Hypoxic Viable Tissue in Human Chronic Cerebral Ischemia Because of Unilateral Major Cerebral Artery Steno-Occlusive Disease

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Background and Purpose—Positron emission tomography (PET) with radiolabeled 2-nitroimidazoles directly detects hypoxic but viable tissue present in an acute ischemic area in the human brain. This study using PET with 1-(2-18F-fluoro-1-[hydroxymethyl]ethoxy) methyl-2-nitroimidazole (18F-FRP170) aimed to determine whether tissue with an abnormally elevated uptake of 18F-FRP170 exists in human chronic cerebral ischemia because of unilateral atherosclerotic major cerebral artery steno-occlusive disease.

Methods—18F-FRP170 PET was performed, and cerebral blood flow and metabolism were assessed using 15O-gas PET in 20 healthy subjects and 52 patients. A region of interest (ROI) was automatically placed in 3 segments of the middle cerebral artery territory in both cerebral hemispheres with a 3-dimensional stereotaxic ROI template using SPM2, and each PET value was determined in each ROI. The ratio of values in the affected versus contralateral hemispheres was calculated for the 18F-FRP170 PET image.

Results—A significant correlation was observed between oxygen extraction fraction and 18F-FRP170 ratios ($\rho=0.509; P<0.0001$) in a total of 156 ROIs in 52 patients. The specificity and positive-predictive value for a combination of an elevated oxygen extraction fraction and a moderately reduced cerebral oxygen metabolism for detection of an abnormally elevated 18F-FRP170 ratio (19 ROIs: 12%) were significantly greater than those for the individual categories (elevated oxygen extraction fraction, moderately reduced cerebral oxygen metabolism, or reduced cerebral blood flow).

Conclusions—Tissues with abnormally elevated uptake of 18F-FRP170 exist in human chronic cerebral ischemia characterized by a combination of misery perfusion and moderately reduced oxygen metabolism because of unilateral atherosclerotic major cerebral artery steno-occlusive disease. (Stroke. 2015;46:1250-1256. DOI: 10.1161/STROKEAHA.114.008238.)

Key Words: cerebrovascular disease ▪ positron-emission tomography

Cerebrovascular autoregulatory mechanisms act via dilation of precapillary resistance vessels to maintain cerebral blood flow (CBF) in the context of reductions in cerebral perfusion pressure.1,2 However, autoregulatory capacity is not sufficient to compensate for severe reductions in cerebral perfusion pressure, thereby leading to a decline in CBF. In this context, referred to as misery perfusion,3 cerebral oxygen metabolism is dependent on a progressive increase in oxygen extraction fraction (OEF).4 When CBF is further reduced beyond compensation of the increase in OEF, cerebral oxygen metabolism begins to decline, leading to the irreversible brain damage that characterizes cerebral infarction.

In acute ischemic stroke, the ischemic penumbra is defined as peri-infarct tissue that is functionally impaired but structurally intact and remains potentially salvageable.5,6 Positron emission tomography (PET) using 15O identifies areas of misery perfusion in a patient with acute ischemic stroke.7,8 18F-fluoromisonidazole (FMISO) is a PET marker of hypoxic but viable tissue that exists in an acute ischemic area in the human brain,9–12 and areas with uptake of the tracer reportedly are metabolically compromised tissue at risk of infarction after acute ischemic stroke.9,10,12 The mechanism of selective retention of 2-nitroimidazoles, including FMISO, in hypoxic tissue is not clearly understood but may involve nitroreductases. Nitroimidazole molecules enter cells by passive diffusion and undergo nitroreduction to products that are covalently bound to intracellular macromolecules. These products are reoxidized and diffuse out of the cells under normoxic conditions, whereas remaining trapped by macromolecules within cells under hypoxic conditions.13–15 Therefore,
PET with radiolabeled 2-nitroimidazoles may allow detection of hypoxic tissue, although the products of the tracer also remain trapped when cells are no longer hypoxic after recovery of perfusion.

