) from Harlan Winkelmann (Borchen, Germany) weighing 305 to 330 g were anesthetized with 5% isoflurane and maintained with 2.5% isoflurane in 65%/35% nitrous oxide/oxygen. Throughout the surgical procedure, body temperature was maintained at 37°C with a thermostatically controlled heating pad. Before PET imaging, animals were prepared for ischemia induction by macrosphere model as described elsewhere. Briefly, the right common carotid artery, internal carotid artery, and external carotid artery were exposed through a midline incision of the neck and the external carotid artery and the pterygopalatine branch of the internal carotid artery were ligated. A PE-50 catheter was filled with saline and 4 TiO2 macrospheres (diameter of 0.315–0.355 mm; BRACE, Alzenau, Germany). This catheter was inserted into the common carotid artery, advanced to the origin of the pterygopalatine artery, and fixed in place. After placing the rats within the micro-PET scanner and running baseline measurements, macrospheres were injected through a saline-filled catheter placed in the internal carotid artery to occlude middle cerebral artery; n = 13). After PET imaging, the catheter was removed and the animals were allowed to recover.

Positron Emission Tomography

PET imaging was performed on a microPET Focus 220 scanner (Concorde Microsystems, Inc, Knoxville, TN; 63 image planes; 1.5-mm full width at half maximum) as described elsewhere. After a 10-minute transmission scan for attenuation correction, rats received an intravenous bolus injection of [15O]H2O (1.6–2.4 mCi/rat) before as well as 5, 30, and 60 minutes after MCAO without changing the position of the animals in the PET scanner. Emission data acquired were 2 minutes. Thereafter, an intravenous bolus of FDG (1.4–2.0 mCi/rat in 500 µL) was injected into the tail vein at 75 minutes after embolization of macrospheres, and emission data were acquired for 60 minutes. After histogramming in timeframes of 6×30 seconds, 3×60 seconds, 3×120 seconds, and 12×240 seconds and Fourier rebinning, data were reconstructed using a 2-dimensional filtered back projection. An image derived input function was extracted from the FDG-PET data and parametric images of the kinetic constants were determined by voxelwise application of a 2-tissue-compartment kinetic model with 4 rate constants (K1, transport from blood to brain; k2, transport from the brain to blood; k3, phosphorylation; k4, dephosphorylation). K1 is related to rCBF by K1=rCBF×(1−e−П/PS), where PS is the permeability surface product of FDG. In hypoperfused tissue, (rCBF<PS): K1≈rCBF.

The metabolic rate constant or net influx rate constant is given by K1−DI=[K1−CM]/(K1−CM)+b. In the autoradiographic method, the cerebral metabolic rate of glucose consumption CMRg is given by: CMRg=LC,Ki × Cg, where LC is the lumped constant and Cg the plasma glucose level.

Characterization of Tissue With K1 and Ki

Taking parametric images of K1 and Ki, each image voxel can be displayed in a K1–Ki diagram. We hypothesized that tissue condition was related to its location in the K1–Ki diagram. Healthy tissue (contralateral hemisphere) could be characterized by the center of mass (CM) and the SD. Affected tissue was shifted with respect to the healthy tissue by a shift to lower Ki and higher Ki.

With K1>m K1+b (m=K1−CM+0.75 SD,Ki−0.75 SD,Ki−0.04, b=−0.04 m) had a high risk of being infarcted after 24 hours. Furthermore, we could divide the tissue being infarcted after 24 hours into 2 groups: (1) early viable tissue with Ki≥K1−CM+0.75 SD,Ki; and (2) early infarct core (Ki≤K1−CM+0.75 SD,Ki).

Radiochemistry

[15O]H2O was synthesized by the reduction of [15O]O2 with H2 gas (catalyzed by Pd black at 140°C). The synthesized [15O]H2O gas, trapped in a 500 µL saline solution, was used for animal experiments. FDG was produced as described previously.

Magnetic Resonance Imaging

Twenty-four hours after induction of ischemia, 8 rats were anesthetized with isoflurane and T2-weighted MRI on a 4.7-T BioSpec system (Bruker BioSpin, Ettlingen, Germany) was performed as described elsewhere.

