Regulation of plasma and tissue concentraion of a spin probe, Oxo63, for pO_2 mapping in mice

K-I. Matsumoto[1], S. English[1], K-I. Yamada[1], A. Thirumaran[1], N. Devasahayam[1], J. A. Cook[1], J. B. Mitchell[1], S. Subramanian[1], M. C. Krishna[1]

[1]Radiation Biology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD, United States

Synopsis

The in vivo pharmacokinetics of the spin probe, Oxo63, after bolus and/or continuous intravenous infusion was investigated in C3H mice to determine a suitable dose of Oxo63 for Electron Paramagnetic Resonance (EPR) based oxygen mapping. Steady blood and tissue concentration of Oxo63 will lead to reliable quantification of pO_2 by Continuous Wave (CW) / time-domain Electron Paramagnetic Resonance Imaging (EPRI) and Overhauser-enhanced MRI (OMRI). The goal was to attain stable tissue Oxo63 concentration at optimal levels for quantitative oxygen mapping. Continuous infusion following a bolus injection was found to be effective to obtain stable plasma concentration and image intensity.

A number of techniques currently provides tissue oxygen tension or are under development. Oxymetric imaging of small animals have been carried out employing the paramagnetic contrast agent Oxo63 using CW, time domain EPRI and OMRI methods. CW EPRI, in the spectral-spatial mode, can directly measure the EPR line width, which depends on oxygen concentration. Time-domain EPRI can obtain oxygen dependent T_2^* by analyzing the free induction decay. OMRI is a double resonance technique, which utilizes paramagnetic contrast agents to enhance the MR image intensity based on electron T_2 and proton density. OMRI combines the strengths of EPR and Nuclear Magnetic Resonance (NMR) to generate high-resolution MRI, via the Overhauser effect, fundamentally known as Dynamic Nuclear Polarization (DNP). The change in the MRI intensity obtained at two different EPR power levels are used to estimate the EPR spectral line width of the contrast agent, which in turn can be correlated to pO_2 after suitable calibrations.

The spin-spin interaction of the spin probe and the oxygen molecule are reflected in the relaxation time. However, the EPR relaxation time depends not only on oxygen concentration but also several factors, for example, concentration of the spin probe itself, anisotropy of the probe molecule, and/or instrumental conditions. Therefore, we have to eliminate other factors except the effect of the oxygen concentration. A constant tissue concentration of spin probe would be useful to image a subject and assess the effects of their experimental treatment over time; accumulation or elimination of the compound in specific anatomical regions could translate to and be mistaken for changes in local pO_2, especially in OMRI based oxymetry, since the enhancement factors depend both on the spin probe concentration and pO_2. For quantitative assessment of pO_2, the blood and tissue concentration of Oxo63 should be regulated to remain unaltered during measurement.

Materials and Methods

Oxo63 was obtained from Nycomed Innovation AB (Malmo, Sweden). Female C3H mice (22–27 g) were used. The mouse was anesthetized by isofurane. The tail vein was cannulated to inject Oxo63 solution. The jugular vein was cannulated by a PE-10 tube. The jugular vein cannula was passed through the X-band EPR cavity and the outer end of the cannulation tube was connected to a 1 mL syringe. The cannulation line and the syringe were filled with heparinized saline previously. Isotonic Oxo63 aqueous solution was administered through a tail vein cannulation by bolus injection (0.75 µmol/g b.w.) or combination of bolus and continuous injection (0.75 µmol/g b.w. followed by 0.06 µmol/min/g b.w.). Immediately after the injection, blood was drawn up to the X-band EPR cavity from juggler vain cannulation, and EPR signal was measured. The blood was pumped back into the juggler vain immediately after measurement. The measurements were repeated by several draw-measure-pump-back sequences until 60 min after injection of Oxo63 solution. The EPR conditions were as follows; microwave frequency: 9.4 GHz, microwave power: 0.5 mW, magnetic field modulation frequency: 100 kHz, magnetic field modulation amplitude: 0.1 Gauss, time constant: 0.008 sec.