In chronic cerebral ischemia because of severe stenosis of the cervical internal carotid artery (ICA), preoperatively impaired cognitive function occasionally improves after carotid endarterectomy; the reversible cognitive impairment is related to a state of reduction in metabolism because of moderate, but potentially reversible, downregulation of cortical neurotransmitter receptors in response to more severe reduction in brain perfusion because of ICA stenosis, and the cognitive improvement is associated with postoperative normalization of the cerebral metabolism followed by postoperative recovery of cerebral perfusion. These findings suggest that functionally impaired but structurally intact tissue may exist in areas of chronic cerebral ischemia with a combination of misery perfusion and reduced cerebral metabolism and that such tissue may be viable under hypoxic conditions. To our knowledge, there is only 1 previous study that has imaged hypoxic tissue in the context of human chronic cerebral ischemia. Although a high OEF is an indirect marker of hypoxic tissue, a new radiolabeled 2-nitroimidazole, 1-(2-18F-fluoro-1-[hydroxymethyl]ethoxy)-methyl-2-nitroimidazole (18F-FRP170), has been recently developed to directly image accumulation of the tracer in malignant brain tumors. The 18F-FRP170 clearly detects viable tissues under hypoxic conditions as an accumulation of the tracer in malignant brain tumors. Therefore, the purpose of this study, using 15O-gas and 18F-FRP170 PET, was to demonstrate the presence of tissue with abnormally elevated uptake of 18F-FRP170 in the context of human chronic cerebral ischemia because of unilateral atherosclerotic major cerebral artery steno-occlusive disease.

Subjects and Methods
Healthy Subjects
This study evaluated 20 healthy male subjects aged 30 to 67 years (mean, 55 years) who underwent screening based on past history, physical examination, and neurological and cognitive testing. The subjects had no past history of hypertension, diabetes mellitus, atrial fibrillation, or pulmonary disease, and magnetic resonance (MR) imaging did not reveal any organic lesions, leukoaraiosis, or asymptomatic lacunar infarction.

Patients
This study also included 52 patients (18 women and 34 men) aged 42 to 82 years (mean, 62 years) with unilateral middle cerebral artery (MCA) or ICA steno-occlusive disease. All patients had experienced prior cerebral ischemic events. Conventional magnetic resonance imaging was performed in all patients, and no infarct in the basal ganglia, internal capsula, or cerebral cortex was observed in any of the patients; 40 patients exhibited the rosary-like infarcts in the basal ganglia, internal capsula, or cerebral cortex was observed in any of the patients; 40 patients exhibited the rosary-like infarcts located at the corona radiate or the subcortical white matter in the centrum semiovale or the anterior or posterior watershed zone, which were defined as subcortical border zone infarction; and the remaining 12 did not have any infarction. Twenty-seven patients had transient ischemic attacks with (15 patients) or without (12 patients) definite subcortical border zone infarction on magnetic resonance imaging. The remaining 25 patients had minor complete strokes with definite subcortical border zone infarction on magnetic resonance imaging. Cerebral angiography with arterial catheterization or MR angiography demonstrated ICA stenosis (>70%) in 8 patients, ICA occlusion in 27 patients, MCA stenosis (>50%) in 10 patients, and MCA occlusion in 7 patients. No patient had occlusion or stenosis of >50% in the contralateral ICA or MCA.

The study protocol was approved by the local ethics committee, and all subjects gave written informed consent before the study.

Positron Emission Tomography
PET studies were performed using a SET-3000GCT/M scanner (PET/CT; Shimadzu Corp). This modality uses gadolinium silica oxide detectors and provides 59 slices with 2.6-mm slice thickness. The axial field of view was 156 mm, and the spatial resolution was 3.5 mm full width half maximum at 1 cm in-plane and 4.2 mm full width half maximum at center axially. The scanner was operated in static scan mode with dual-energy window acquisition for scatter correction. The coincidence time window was set to 10 ns. A shield module consisting of 7-mm thick lead plates attached to the gantry bed and covering the breast and shoulder of the subject was used to reduce the counting rate of random coincidence and scatter coincidence attributable to radioactivity outside the field of view.

Before the emission scans, a transmission scan (3 minutes) with a 150Cs point source was performed with a bismuth germanate transmission detector ring coaxially attached to the gadolinium silica oxide emission detector ring. CBF was determined although the subject continuously inhaled C15O2 through a mask. Measurements of cerebral metabolic rate of oxygen (CMR(O2)) and OEF were obtained during continuous inhalation of 15O2. Data were collected for 5 minutes. A single bolus of C15O2 was used to measure cerebral blood volume. CBF, CMRO2, and OEF were calculated using the steady state method, and CMRO2 and OEF were corrected by cerebral blood volume.

The 18F-FRP170 was synthesized using on-column alkaline hydrolysis according to previously described methods. The final formulation for injection was prepared in normal saline containing 2.5% v/v ethanol using solid-phase extraction techniques. At 60 minutes after intravenous injection of 150 to 370 MBq of 18F-FRP170, data were collected for 10 minutes.

Patients underwent PET studies >2 months after the last ischemic event, and the interval between 15O-gas PET and 18F-FRP170 PET ranged from 1 to 4 days.

