Nitroxide probes as redox sensors for imaging of cancer *in vivo*

Rumiana Bakalova,[1] Zhivko Zhelev,[1] Sayaka Shibata,[1] Lubomir Spasov,[2] Ichio Aoki[1]

[1]Molecular Imaging Center, NIRS, 4-9-1 Anagawa, Inage-ku, Chiba 263-8555, Japan; [2]Medical Faculty, Sofia University "St. Kl. Ohridski", Sofia, Bulgaria

INTRODUCTION: Over 50 years experience in free radical biology and medicine shows that the normal cells of healthy mammalians are characterized with low steady-state level of reactive oxygen species (ROS) and some constant (reference) level of reducing equivalents.[1] Increasing of ROS above the critical level provokes genomic instability and uncontrolled proliferation. The normal cells become malignant. It is widely accepted that the balance between ROS and reducing equivalents in cells and tissues determines their redox activity. The evaluation of tissue redox activity has as a large prognostic value for cancer therapy and could significantly help planning of tolerant treatment and increasing quality of life of the patients.
The present study is directed to development of rapid and simple methodology for non-invasive evaluation of this parameter *in vivo* (in intact animals), that allows a differentiation of cancer development from normal (healthy) condition in a single measurement. The method is based on redox cycle of nitroxide probes and their MRI (magnetic resonance imaging) contrast properties, which makes them useful molecular sensors for tissue redox activity

EXPERIMENTAL METHODS: The mice (Balb6) were separated in two experimental groups - healthy mice (controls) and cancer-bearing mice (with brain neuroblastoma). The mice were used 8-9 days after inoculation of cancer cells (Neuro2a) in the brain. They were subjected to anesthesia (1.5% isoflurane). The tail vein was canulated for drug administration. The MR imaging of mouse brain was started. After the 5th frame (scan-time ~1 min 40 sec) during continuous scanning, the nitroxide probe was injected (100 μL per 25 g b.w.; single fast injection – within 15-20 sec) and the MR imaging continued up to 40-80 frames (total scan-time ~14-25 min). Two regions of interests (ROI) were selected – brain tissue (cortex) and soft tissues surrounding the brain.
Three nitroxide probes were used as redox-sensitive MRI contrast agents: (i) strongly hydrophobic, cell and BBB (blood-brain barrier) permeable, DNA-annealing SLENU; (ii) slightly hydrophobic, cell and BBB permeable TEMPOL; (iii) hydrophilic, cell and BBB non-permeable CMP. The nitroxide radical (which is characterized by T_1 contrast properties) participates in electron-transfer reactions (with cellular oxidants and reducers) with formation of non-contrast intermediate products (Figure 1). The rate constants of these reactions determine the MRI signal decay of nitroxide probe in living cells and tissues. For example, in healthy mammalians, the half-life of MRI signal decay of nitroxide probe in the selected ROI (for example, brain tissue) is considered as a reference steady-state level of tissue redox activity in norm. In cancer-bearing mammalians (in moderate or terminal stage of cancer), the half-life of MRI signal decay in the same or similar ROI is rather different from the subsequent reference level in norm. Thus, the half-life of MRI signal decay could be used as a diagnostic marker for tissue redox activity.[2]

RESULTS AND DISCUSSION: Figure 1 (left part) shows a typical kinetics of MRI signal intensity of nitroxide SLENU in the brain of control mouse. The signal increased slightly after injection, followed by rapid decay. The first 5 frames (before injection of SLENU) were used for calculation of the averaged baseline level. All data were normalized to the baseline. The data were processed with *ImajeJ* software. The histograms on Figure 1 represent the normalized data from 6 animals. In control mice, the half-life ($\tau_{1/2}$) of MRI signal decay was about 1 min or 2 min 20 sec in the brain or surrounding tissues, respectively. These $\tau_{1/2}$ values could be considered as reference values for the normal redox activity of the respective tissues. In the control group, the profile of the histograms and $\tau_{1/2}$ values are indicative of a high reducing activity of the brain and surrounding tissues to the nitroxide radical.
In cancer-bearing mice, the profiles of time-dependent MRI signal dynamics of SLENU in the brain and surrounding tissues (Figure 1 – right part) were completely different from the reference profiles. The MRI signal intensity increased after the injection of nitroxide and remained high and stable over 14 min, without decay.The histograms were same in the cancer hemisphere of the brain, "normal" (non-cancer) hemisphere, and "normal" surrounding tissues. These profiles and $\tau_{1/2}$ values are indicative of a low reducing activity of the brain and surrounding "normal" tissues of cancer-bearing animals to the nitroxide probe. The "normal" tissues arround the tumor have a different metabolic activity from the pre-cancer state, making them more sensitive to damage.
It was also observed that CMP, which is hydrophilic and non-permeable for cellular membrane and BBB, was not appropriate for MR imaging of tissue redox activity *in vivo*. The most reproducible results were