Histology

Twenty-four hours after MCAO, 3 animals (2 without MRI investigation) were decapitated under deep anesthesia. The brains were stained with hematoxylin and eosin according to the staining protocol given by Merck (Merck KGaA, Darmstadt, Germany). Animals without hematoxylin and eosin staining were not euthanized after 24 hours but included into a different study on the long-term development of stroke.

Image Analysis

For coregistration of PET, MRI, and histological images, the image analysis software VINCI (Max Planck Institute for Neurological Research, Cologne, Germany) was used. Additional coregistration to an anatomic data set of a 3-dimensional rat brain atlas constructed from the brain slices presented by Swanson was performed.

Infarct Volume Quantification

On T2-weighted images of MRI, the lesion volume in millimeters cubed was detected by computer-aided manual tracing of the hyperintense lesions using the image analysis software VINCI. The edema-corrected infarct volume was quantified as described elsewhere.

Volume of Interest Grid

Based on the 3-dimensional anatomic data set, volumes of interest (VOIs; 2×2×2 mm3) were placed in the ipsilateral and corresponding positions of the contralateral hemisphere (Supplemental Figure I; http://stroke.ahajournals.org). rCBF was assessed as percentage injected dose of [15O]H2O averaged over 2 minutes acquisition time. For the analysis of the rCBF development over time, rCBF was calculated as percentage of baseline rCBF in [15O]H2O-PET. Absolute values of rCBF evaluated by [15O]H2O-PET at 60 minutes and K1 of FDG-PET were compared.

Statistical Analysis

Statistical analysis was performed with SigmaPlot 11 (Systat Software Inc). Two-way analyses of variance for repeated measurements with a post hoc test of Holm-Sidak were used to analyze the rCBF development in each animal (factors: time [baseline, 5 minutes, 30 minutes, 60 minutes] and VOI [infarcted VOIs versus corresponding contralateral VOIs]). Statistical significance was set at ≤5% (P<0.05).

Results

Postmortem, 3 animals showed no occlusion of the middle cerebral artery mainstem after the injection of macrospheres and were excluded from the study.

rCBF Development After MCAO

After the injection of 4 macrospheres, all animals (n=10) showed a uniform response of rCBF within the first 60
minutes. The rCBF development in the ipsi- and contralateral hemisphere of 2 representative animals is displayed in Figure 1. In all animals, rCBF in the infarct core (later determined histologically) significantly decreased within the first 30 minutes after MCAO (at 5 minutes to 43%–76% and at 30 minutes to 38%–65% of baseline rCBF). This was reflected by a significant main effect of factor time (F[3,30–11022/11021174.68; P < 0.001). Post hoc comparison revealed that within the infarcted VOIs, the time points 5, 30, and 60 minutes were significantly different from baseline rCBF. After 30 minutes, rCBF remained stable; there was no relevant difference in rCBF between 30 and 60 minutes (Figure 1). The rCBF of the corresponding VOIs of the contralateral hemisphere stayed between 90% and 110% of the baseline level.

Comparison of [15O]H2O-PET and K1 of FDG-PET

K1 is the rate constant for FDG transport from blood into brain tissue. In hypoperfused tissue, K1 is related to rCBF. Both, [15O]H2O-PET at 60 minutes and K1 of FDG-PET at 75 minutes showed very similar patterns of the ischemic tissue (Figure 2A–B). In each animal, rCBF within the ischemic hemisphere determined by [15O]H2O-PET at 60 minutes correlated well to the K1 of FDG-PET (r = 0.86 ± 0.09; P < 0.001; exemplary correlation of 1 animal in Figure 2C). In the overall analysis of all VOIs in the ipsi- and contralateral hemispheres of all animals, the relation of [15O]H2O-PET at 60 minutes and K1 of FDG-PET could be fitted by the function $K1 = a \times (H2O-PET) \times (1 - e^{-b/(a \times H2O-PET)})$ with $a = 32.69 \pm 1.33$ mL/cm³/min and
Characterization of Infarct

In all animals, we determined the infarct volume after 24 hours either histologically (n=3; 323.7±145.0 mm³; Figure 1A) or by MRI (n=8; 360.8±93.7 mm³; Figure 1B; exemplary coronal section of 2 animals in Figure 1, bottom). Ex vivo, we assessed the position of the TiO₂ spheres in the circle of Willis, which could be found within the internal carotid artery/middle cerebral artery vessel arborization blocking the proximal middle cerebral artery (examples of 2 animals in Figure 1, bottom).