Data Analysis
All PET images were transformed into the standard brain size and shape by linear and nonlinear transformation using SPM2 for anatomical standardization. Thus, brain images from all subjects had the same anatomic format. Three hundred and eighteen constant regions of interest (ROIs) were automatically positioned in both cerebral hemispheres using a 3-dimensional stereotaxic ROI template with SPM2 (FUJIFILM RI Pharma Co. Ltd, Tokyo, Japan). The ROIs were grouped into 10 segments (callosomarginal, pericallosal, precentral, central, parietal, angular, temporal, posterior, hippocampus, and cerebellar) in each hemisphere according to the arterial supply. Of these 10 segments, the precenral and central segments were combined and defined as an ROI of the frontal cortex perfused by the MCA (ROIf-MCA); the parietal and angular segments were combined and defined as an ROI of the parietal cortex perfused by the MCA (ROIp-MCA); the temporal segments were combined as an ROI of the temporal cortex perfused by the MCA (ROIt-MCA); Figure 1 in the online Data Supplement. CBF, CMR02, and OEF on 15O-gas PET images were measured in the ROIs_MCA, ROI,MCA, and ROIs,MCA in the cerebral hemisphere ipsilateral to the lesion. Radioactive counts on 18F-FRP170 PET images were measured in the bilateral ROIs_MCA, ROI,MCA, and ROIs,MCA, and ROI,MCA, the ratio of the value in the affected cerebral hemisphere to that in the contralateral cerebral hemisphere was then calculated for each ROI in 18F-FRP170 PET images.

Healthy subjects were assigned to 1 of 2 groups, each consisting of 10 subjects who underwent 15O-gas PET or 18F-FRP170 PET assessments. In the former group, CBF, CMRO2, and OEF were measured in the bilateral hemisphere ROIs. In the latter group, the 18F-FRP170 ratio was calculated when the left cerebral hemisphere was defined as the affected side; mean and SD of the 18F-FRP170 ratio were then...
calculated in each ROI (ROIsf-MCA, ROIsp-MCA, or ROIst-MCA). Of these 3 MCA ROIs, the highest value of the mean+2 SDs of 18F-FRP170 ratio was determined. Any patient with an MCA ROI with 18F-FRP170 ratio greater than the highest value was defined as having an abnormally elevated 18F-FRP170 ratio.

In addition, mean data in the whole MCA territory (ROIs whole-MCA= mean value of [ROIsf-MCA+ROIsp-MCA+ROIst-MCA]) in healthy subjects and patients were calculated and analyzed in the same manner as that for each MCA ROI (ROIsf-MCA, ROIsp-MCA, or ROIst-MCA).

**Statistical Analysis**

Data are expressed as the mean±SD. Differences in various parameters between the controls and patients were evaluated using the Mann–Whitney U test. Correlations between various parameters were determined using the Spearman’s rank correlation coefficient. Statistical significance was set at the P<0.05 level. To verify an assumption that the 18F-FRP170 ratio is abnormally elevated when the CBF or CMRO2 is reduced or the OEF is elevated and to investigate which of these 3 parameters or which combination is more strongly associated with an abnormally elevated 18F-FRP170 ratio, the accuracy of using CBF, CMRO2, or OEF to detect an abnormally elevated 18F-FRP170 ratio was determined using a receiver operating characteristic (ROC) curve. When a CBF or CMRO2 in an MCA ROI in a patient was less than the cutoff point lying closest to the left upper corner of the ROC curve for detection of an abnormally elevated 18F-FRP170 ratio, the ROI was categorized as having a reduced CBF or CMRO2, respectively; when a OEF in an MCA ROI in a patient was greater than the cutoff point lying closest to the left upper corner of the ROC curve for detection of an abnormally elevated 18F-FRP170 ratio, the ROI was categorized as having an elevated OEF. Exact 95% confidence intervals of sensitivity, specificity, positive-, and negative-predictive values were computed using the binomial distributions. The differences in sensitivity, specificity, positive-, or negative-predictive values between the categories of reduced or elevated PET value were analyzed using the 95% confidence intervals.

