Supplementary Materials

Nitroxyl Radicals as Low Toxic Spin-Labels for Non-Invasive Magnetic Resonance Imaging of Blood-Brain Barrier Permeability for Conventional Therapeutics

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In vivo MRI measurements

MRI measurements were performed on 7.0 Tesla horizontal magnet (Kobelco and Jastec, Japan) interfaced to a Bruker Avance console (Bruker BioSpin, Germany) and controlled with ParaVision 4.0.1 (Bruker BioSpin, Germany).

Mice (C57Bl/6, ~25 g) were anesthetized by isoflurane (1.2%) and placed in a head holder (Rapid Biomedical, Germany), stomach-side down and fixed had. A respiration sensor (SA Instruments, NY, USA) was placed on the back of the mice. A non-magnetic temperature probe (FOT-M and FTI-10, FISO Technology, Germany) was used to monitor the rectal temperature of the mice. The tail vein was cannulated by polyethylene tube (PE-10, Becton-Dickinson, NJ, USA) for the injection of drug. The mouse was then placed in the ^{1}H- volume radio-frequency (RF) resonator (Brucker BioSpin) with surface RF receiver (Rapid Biomedical, Germany), which was previously warmed up using a body temperature controller (Rapid Biomedical). The resonator units, including the mouse, were placed in the magnet bore. The mouse body temperature was kept at 37 +/- 1 ^{o}C during the MR measurements. Before the measurements after drug injection, five control images of the mouse brain were taken with the following parameters: T_1-weighted incoherent gradient-echo sequence (fast low-angle shot; FLASH), repetition time (TR) = 75 ms; echo time (TE) = 3.5 ms; flip angle (FA) = 45 degrees; field of view (FOV) = 3.2 x 3.2 cm; number of averages = 4; scan time = 19.6 seconds; matrix = 64 x 64; slice thickness = 1.0 mm; number of slices = 4. We selected coronal slice orientations with a 500 um x 500 um x 1000 um nominal voxel resolution. A solution of SLENU or TEMPOL in DMSO (15 μmol per 25 g mouse) was injected via the tail vein, 1 min 40 sec after starting the scan. T_1-weighted images were acquired continuously within 20 min.

Mice, injected with DMSO only (in the same volume) served as controls.

The MRI data were analyzed using the ImageJ (National Institute of Health, MD, USA) software.

All experiments were conducted in accordance with the guidelines of the Physiological Society of Japan and were approved by the Animal Care and Use Committee of the National Institute of Radiological Sciences, Chiba, Japan.

***In vitro* EPR measurements**

SLENU and TEMPOL were first dissolved in DMSO to prepare 200 mM stock solutions. These 200 mM solutions were diluted with PBS, containing 1% bovine serum albumin to prepare 2 mM standard solutions. Each solution was put into a glass capillary and their X-band EPR spectra were measured on X-band EPR instrument (JEOL, Akishima, Japan) with a TE-mode cavity. The capillary tube was positioned in the center of the TE-mode cavity using special sample holder. The measurements were made under the following conditions: microwave frequency = 9.4 GHz, magnetic field strength = 336 mT, microwave power = 2.0 mW, field modulation frequency = 100 kHz, field modulation amplitude = 0.063 mT, time constant = 0.01 s, sweep width = 10 mT, scan time (sweep time) = 1 min.

In parallel, 2 μL of 200 mM stock solutions of SLENU or TEMPOL (in DMSO) were added to 198 μL freshly isolated blood (with heparin) and EPR measurements were provided at the parameters mentioned above within 30 min scan time.

Figure 1S. MRI signal dynamic of TEMPOL in the brain after i.v. injection in mice. Each image was obtained within 20-sec interval using gradient-echo MRI. In the images, the red colour represents an extraction of the signal between every single slide and the averaged baseline signal (first 5 slides – before injection). In the chart, the red and black colours represent MRI signal dynamic in the brain or entire area, respectively. Representative image from three independent experiments is shown in the figure.