Tissue Characterization and Prediction of Infarct Outcome

Acute ischemic tissue could be characterized using the net FDG influx rate constant $K_i$ and the rate constant for FDG transport from blood into brain $K_1$. From comparison with the infarct at 24 hours after MCAO, we found that tissue fate could be predicted in the following way: if $K_i < mK_1 + b$ (see “Materials and Methods”) at 60 minutes after MCAO, this region will be infarcted 24 hours later (Figure 3A–B). Furthermore, this tissue with malprediction could be divided into 2 classes: (1) immediately damaged tissue with $K_i \leq Ki_{CM} + 0.75 SD_{Ki}$ constituting the early infarct core; and (2) still viable tissue ($K_i > Ki_{CM} + 0.75 SD_{Ki}$), which was predominantly but not only localized between healthy tissue and early infarct core. In some individuals, this early viable tissue covered >70% of the later infarct volume.

The infarct size predicted with the $K_1-K_i$ diagram (375.8±102.3 mm³) and the edema-corrected infarct size determined by T2-weighted MRI (360.8±93.7 mm³; $P>0.5$) correlated well ($r=0.85; P<0.05$; Figure 3C).

Discussion

In this study, we performed remote occlusion of the middle cerebral artery in rats within the $^{18}$O-PET scanner leading to focal cerebral ischemia. In our macrosphere model, the intra-arterial injection of 4 TiO₂ spheres leads to permanent MCAO and mimics arterioarterial embolism of “hard” atherosclerotic plaque material.19 The macrosphere model seems to be a relevant model for studying the pathophysiology of stroke, because arterioarterial embolism is 1 of the most frequent causes of stroke in humans.20–22 We have recently characterized early cortical rCBF dynamics in this model by real-time imaging using Laser Speckle Contrast Imaging.23 Moreover, this model easily allows remote vessel occlusion within MRI12,24 and, as we show here, PET scanners.

The injection of macrospheres led to an occlusion of the middle cerebral artery mainstem and resulted in large infarcts. Using $[^{15}$O]H₂O-PET, we observed the development of an ischemic core within the first 30 minutes after induction of ischemia with no relevant changes during the next 30 minutes, which is in accordance with other permanent rat stroke models such as photothrombotic model,25 distal MCAO,26,27 and intraluminal suture model.28,29

The rCBF measurements within the ipsilateral hemisphere quantified by $[^{15}$O]H₂O-PET at 60 minutes, and by $K_1$ of FDG, correlated well in each animal. Thus, the $K_1$ parameter of FDG is a reliable measure for rCBF in hypoperfused tissue. From fitting of the theoretical function of the relation of $K_1$
to rCBF, the average permeability surface product of FDG was determined as 0.1906 ± 0.0057 mL/cm²/min, which is in line with the values reported in the literature. The major finding of the present study is that the 2 kinetic parameters \( K_1 \) and \( K_i \) of FDG have a high diagnostic potential for the analysis of acute ischemic tissue. In hypoperfused regions, \( K_1 \) is approximately equal to cerebral blood flow, that is, tissue with substantial reduction of cerebral blood flow can be identified by a reduction of \( K_1 \). The FDG net influx rate \( K_i \), which depends on transport as well as phosphorylation of FDG, is a measure for tissue viability. The differences of FDG and glucose with respect to transport and phosphorylation have the effect that \( K_i \) of FDG is elevated in regions with preserved glucose consumption and reduced transport (cerebral blood flow, \( K_1 \)). We observed that part of the tissue infarcted after 24 hours had normal or elevated \( K_i \) at 1 hour after MCAO. In these regions, the reduced blood flow and thereby glucose supply is compensated by an increase of phosphorylation rate to maintain cellular energy consumption.

