Supplementary Materials

Imaging of cancer by red/ox mediated mechanism: a radical diagnostic approach

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Chemicals

Nitroxyl-labeled nitrosoureas - {1-ethyl-3-[4-(2,2,6,6-tetramethylpiperidine-1-oxyl)]-1-nitrosourea} (SLENU) were synthesized and purified according to Gadjeva et al. (with slight modifications) [Int. J. Pharmacol. 212 (2001) 257-266; Eur. J. Med. Chem. 37 (2002) 295-300].

Deionized water (deionization by the Milli-Q system) was used for all experiments. Other chemicals used were of analytical or HPLC grade.

Cancer model

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.

Nude mice Balb6 were used. The mice were separated in two groups: healthy mice (controls; n=6) and mice with brain neuroblastoma (cancer-bearing mice; n=7). In both groups, the mice were same age, almost same weight (24+/-2 g), and maintained under same conditions.

The cancer model was developed using Neuro2a cells. The cancer cells ($0.5x10^5$ cells in 10 μL) were inoculated in one hemisphere of the brain. Neuro2a cells initiate a development of brain neuroblastoma without significant angiogenesis within ~10 days after inoculation (Figure 3S). It allows to neglect the MRI signal enhancement after injection of nitroxide, coming from the blood vessels, which is not indicative for tissue redox activity.

The mice were subjected to MRI measurements on the 7^{th}-8^{th} day after inoculation.

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).

Nude mice (Balb6, ~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 in DMSO (100 mM stock-solution) was injected via the tail vein (100 µL per 25 g mouse) a, 1 min 40 sec after starting the scan. T_1-weighted images were acquired continuously within ~14 min. Mice, injected with DMSO only (in the same volume) served as controls.

A “dip” in MRI signal was seen in all kinetic curves at time 2-3 min. It is a result of two processes going together: (i) penetration and accumulation of nitroxide probe in the respective tissue, which is accompanied with fast MRI signal enhancement; and (ii) subsequent reduction/oxidation of nitroxide into the cells, which it is accompanied with fast MRI signal decrease (if the reduction dominates over oxidation) or with MRI signal enhancements (if the oxidation dominates over reduction). The amplitude of the “dip” depends on the time of injection of nitroxide probe. At slow injection (~30-40 sec) the “dip” disappeared.

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

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In vitro EPR measurements

SLENU was injected intravenously in mice. Five-ten minutes after injection, the brain was extracted, perfused by saline solution and homogenized with 4-fold volume of PBS. Hundred μL of sodium ferricyanide (10 mM stock-solution) were added to 400 μL of tissue homogenate and incubated within 15 min as it was described in Hyodo et al. (*Cancer Res.* 2006, **66**, 9921). The ferricyanide quantitatively converts the hydroxylamine, produced as a result of *in vivo* reduction, back to the oxidized form (Krishna et al., *Proc. Natl. Acad. Sci. USA* 1992, **89**, 5537). The sample (100 μL) was places into a glass capillary and X-band EPR spectra was measured on X-band EPR instrument (Brucker) 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.

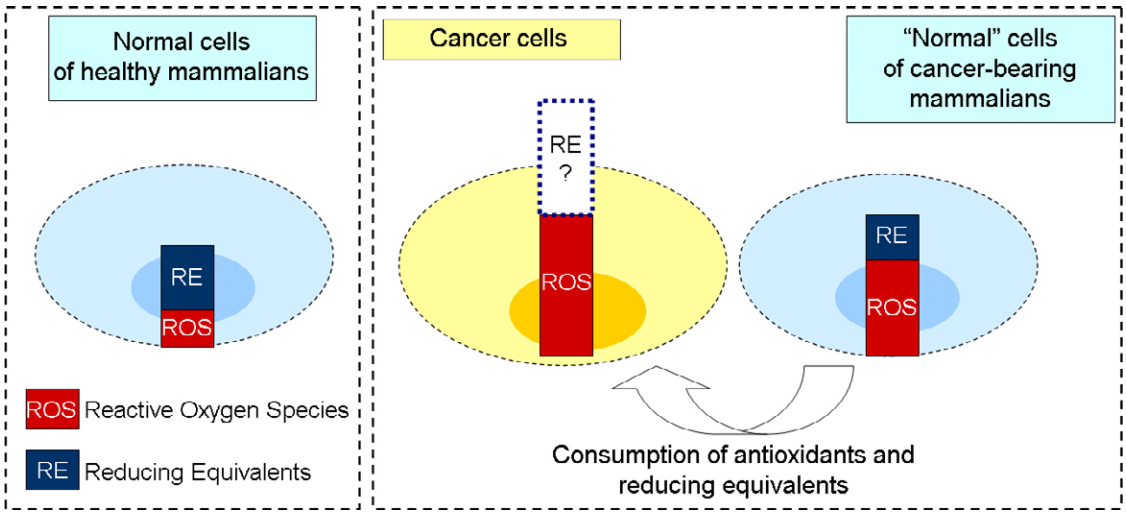
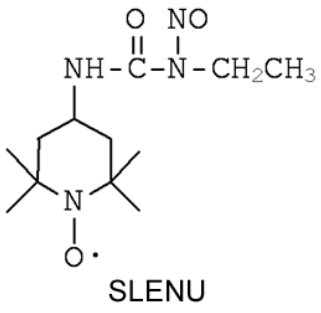


Figure 1S. Comparative levels of reactive oxygen species (ROS) and reducing equivalents (RE) in normal (healthy) cells, cancer cells and "normal" cells of cancer-bearing mammalians.



SLENU

Figure 2S. Nitroxide-labeled nitrosourea

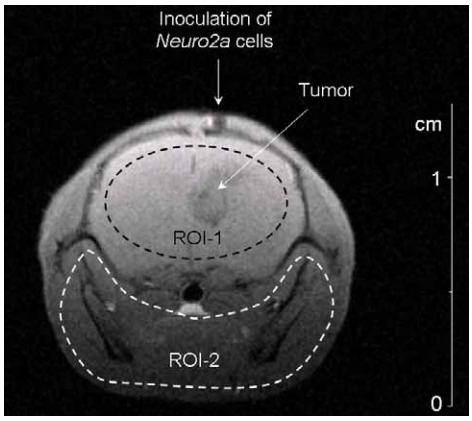


Figure 3S. MR image (spin-echo sequence) of tumor in mouse brain obtained 8 days after inoculation of cancer cells in Balb6 nude mouse. The dotted lines indicate the regions of interests (ROI-1 – brain tissue; ROI-2 – surrounding tissues).