**Results**

Mean, SD, and range of 18F-FRP170 ratio, CBF, CMRO2, and OEF in ROIsf-MCA, ROIsp-MCA, and ROIst-MCA in 10 healthy subjects and 52 patients are shown in Table 1. 18F-FRP170 ratios did not differ between healthy subjects and patients in all 3 MCA ROIs. CBF and CMRO2 were significantly lower in patients than in healthy subjects in all 3 MCA ROIs, whereas OEF was significantly greater in patients than in healthy subjects in ROIsf-MCA and ROIst-MCA. OEF did not differ between healthy subjects and patients. The mean+2

<table>
<thead>
<tr>
<th>Table 1. Positron Emission Tomographic Values Obtained From Healthy Subjects and Patients in ROIsf-MCA, ROIsp-MCA, and ROIst-MCA</th>
<th>Healthy Subjects (n=10* or 20†)</th>
<th>Patients (n=52‡)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ROIsf-MCA</strong></td>
<td>18F-FRP170 ratio</td>
<td>Mean 1.000</td>
<td>1.013</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>0.047</td>
<td>0.058</td>
<td>0.047</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>0.935–1.057</td>
<td>0.845–1.182</td>
<td>N.S.</td>
</tr>
<tr>
<td><strong>CBF, mL/100 g per min</strong></td>
<td>Mean 46.8</td>
<td>38.6</td>
<td>46.8</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>7.2</td>
<td>7.6</td>
<td>7.2</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>4.34</td>
<td>3.07</td>
<td>4.34</td>
</tr>
<tr>
<td><strong>ROIsp-MCA</strong></td>
<td>18F-FRP170 ratio</td>
<td>Mean 1.000</td>
<td>1.017</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>0.047</td>
<td>0.063</td>
<td>0.047</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>2.78–3.83</td>
<td>1.76–3.85</td>
<td>0.0477</td>
</tr>
<tr>
<td><strong>CMRO2, mL/100 g per min</strong></td>
<td>Mean 40.8</td>
<td>42.6</td>
<td>40.8</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>4.4</td>
<td>5.3</td>
<td>4.4</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>4.4</td>
<td>5.3</td>
<td>4.4</td>
</tr>
<tr>
<td><strong>ROIst-MCA</strong></td>
<td>18F-FRP170 ratio</td>
<td>Mean 1.000</td>
<td>1.011</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>0.046</td>
<td>0.057</td>
<td>0.046</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>0.935–1.076</td>
<td>0.889–1.172</td>
<td>N.S.</td>
</tr>
<tr>
<td><strong>CBF, mL/100 g per min</strong></td>
<td>Mean 47.2</td>
<td>39.1</td>
<td>47.2</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>6.3</td>
<td>7.3</td>
<td>6.3</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>36.6–57.4</td>
<td>24.2–50.3</td>
<td>0.0019</td>
</tr>
<tr>
<td><strong>CMRO2, mL/100 g per min</strong></td>
<td>Mean 3.60</td>
<td>3.13</td>
<td>3.60</td>
</tr>
</tbody>
</table>
| **Mean** | 0.45 | 0.48 | 0.45 | 0.48 | 0.45 | 0.48 | (Continued)
Figure 1 compares the $^{18}$F-FRP170 ratio and CBF in each ROI from the patients, with no significant correlation identified between the two parameters. The sensitivity, specificity, positive-, and negative-predictive values for CBF at the cutoff point lying closest to the left upper corner of the ROC curve for detecting an abnormally elevated $^{18}$F-FRP170 ratio are shown in Figure II in the online-only Data Supplement and Table 2. The cutoff point was 35.9 mL/100 g per minute (Figure 1) and an ROI with CBF less than this value was categorized as having a reduced CBF. The value represents mean$-1.5$ SD (for ROIsf-MCA and ROIsp-MCA) or mean$-1.8$ SD (for ROIst-MCA) of the control value obtained from healthy subjects. The lowest CBF of ROIs with an abnormally elevated $^{18}$F-FRP170 ratio was 23.7 mL/100 g per minute.

Figure 2 compares $^{18}$F-FRP170 ratio and CMRO2 in each ROI from the patients. Again, no significant correlation was identified between the two parameters. The sensitivity, specificity, positive-, and negative-predictive values for CMRO2 at the cutoff point lying closest to the left upper corner of the ROC curve for detecting an abnormally elevated $^{18}$F-FRP170 ratio are shown in Figure II in the online-only Data Supplement and Table 2. The cutoff point was 3.31 mL/100 g per minute (Figure 2) and an ROI with CMRO2 less than this value was categorized as having a reduced CMRO2. The value represents mean$-0.4$ SD (for ROIsf-MCA and ROIsst-MCA) or mean$-0.6$ SD (for ROIsp-MCA and ROIsst-MCA) of control. Furthermore, when the cutoff point was moved in decrements from 3.31 mL/100 g per minute of CMRO2, the sensitivity and positive-predictive values became 0% at a cutoff point of 2.51 mL/100 g per minute (Figure 2), which represents mean$-2.4$ SD (for ROIsf-MCA and ROIsst-MCA) or mean$-2.0$ SD (for ROIsst-MCA) of control. When CMRO2 <2.51 mL/100 g per minute or between 3.31 and 2.51 mL/100 g per minute was categorized as severely or moderately reduced, respectively, the specificity for a moderately reduced CMRO2 for detection of an abnormally elevated $^{18}$F-FRP170 ratio was significantly greater than that for a reduced CMRO2 (Table 2).