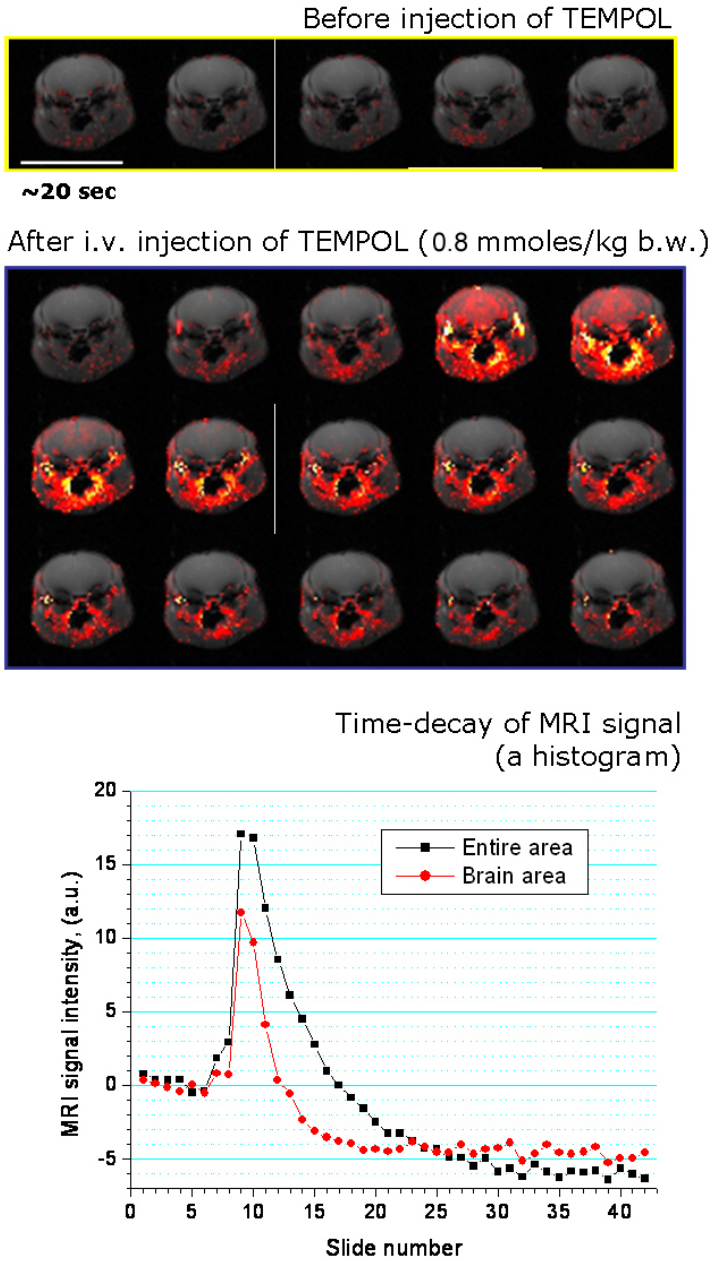
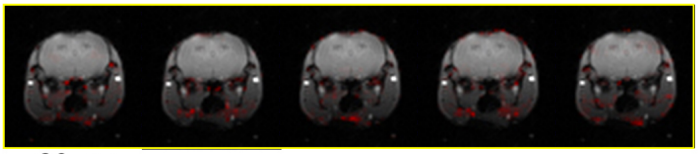


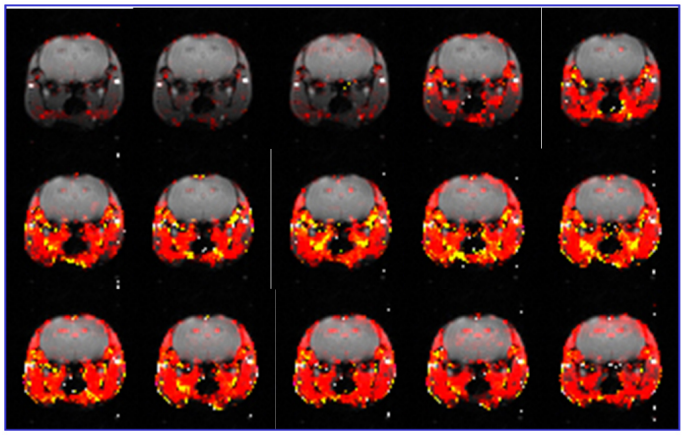
Figure 2S. MRI signal dynamic of Carbamoyl-PROXYL in the brain after i.v. injection in mice. Each image was obtained within 20-sec interval using gradient-echo MRI. In the images, the red colour represents an extraction of the signal between every single slide and the averaged baseline signal (first 5 slides – before injection). In the chart, the red and black colours represent MRI signal dynamic in the brain or entire area, respectively. Representative image from three independent experiments is shown in the figure.

Before injection of Carbamoyl-PROXYL



~20 sec

After injection of Carbamoyl-PROXYL (0.8mmoles/kg b.w.)



Time-decay of MRI signal
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