obtained using SLENU, followed by TEMPOL.

CONCLUSIONS: In conclusion, the present study demonstrates a development of new diagnostic approach for carcinogenesis based on the different tissue redox activity of normal and cancer-bearing mammalians and its imaging by cell permeable nitroxide probes and MRI. There is a very clear difference between MRI signal dinamics of SLENU and TEMPOL in healthy and cancer-bearing brain, which is indicative of the different metabolic activity of both tissues. The half-life of MRI signal decay is an appropriate diagnostic marker for carcinogenesis and a prognostic marker for the efficiency of cancer therapy. The described methodology is also applicable in isolated tissue specimens (e.g., biopsy specimens).

REFERENCES:

1. D. Trachootham, J. Alexandre and P. Huang, *Nat. Rev. Drug Discovery.* 2009, 8, 579.
2. Z. Zhelev, R. Bakalova, I. Aoki, V. Gadjeva and I. Kanno, *Mol. BioSystems* 2010, 6, 2386.

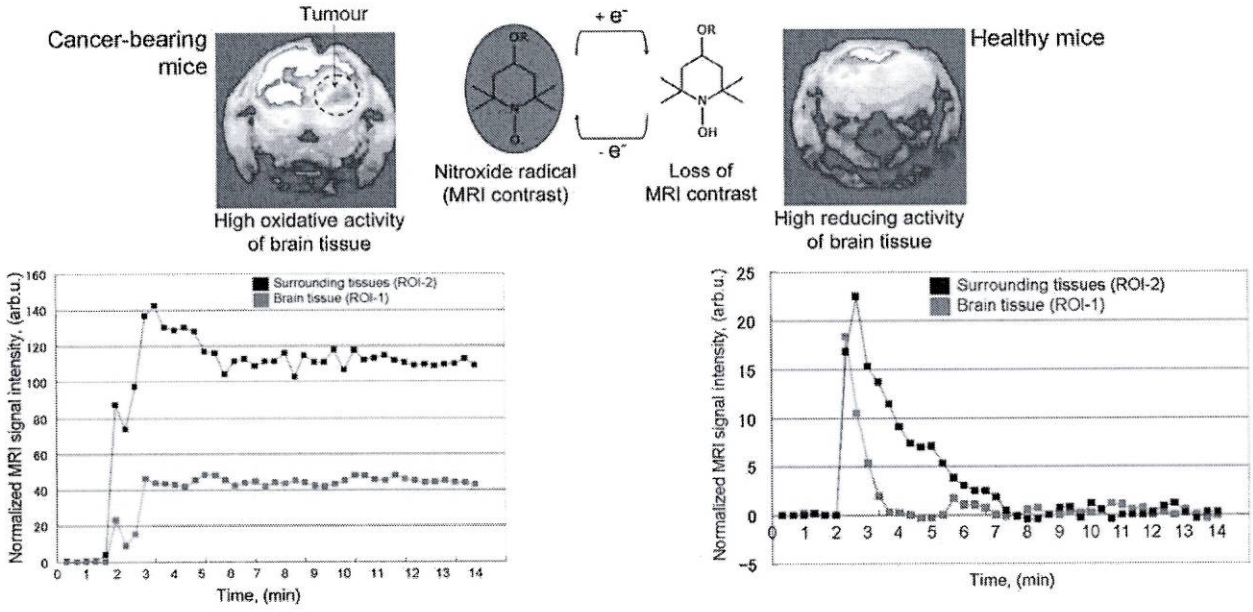

Figure 1. Dynamics of MRI signal enhancement by nitroxide probe in the brain and surrounding (soft) tissues of healthy and cancer-bearing mice.