CW and time-domain EPRI and OMRI were carried out by same dose described above. Data acquisition was started 5 min after an initial bolus injection of Oxo63. Details of each imaging technique have been reported elsewhere (Krishna et al. 2002, Subramanian et al. 2002, Matsumoto et al. 2003).

Results and Discussion

Plasma concentration of Oxo63 started going down immediately after a bolus injection. Continuous infusion following a bolus injection was, however, effective to obtain stable plasma concentration. The time-domain EPRI, which has good temporal resolution, (less than 1 min for a pO_2 image) showed that a stable plasma concentration was able to keep tissue image contrast at a plateau level after 20 min delay, and then tissue image contrast was brought up gradually. The OMRI (2-5 min for an image) showed similar results with higher anatomical resolution. CW-EPRI, which requires 10-15 min for an image, is expected to give much better quantifiable oxymetry information, when the spin concentration remains unaltered during the measurement. Maintaining a steady blood and tissue concentration of the spin probe Oxo63 by the above procedure will lead to reliable quantification of pO_2 by CW / time-domain EPR and OMRI.

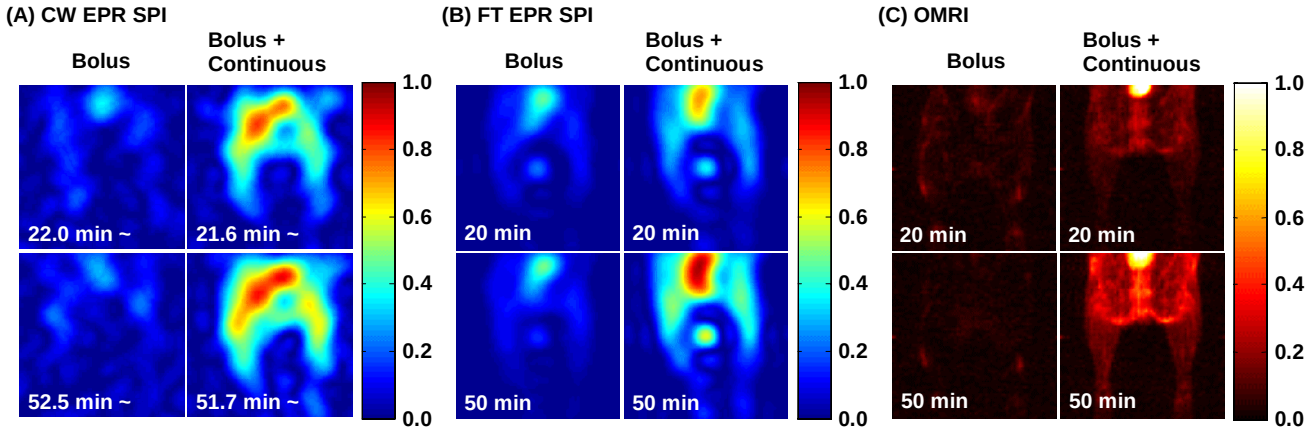

Fig. 1 Comparison of bolus and continuous injection by means of CW EPRI, time-domain EPRI, and OMRI. (A) CW EPR Single-Point Imaging. (B) time-domain EPR Single-Point Imaging. (C) OMRI. The left column of each figure shows the image after bolus injection (0.75 µmol/g b.w.), and the right column shows combination of bolus and continuous injection (0.75 µmol/g b.w. followed by 0.06 µmol/min/g b.w.).

Reference

1. Krishna MC, English S, Yamada K, Yoo J, Murugesan R, Devasahayam N, Cook JA, Golman K, Ardenkjaer-Larsen JH, Suburamanian S, Mitchell JB. Proc Natl Acad Sci USA 2002;99:2216–2221.
2. Subramanian S., Yamada K, Irie A, Murugesan R, Cook JA, Devasahayam N, Van Dam GM, Mitchell JB, Krishna MC. Magn Reson Med 2002;47:1001–1008.
3. Matsumoto K, Chandrika B, Lohman JAB, Mitchell JB, Subramanian S, Krishna MC. Magn Reson Med 2003;50:865–874.