Figure 3 compares the $^{18}$F-FRP170 ratio and OEF in each ROI from the patients. The correlation between the 2 was significant ($P<0.0001$), with a correlation coefficient of 0.509. The sensitivity, specificity, positive-, and negative-predictive values for OEF in the cutoff point lying closest to the left upper corner of the ROC curve for detecting an abnormally elevated $^{18}$F-FRP170 ratio are shown in Figure II in the online-only Data Supplement and Table 2. The cutoff point was 46.3% (Figure 3), and an ROI with OEF greater than this value was categorized as having an elevated OEF. The value represents mean$+1.6$ SD (for ROIsf-MCA) or mean$+1.3$ SD (for ROIsf-MCA and ROIsst-MCA) of control. Further, the specificity and positive-predictive value for a combination of an elevated OEF and a moderately reduced CMRO2 for detection of an abnormally elevated $^{18}$F-FRP170 ratio were significantly greater than those for the individual categories (elevated OEF, moderately reduced CMRO2, or reduced CBF); significant differences in the sensitivity and negative-predictive value were not observed among the combined category and the individual categories (Figure 3; Table 2).

Mean data in the ROIswhole-MCA in healthy subjects and patients and analysis in the same manner as that for each MCA ROI are presented in Tables I and II; Figures III, IV, and V in the online-only Data Supplement.

Representative PET images in 1 patient with an abnormally elevated $^{18}$F-FRP170 ratio are shown in Figure 4.

Discussion

This study used $^{15}$O-gas and $^{18}$F-FR 170 PET to demonstrate that tissues with abnormally elevated uptake of $^{18}$F-FRP170, a direct marker of hypoxic but viable tissue, are present in human chronic cerebral ischemia with a combination of misery perfusion and moderately reduced oxygen metabolism because of unilateral atherosclerotic major cerebral artery steno-occlusive disease.

The rosary-like infarcts located at the subcortical white matter in the centrum semiovale or the anterior or posterior watershed zone are associated with hemodynamic impairment in ICA occlusive diseases.35 The same pattern infarcts located at the corona radiate seems to be related to hemodynamic impairment in MCA occlusive diseases.36 These infarcts were defined as subcortical border zone infarction and this study tried to enroll patients with such infarcts to investigate relationship between misery perfusion and $^{18}$F-FRP 170 PET findings.

PET with $^{18}$F-FMISO has been commonly used to detect hypoxic tissue.37–39 However, $^{18}$F-FMISO has various limitations, such as slow accumulation in hypoxic tissues, low target-to-background contrast, and significant amounts of radioactive metabolic products.28,40 The $^{18}$F-FMISO agent is
relatively lipophilic, whereas high hydrophilicity is associated with rapid blood clearance and high target:background ratios. In contrast, the 18F-FRP 170 used in this study has high image contrast, fast clearance, and readily crosses the blood–brain barrier; therefore, it is more suitable for visualizing hypoxic brain tissue than 18F-FMISO. Interestingly, a study using intratumoral oxygen pressure measurements with microelectrodes during resection of malignant glioma has directly demonstrated that an accumulation on 18F-FRP 170 PET represents viable tissue under the hypoxic condition. Although hypoxic tissue exhibiting increased uptake of 18F-FMISO may be doomed to die in acute stroke, a recent study using diffusion/perfusion MR or computed tomographic perfusion imaging and 18F-FMISO in acute ischemic stroke demonstrated that 18F-FMISO trapping overlapped the ischemic core presented as high intensity on diffusion MR, as well as the ischemic penumbra. PET studies using 15O in acute stroke also often show a high OEF in the ischemic core, as well as the ischemic penumbra, suggesting that the ischemic core under such conditions may remain viable at the time when PET is performed, although it is likely to die soon after that. Thus, cerebral tissue with increased uptake of 18F-FMISO in acute ischemia may represent a situation where increased OEF is combined with reduced CMRO2, which corresponded with our results using 18F-FRP 170 PET in chronic ischemia.

In this study, although a positive correlation was observed between the OEF and 18F-FRP170 ratios, the area with an elevated OEF did not exhibit an elevated 18F-FRP170 ratio when the area had a normal CMRO2. Several investigators showed that the degree of 18F-FMISO uptake is often greater in the ischemic core than in the ischemic penumbra in acute ischemia. Oxygen metabolism is theoretically reduced to a greater degree in the core versus penumbra. Thus, reduced CMRO2, in addition to increased OEF may be an essential characteristic of hypoxic tissue in cerebral ischemia. In contrast, areas with severely reduced CMRO2 did not exhibit elevated 18F-FRP170 ratios, suggesting that the cerebral tissue in these areas might be nonviable.

