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\(_2\) microspheres. 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\(\pm\)102.3 mm\(^3\)) correlated significantly with the infarct size determined by MRI after 24 hours (360.8\(\pm\)93.7 mm\(^3\); \(r=0.85\)).

Conclusions—Hypoperfused tissue can be identified by decreased \(K_l\) 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
sequential $[^{15}O]$H$_2$O-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 TiO$_2$ macroospheres (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, macroospheres 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 $[^{15}O]$H$_2$O (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 were acquired for 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 macroospheres, 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-tissues-compartment kinetic model with 4 rate constants ($K_1$, transport from blood to brain; $k_2$, transport from the brain to blood; $k_3$, phosphorylation; $k_4$, dephosphorylation). $K_i$ is related to rCBF by $K_i=\text{rCBF} \times (1 - e^{-PS_iCBF})$, where $PS$ is the permeability surface product of FDG. In hypoperfused tissue, $(rCBF < PS): K_i = rCBF$. The metabolic rate constant or net influx rate constant is given by $K_i = K_i \times (k_2 \times k_3)$. In the autodigraphic method, the cerebral metabolic rate of glucose consumption CMR$_{glc}$ is given by: CMR$_{glc} = \frac{LC \times C_p}{C_m \times C_p}$, where LC is the lumped constant and $C_p$ the plasma glucose level.

**Characterization of Tissue With $K_i$ and $K_i$**

Taking parametric images of $K_i$ and $K_i$, each image voxel can be displayed in a $K_i$–$K_i$ diagram. We hypothesized that tissue condition was related to its location in the $K_i$–$K_i$ 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 $K_i$ and higher $K_i$. From comparison with the infarct measured by 2-weighted MRI 24 hours after MCAO, the following line could be defined that separates healthy from later infarcted tissue in the early $K_i$–$K_i$ diagram: tissue with $K_i > m \times K_i + b$ ($m = (K_iCM + 0.75) \times (0.75 SD_{K_i} + 0.75 SD_{K_i} - 0.04)$, $b = -0.04$) 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 $K_i > K_iCM + 0.75 SD_{K_i}$; and (2) early infarct core ($K_i = K_iCM + 0.75 SD_{K_i}$).

**Radiochemistry**

$[^{15}O]$H$_2$O was synthesized by the reduction of $[^{15}$O$]$O$_2$ with H$_2$ gas (catalyzed by Pd black at 140°C). The synthesized $[^{15}$O$]$H$_2$O 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 T$_2$-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 T$_2$-weighted images of MRI, the lesion volume in millimeters cubed was detected by computer-aided manual tracing of the hypertensive 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 mm$^3$) 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 $[^{15}$O$]$H$_2$O averaged over 2 minutes acquisition time. For the analysis of the rCBF development over time, rCBF was calculated as percentage of baseline rCBF in $[^{15}$O$]$H$_2$O-PET. Absolute values of rCBF evaluated by $[^{15}$O$]$H$_2$O-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 macroospheres and were excluded from the study.

rCBF Development After MCAO

After the injection of 4 macroospheres, 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 ($F[3,30–129]=174.68; P<0.001$). 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 ($<3\%$) 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 [$^{15}$O]H$_2$O-PET and $K_1$ of FDG-PET**

$K_1$ is the rate constant for FDG transport from blood into brain tissue. In hypoperfused tissue, $K_1$ is related to rCBF. Both, [$^{15}$O]H$_2$O-PET at 60 minutes and $K_1$ 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 [$^{15}$O]H$_2$O-PET at 60 minutes correlated well to the $K_1$ of FDG-PET ($r=0.86\pm0.09; P<0.001$; 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 [$^{15}$O]H$_2$O-PET at 60 minutes and $K_1$ of FDG-PET could be fitted by the function $K_1=a\times(H_2O\text{-PET})\times(1-exp[-b/(a\times H_2O\text{-PET})])$ with $a=32.69\pm1.33$ mL/cm$^3$/min and

![Figure 1](http://stroke.ahajournals.org/)
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 \( K_i \equiv K_{i,CM} + 0.75 SD_{K_i} \) constituting the early infarct core; and (2) still viable tissue (\( K_i > K_{i,CM} + 0.75 SD_{K_i} \)), 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 \( \beta \)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. 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. We have recently characterized early cortical rCBF dynamics in this model by real-time imaging using Laser Speckle Contrast Imaging. Moreover, this model easily allows remote vessel occlusion within MRI 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 \([^{15}O]H_2O\)-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, distal MCAO, and intraluminal suture model.

The rCBF measurements within the ipsilateral hemisphere quantified by \([^{15}O]H_2O\)-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.30

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, $K_1$).14 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.31,32 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.33 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.34–36

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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SUPPLEMENTAL MATERIAL
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.
ラット塞栓性脳卒中モデルにおいて [18F]-2-フルオロ-2-デオキシ-D-グルコース陽電子断層撮影法が低灌流を同定し組織の運命を予測する可能性

Potential of Early [18F]-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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背景および目的:
局所脳虚血の in vivo での病態生理過程を研究するには実験的脳卒中モデルが不可欠である。本研究では、ラットの塞栓性脳卒中モデルを用いて、(1) [15O]H2O および [18F]-2-フルオロ-2-デオキシ-D-グルコース (FDG) を用いた陽電子断層撮影 (PET) で初期の局所脳血流および代谢の特徴を明らかにし、(2) 組織の運命を予測するための潜在的パラメータを同定した。

方法:
4 つの TiO2 macrosphere の注入口に TiO2 macrosphere に注入し、10 匹の Wistar ラットに中大脳動脈の遠位閉塞を誘発した。連続的な [15O]H2O-PET (ベースライン、中大脳動脈閉塞の 5 分、30 分、60 分後) および FDG-PET の測定 (中大脳動脈閉塞の 75 分後) を行った。[15O]H2O-PET データおよび FDG 動態パラメータを 24 時間時点の MRI および組織像と比較した。

結果:
局所脳血流量は中大脳動脈閉塞から 30 分以内に大幅に低下し (ベースライン局所脳血流量の 41 ~ 58%, p < 0.001), 30 ~ 60 分の間には有意な変化は認められなかった。60 分の時点では、局所脳血流量は全動物において FDG の一方向性輸送パラメータ KI とよく相関していた (r = 0.86 ± 0.09; p < 0.001)。FDG の KI および正味流入速度定数 Ki を考慮に入れることにより組織の運命を正確に予測できた。FDG-PET により予測された梗塞容積 (375.8 ± 102.3 mm3) は、24 時間後の MRI により測定された梗塞サイズ (360.8 ± 93.7 mm3; r = 0.85) と統計学的に有意に相関していた。

結論:
FDG の KI 低下により、低灌流組織を同定できる。KI および Ki を用いて急性虚血組織の特徴を十分に明らかにすることができ、梗塞のコアと初期生存組織を識別することができる。FDG-PET は広く普及していることから、本研究の所見を虚血の早期診断のため臨床に応用するのは容易である。

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図2

代表的な動物において MCAO から 60 分後の[15O]H2O-PET (A), FDG の KI (B), および rCBF と KI の相関 (C) により明確にされた同側半球の rCBF。全動物の同側および対側半球における FDG の KI および rCBF により、これらのパラメータの理論上の関係が確認されている (D)。rCBF: 局所脳血流量、PET: 陽電子断層撮影法、MCAO: 中大脳動脈閉塞、FDG: [18F]-2-フルオロ-2-デオキシ-D-グルコース。