Supplement

Transient Stroke Model

The rats were anesthetized with 1.5% isoflurane in 70% N2O/30% O2 (flow rate, 1.0 L/min). The right middle cerebral artery (MCA) was then occluded using the intraluminal filament technique, as previously described [1]. In brief, the common carotid and proximal pterygopalatine arteries were ligated using a 4-0 silk suture. Thereafter, the superior thyroid and occipital branches of the external carotid artery were transected. Finally, a silicon rubber-coated 5-0 nylon monofilament (tip diameter, 0.31 ± 0.02 mm) was advanced 17–8 mm to the bifurcation along the internal carotid artery to occlude the MCA. After 60 minutes of MCA occlusion, the filament was carefully withdrawn to induce vascular recanalization.

MRI and ^18^F-FDG PET Acquisition Methods

During MRI examination, the rats were positioned on a custom-made cradle and anesthetized with 1.5%–2.0% isoflurane inhalation through a mask. A body temperature of 37°C was maintained using a temperature-controlled water blanket. The tail vein and femoral artery were cannulated with polyethylene catheters for MR contrast agent injection and blood sampling, respectively.

During PET examination, while continuing to keep the rat warm, 6.5 ± 1.0 MBq in 0.2 mL of fluorodeoxyglucose (FDG) was administered via the tail vein under anesthesia (1.5% isoflurane in 100% O2 gas). Static PET images were acquired for 20 minutes in a 1–5 coincident in a single field of view. PET images were reconstructed using Tera-Tomo three dimensional in the full detector mode. Three-dimensional volume of interest analysis of the reconstructed images was performed using the InterView Fusion software package (Mediso) and standard uptake value (SUV) analysis.

Reference

1. Liu F, Lu J, Manaenko A, Tang J, Hu Q. Mitochondria in ischemic stroke: new insight and implications. Aging Dis 2018;9:924-937
Measurement of Image Parameters

To measure hyperoxia-induced $\Delta R_1$ (hyperO2$\Delta R_1$), normoxic (20% oxygen) and hyperoxic (100% oxygen) gases were infused through a respiratory mask without mechanical ventilation. The gas flow was maintained at a constant rate (1 L/min at 1 atm) during the experiment, with the inhaled gas mixture manually switched using a multichannel flowmeter. HyperO2$\Delta R_1$ was calculated according to the difference in $R_1$ values between hyperoxic and normoxic breathing.

The $R_1$ relaxation rate was calculated according to the following equation:

$$M(t) = M_0(1 - \alpha \cdot e^{-R_1TR}) + C$$

where $M(t)$ is the signal intensity at a particular repetition time (TR) or echo time (TE), $M_0$ is the steady-state magnetization including spin density and $T_1$ recovery, $\alpha$ is the efficacy of incomplete saturation of longitudinal magnetization, and $C$ is a constant. Then, hyperO2$\Delta R_1$ was calculated according to the difference in $R_1$ values between hyperoxic and normoxic conditions.

The apparent diffusion coefficient (ADC) was quantified using the following equation:

$$SI(b) = SI(0) \cdot e^{-ADC \cdot b \text{ value}}$$

where $SI$ is the signal intensity at a certain $b$ value and ADC is the ADC.

Cerebral blood flow and cerebral blood volume were measured using dynamic susceptibility contrast-enhanced MRI after converting the MR signal intensity to gadolinium concentration according to the indicator dilution theory.

The SUV of the $^{18}$F-FDG was calculated using the following equation:

$$SUV = \frac{\text{tissue radioactivity concentration}}{\text{injected activity}} \cdot \frac{\text{body weight (g)}}{\text{injection dose (Bq)}} \cdot e^{-\frac{\text{duration (min)}}{\text{half-life}}}$$

where $RI$ is the tissue radioactivity intensity of $^{18}$F-FDG, duration (60 minutes) is the time between tracer injection and PET scan initiation, and the half-life of radioactive $^{18}$F is 109.77 minutes.

Image parameters were quantified using the Analysis of Functional NeuroImages (AFNI, National Institutes of Health) program and ImageJ (National Institutes of Health).
Supplementary Table 1. Results of Post-Hoc Analysis Across Infarct, Ischemia, and Non-Ischemia Areas for Each Imaging Parameter and Time Point

| Imaging Parameters | Time Points (Hours) | P Value from Friedman Test | P Value from Post-Hoc Analysis (Wilcoxon Signed-Rank Test) | Infarct vs. Ischemia | Infarct vs. Non-Ischemia | Ischemia vs. Non-Ischemia |
|--------------------|---------------------|----------------------------|-------------------------------------------------------------|----------------------|--------------------------|---------------------------|
| HyperO2ΔR1         | 2.5                 | 0.006*                     | 0.028                                                       | 0.027                | 0.046                    |                           |
|                    | 4.5                 | 0.003*                     | 0.028                                                       | 0.028                | 0.039                    |                           |
|                    | 6.5                 | 0.032*                     | 0.028                                                       | 0.046                | 0.172                    |                           |
| ADC                | 2.5                 | 0.002*                     | 0.028                                                       | 0.028                | 0.028                    |                           |
|                    | 4.5                 | 0.002*                     | 0.028                                                       | 0.028                | 0.028                    |                           |
|                    | 6.5                 | 0.002*                     | 0.027                                                       | 0.028                | 0.028                    |                           |
| 18F-FDG            | 2.5                 | 0.002*                     | 0.028                                                       | 0.028                | 0.028                    |                           |
|                    | 4.5                 | 0.009*                     | 0.028                                                       | 0.028                | 0.116                    |                           |
|                    | 6.5                 | 0.115                      |                                                             |                      |                          |                           |
| CBF                | 2.5                 | 0.009*                     | 0.028                                                       | 0.028                | 0.173                    |                           |
|                    | 4.5                 | 0.042*                     | 0.249                                                       | 0.028                | 0.917                    |                           |
|                    | 6.5                 | 0.513                      |                                                             |                      |                          |                           |
| CBV                | 2.5                 | 0.011*                     | 0.028                                                       | 0.028                | 0.463                    |                           |
|                    | 4.5                 | 0.311                      |                                                             |                      |                          |                           |
|                    | 6.5                 | 0.607                      |                                                             |                      |                          |                           |

Post-hoc analysis was performed in case of statistical significance using Friedman tests. *Statistically significant, Friedman test. CBF = cerebral blood flow, CBV = cerebral blood volume, FDG = fluorodeoxyglucose, HyperO2ΔR1 = hyperoxia-induced ΔR1.
Supplementary Fig. 1. Comparisons of $^{18}$F-FDG uptake, CBF, and CBV in three areas (infarct, ischemia, and non-ischemia). $^{18}$F-FDG uptake, CBF, and CBV did not show consistent differences between the three areas across the three time points, by *Statistical significance between infarct and ischemia, †Statistical significance between infarct and non-ischemia, ‡Statistical significance between ischemia and non-ischemia. CBF = cerebral blood flow, CBV = cerebral blood volume, FDG = fluorodeoxyglucose, H = hours.