These findings are in accordance with the increase of the oxygen extraction fraction that has been observed in the early ischemic tissue and defines the ischemic penumbra. The decrease of oxygen supply as a consequence of the reduced cerebral blood flow is compensated by an increase of oxygen extraction fraction to maintain oxygen consumption. In the same manner, hexokinase activity (and thereby \( K_i \) of FDG) is increased to maintain glucose consumption in regions with low cerebral blood flow. Thus, we provide a new method for the identification of the ischemic penumbra purely based on FDG-PET data. Although oxygen extraction fraction measurements require an onsite cyclotron due to the short half-life of \( ^{15} \)O (2 minutes), FDG is commercially available and the method is applicable more generally (even clinically) than the oxygen extraction fraction method. Therefore, our method extends the opportunity for the detection of an ischemic penumbra to the whole PET community. It was so far limited to PET centers with an onsite cyclotron.

To our knowledge, the power of FDG-PET for the diagnosis of acute ischemia has not been reported. The results we present here can only be obtained using kinetic modeling of the PET data. In particular, the explicit determination of \( K_1 \) is required. Kinetic modeling was not performed in any of the published FDG studies in acute ischemia.

In conclusion, in the macrosphere model for embolic stroke, functionally relevant alterations in rCBF occur between 5 and 30 minutes after induction of ischemia. Kinetically modeled FDG-PET is a reliable method for measuring rCBF in acute stroke and allows the identification of primarily affected but still viable tissue that is accessible to therapeutic interventions. The relation to diffusion/perfusion weighted MRI in ischemic tissue remains to be investigated.

**Disclosures**

None.

**References**


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S1: Volume of interest (VOI) analysis: The position of VOIs (2x2x2 mm³) is displayed on paramedial (A), intermediate (B) and lateral (C) sagittal brain sections of one hemisphere.
S2: The individual order of VOIs with the associated rCBF at 60 minutes is displayed as percentage of baseline and presented for the ipsilateral (rCBFi (%)) and contralateral (rCBFc (%)) hemisphere of two representative animals (figure 1). Ipsi- and contralateral VOIs were individually sorted by the rCBFi at 60min, shown as percentage of baseline in ascending order.
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Abstract

Background and Objectives: The local ischemic pathophysiology in vivo is a fundamental question for experimental stroke models. In this study, we used a rat embolic stroke model to (1) study the regional blood flow and metabolism using \(^{15}\text{O}\)H\(_2\)O and \(^{18}\text{F}\)-2-Deoxy-2-F-fluoro-D-glucose (FDG) PET at early time points after middle cerebral artery occlusion (MCAO) and to (2) identify tissue that would undergo infarction and its possible survival.

Methods: Four TiO\(_2\) microspheres were injected into 10 Wistar rats to induce focal ischemia. Continuous \(^{15}\text{O}\)H\(_2\)O-PET, FDG-PET, and 2D-positron emission tomography (PET) dynamic parameters were measured at various time points after MCAO induction (1 h, 1, 2, 4, and 9 h).

Results: Local blood flow decreased within 30 min after MCAO (41% to 58% of baseline, \(p < 0.001\)). 30 to 60 min after MCAO, there was a strong correlation between FDG's one-directional transport (K1) and rCBF (\(r = 0.86 \pm 0.09; \text{p} < 0.001\)). After 1 h, the correlation significantly decreased (\(r = 0.53 \pm 0.09; \text{p} < 0.001\)). After 2 h, no correlation was observed (\(r = 0.25 \pm 0.09; \text{p} < 0.001\)). After 4 h, a weak correlation was observed (\(r = 0.13 \pm 0.09; \text{p} < 0.001\)). After 9 h, there was no correlation (\(r = 0.00 \pm 0.09; \text{p} < 0.001\)).

Conclusion: To predict tissue fate, it is important to identify regions that are still perfused with blood flow. \(^{18}\text{F}\)-FDG uptake can be used in combination with \(^{15}\text{O}\)H\(_2\)O-PET and rCBF to identify regions that are still perfused with blood flow. This will help to identify regions that are likely to survive and those that are likely to infarct.

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