Kuroda et al suggested that reduced CBF in the normal-appearing cerebral cortex includes 2 pathophysiologically different conditions: misery perfusion because of hemodynamic compromise and matched hypometabolism because of border zone infarction. 18F-FRP170 ratio may be elevated in the former condition. In contrast, for the latter condition, border zone infarction may cause selective neuronal damage in the normal-appearing cerebral cortex beyond the regions of infarcts, resulting in reduced metabolism in the cerebral cortex. In addition, metabolism in the cerebral cortex with border zone infarction may be reduced because of diaschisis from the infarction.
Hypoxic tissue presenting as increased uptake of $^{18}$F-FMISO is metabolically compromised and at risk of infarction after acute ischemic stroke.\(^9,^{10,12}\) Our data suggested that cerebral tissue may become hypoxic when oxygen metabolism begins to decline at the end stage of misery perfusion with deterioration of chronic cerebral ischemia because of atherosclerotic major cerebral artery steno-occlusive disease. If this hypothesis is correct, the following research questions are raised. Does hypoxic tissue presenting as increased uptake of $^{18}$F-FRP170 in chronic cerebral ischemia subsequently succumb to irreversible brain damage over time? Does the hypoxic tissue disappear with recovery of CBF and oxygen metabolism after arterial reconstructive surgery? Is the disappearance of hypoxic tissue associated with improvement of cerebral function including cognition? Further studies aimed at answering these questions would be of benefit.

**Conclusions**

This study using $^{15}$O-gas and $^{18}$F-FRP 170 PET demonstrated that tissue with an abnormally elevated uptake of $^{18}$F-FRP170, a direct marker of hypoxic but viable tissue, is present in human chronic cerebral ischemia with a combination of reduced perfusion, moderately reduced oxygen metabolism, and misery perfusion because of unilateral atherosclerotic major cerebral artery steno-occlusive disease.

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**Disclosures**

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Data Supplement (unedited) at:
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http://stroke.ahajournals.org/content/suppl/2016/04/04/STROKEAHA.114.008238.DC2
SUPPLEMENTAL MATERIAL

Hypoxic viable tissue in human chronic cerebral ischemia due to unilateral major cerebral artery steno-occlusive disease
Diagrams showing the regions of interest (ROIs) in a three-dimensional stereotaxic ROI template. The orange (precentral and central segments), blue (parietal and angular segments) and red (temporal segment) ROIs indicate the frontal cortex, the parietal cortex and the temporal cortex perfused by the middle cerebral artery, respectively.
Each receiver operating characteristic curve and each cut-off point lying closest to the left upper corner of the curve used to determine the optimal sensitivity and specificity of CBF, CMRO$_2$ or OEF to detect an abnormally elevated $^{18}$F-FRP170 ratio.
Correlation between CBF and $^{18}$F-FRP170 ratio in mean data in the whole MCA territory ($\text{ROI}_{\text{whole-MCA}} = \text{mean value of} \ [\text{ROI}_{\text{sf-MCA}} + \text{ROI}_{\text{sp-MCA}} + \text{ROI}_{\text{st-MCA}}]$) of patients. The dashed horizontal line denotes mean + 2 SDs of $^{18}$F-FRP170 ratios obtained in healthy subjects and an ROI with $^{18}$F-FRP170 ratio greater than this value is defined as an abnormally elevated $^{18}$F-FRP170 ratio. No significant correlation is identified between the two parameters. The cut-off point of CBF lying closest to the left upper corner of the ROC curve for detection of an abnormally elevated $^{18}$F-FRP170 ratio is 36.2 ml/100 g/min (dashed vertical line) and an ROI with CBF less than this value is categorized as having a reduced CBF. The value represents mean – 1.6 SD of the control value obtained from healthy subjects.
Correlation between CMRO\textsubscript{2} and $^{18}$F-FRP170 ratio in ROI\textsubscript{whole-MCA} in patients. The dashed horizontal line denotes mean + 2 SDs of $^{18}$F-FRP170 ratios obtained in healthy subjects and an ROI with $^{18}$F-FRP170 ratio greater than this value is defined as an abnormally elevated $^{18}$F-FRP170 ratio. No significant correlation was identified between the two parameters. The cut-off point of CMRO\textsubscript{2} lying closest to the left upper corner of the ROC curve for detecting an abnormally elevated $^{18}$F-FRP170 ratio is 3.12 ml/100 g/min (right dashed vertical line) and an ROI with CMRO\textsubscript{2} less than this value is categorized as having a reduced CMRO\textsubscript{2}. The value represents mean – 1.0 SD of control. Further, when the cut-off point is moved in decrements from 3.12 ml/100 g/min of CMRO\textsubscript{2}, the sensitivity and positive-predictive values become 0% at a cut-off point of 2.59 ml/100 g/min (left dashed vertical line), which represents mean – 2.2 SD of control. CMRO\textsubscript{2} less than 2.59 ml/100 g/min or between 3.12 ml/100 g/min and 2.59 ml/100 g/min is categorized as severely or moderately reduced, respectively.
Correlation between OEF and $^{18}$F-FRP170 ratio in ROIwhole-MCA in patients. The dashed horizontal line denotes mean $\pm$ 2 SDs of $^{18}$F-FRP170 ratios obtained in healthy subjects and an ROI with $^{18}$F-FRP170 ratio greater than this value is defined as an abnormally elevated $^{18}$F-FRP170 ratio. The correlation between the two is significant ($P<0.0001$), with a correlation coefficient of 0.672. The cut-off point of OEF lying closest to the left upper corner of the ROC curve for detecting an abnormally elevated $^{18}$F-FRP170 ratio is 46.5% (dashed vertical line) and an ROI with OEF greater than this value is categorized as having an elevated OEF. The value represents mean $\pm$ 1.4 SD of control. Open, half-tone and closed circles denote not reduced CMRO$_2$, moderately reduced CMRO$_2$, severely reduced CMRO$_2$, respectively.
Mean, SD and range of $^{18}$F-FRP170 ratio, CBF, CMRO$_2$, and OEF in ROIs$_{\text{whole-MCA}}$ in 10 healthy subjects and 52 patients are shown in supplementary Table 1. While $^{18}$F-FRP170 ratios do not differ between healthy subjects and patients, CBF and CMRO$_2$ are significantly lower in patients than in healthy subjects and OEF is significantly greater in patients than in healthy subjects. The mean+2 SDs of $^{18}$F-FRP170 ratio obtained in healthy subjects is 1.088. Thus, when $^{18}$F-FRP170 ratio in an ROI$_{\text{whole-MCA}}$ is >1.088, the ROI was defined as having an abnormally elevated $^{18}$F-FRP170 ratio. As a result, of the 52 ROIs in 52 patients, 5 (10%) are classified as having abnormally elevated $^{18}$F-FRP170 ratio.

### Online-only Data Supplemental “Table 1”

Supplemental Table 1. Whole MCA-ROI PET values obtained from healthy subjects and patients.

<table>
<thead>
<tr>
<th></th>
<th>Healthy subjects (N = 10 or 20)</th>
<th>Patients (N = 52)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{18}$F-FRP170 ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1.000</td>
<td>1.014</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>0.044</td>
<td>0.057</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.941-1.057</td>
<td>0.876-1.148</td>
<td>N.S.</td>
</tr>
<tr>
<td>CBF (ml/100 g/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>47.2</td>
<td>38.6</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>6.9</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>36.1-55.7</td>
<td>24.4-46.1</td>
<td>0.0002</td>
</tr>
<tr>
<td>CMRO$_2$ (ml/100 g/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>3.56</td>
<td>3.09</td>
<td>0.0003</td>
</tr>
<tr>
<td>SD</td>
<td>0.45</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>2.97-4.13</td>
<td>2.19-3.69</td>
<td></td>
</tr>
<tr>
<td>OEF (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>39.4</td>
<td>42.3</td>
<td>0.0236</td>
</tr>
<tr>
<td>SD</td>
<td>4.9</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>33.5-46.7</td>
<td>38.1-55.6</td>
<td></td>
</tr>
</tbody>
</table>

*, $^{18}$F-FRP170 ratio of ROI; †, CBF, CMRO$_2$; and OEF of bilateral hemispheric ROIs; ‡, $^{18}$F-FRP170 ratio of ROI and CBF, CMRO$_2$ and OEF of ROI in the hemisphere ipsilateral to lesion.
**Online-only Data Supplemental “Table II”**

Supplemental Table II. Sensitivity, specificity, PPV and NPV for each whole MCA-ROI PET value for detection of an abnormally elevated $^{18}$F-FRP170 ratio.

<table>
<thead>
<tr>
<th></th>
<th>Reduced CBF</th>
<th>Reduced CMRO$_2$</th>
<th>Moderately reduced CMRO$_2$</th>
<th>Elevated OEF</th>
<th>Elevated OEF and moderately reduced CMRO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBF</td>
<td>&lt;36.2 ml/100g/min</td>
<td>&lt;3.12 ml/100g/min</td>
<td>&lt;3.12 ml/100g/min and &gt;2.59 ml/100g/min</td>
<td>&gt;46.5%</td>
<td>&gt;46.5% and 2.59 ml/100g/min &lt; CMRO$_2$ &lt;3.12 ml/100g/min</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100% (5/5)</td>
<td>80% (4/5)</td>
<td>80% (4/5)</td>
<td>100% (5/5)</td>
<td>80% (4/5)</td>
</tr>
<tr>
<td>95% CIs</td>
<td>100-100%</td>
<td>45-115%</td>
<td>45-115%</td>
<td>100-100%</td>
<td>45-115%</td>
</tr>
<tr>
<td>Specificity</td>
<td>74% (35/47)</td>
<td>47% (22/47)</td>
<td>57% (27/47)</td>
<td>94% (44/47)</td>
<td>100% (47/47)</td>
</tr>
<tr>
<td>95% CIs</td>
<td>62-87%</td>
<td>33-61%</td>
<td>43-72%</td>
<td>88-101%</td>
<td>100-100%*</td>
</tr>
<tr>
<td>PPV</td>
<td>29% (5/17)</td>
<td>14% (4/29)</td>
<td>17% (4/24)</td>
<td>63% (5/8)</td>
<td>100% (4/4)</td>
</tr>
<tr>
<td>95% CIs</td>
<td>8-51%</td>
<td>1-26%</td>
<td>2-32%</td>
<td>29-96%</td>
<td>100-100%†</td>
</tr>
<tr>
<td>NPV</td>
<td>100% (35/35)</td>
<td>96% (22/23)</td>
<td>96% (27/28)</td>
<td>100% (44/44)</td>
<td>98% (47/48)</td>
</tr>
<tr>
<td>95% CIs</td>
<td>100-100%</td>
<td>87-104%</td>
<td>90-103%</td>
<td>100-100%</td>
<td>94-102%</td>
</tr>
</tbody>
</table>

PPV, positive-predictive value; NPV, negative-predictive value; *, significantly greater than reduced CBF or moderately reduced CMRO$_2$; †, significantly greater than reduced CBF, moderately reduced CMRO$_2$, or elevated OEF.
The specificity for a moderately reduced CMRO$_2$ for detection of an abnormally elevated $^{18}$F-FRP170 ratio is greater than that for a reduced CMRO$_2$, although the statistical significance of the difference is not observed. The specificity for a combination of an elevated OEF and a moderately reduced CMRO$_2$ for detection of an abnormally elevated $^{18}$F-FRP170 ratio are significantly greater than that for the moderately reduced CMRO$_2$ or reduced CBF; the positive-predictive value for a combination of an elevated OEF and a moderately reduced CMRO$_2$ for detection of an abnormally elevated $^{18}$F-FRP170 ratio are significantly greater than that for elevated OEF, moderately reduced CMRO$_2$, or reduced CBF; significant differences in the sensitivity and negative-predictive value are not observed among the combined category and the individual categories.
Hypoxic Viable Tissue in Human Chronic Cerebral Ischemia Because of Unilateral Major Cerebral Artery Steno-Occlusive Disease

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Abstract

片側脳主幹動脈の閉塞性病変によるヒト慢性脳虚血における低酸素状態の生存組織

背景および目的：放射性標識した2-ニトロイミダゾール

を用いた陽電子放散断層撮影（PET）により、ヒト脳の急性虚血領域において低酸素状態であるが生存している組織が直接検出できる。本研究は、1-2⁻¹⁸F-フルオロ-1-(ヒドロキシメチル)エトキシメチル-2-ニトロイミダゾール(¹⁸F-FRPI70)をPETに用いて、片側脳動態硬化性脳主幹動脈の閉塞性病変によるヒト慢性脳虚血において¹⁸F-FRPI70の取り込みが異常に上昇した組織が存在するか否かを調べた。

方法：健常被験者20例と患者52例に¹⁸F-FRPI70 PETを施行し、¹⁸OガスPETを用いて脳消費を評価した。

图3

症候性の右側中大脳動脈閉塞を有する女性患者（63歳）の

陽電子放散断層撮影（PET）画像。脳虚血量（CBF）は著しく低下しており、脳酸素消費量（CMRO₂）は中等度以上高、酸素摂取率（OEF）は左側大脳半球と比べて右側の頭皮質で増加している。この領域には、1-2⁻¹⁸F-フルオロ-1-(ヒドロキシメチル)エトキシメチル-2-ニトロイミダゾール(¹⁸F-FRPI70)の集積が比較的高い。