Four-dimensional spectral spatial pH mapping of mouse tumour using Continuous Wave-Electron Paramagnetic Resonance imaging (CW-EPRI) and pH sensitive imidazoline nitroxide

Jonathan Goodwin[1], Shunichi Koda[1], Masashi Ohfuchi[1], Anna Pawlak[1], Hironobu Yasui[2], Valery Khramstov[3], and Hiroshi Hirata[1]

[1]Hokkaido University, Sapporo, Hokkaido, Japan, [2]Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido, Japan, [3]Ohio State University

Objectives

Over the past decade a number of advancements have been made in the field of pH measurement in living tissue[1], several of which are based on the measurement of magnetic resonance properties of pH sensitive molecules[2, 3] . In this work we assess the viability of pH sensitive imidazoline nitroxide (RSG)[4], for in-vivo pH mapping of mouse tumour using continuous wave electron paramagnetic resonance imaging (CW-EPR).

Methods

Mouse preparation: Two adult male mice (C3H) aged 6 weeks, were used as tumour model (mouse 1) and control mice (mouse 2), whereby mouse 1 was injected in the right hind leg with a 100µl solution containing ~5 million squamous carcinoma cells. Six days after cell injection, tumour volume measurements were acquired daily for 12 days until an average dimension of ~20 mm was recorded. Mice were then anaesthetized with a 5% isoflurane gas mixture and injected with a 100 mM/200µl RSG/HEPES buffer solution intravenously via tail vein cannulation, immediately prior to scanning. To demonstrate anatomical location of the generated tumour pH map, mouse 1 was sacrificed after EPR imaging using pentobarbital injection while *in-situ* in the scanner bed, and transferred to an MRI system to acquire T2 weighted images for co-registration purposes.

CW-EPR Imaging protocol: A 750Hz CW-EPR scanner[5] was used to perform 4D (1×spectral, 3× spatial) imaging of low field and high field absorption peaks, measured in right hind leg only, using the following scanning parameters: scan time- 0.3 s, modulation amplitude- 0.15 mT, G_{max}- 1.6 mT/cm, B- 1.0 mT, FOV- 5.09 cm and projections- 576.

MRI imaging protocol: Mouse 1 along with the scanner bed was transferred to a Varian 7T MRI animal scanner, which was used to acquire T2 weighted sagital slices (n=30), with the following parameters: TE- 80 ms, TR- 3.5 sec, FOV-10 cm.

Image reconstruction: Low field and high field image files were converted to fortran file format. Using Labview, the two images were superimposed and subtracted to give the difference in spectral position, equivalent to twice the hyperfine coupling constant (HFC). A previously derived HFC / pH calibration curve was used to generate 3D spatial maps of pH distribution in control mouse leg and tumour mouse, from which mean pH distributions were measured.

FOV correction and Image co-registration: Differences in EPR acquired pH maps and 7T MRI images of mouse 1 were adjusted for orientation and FOV differences using in-house developed software[6]. Corrected images were converted to NFTI format and co-registered using SPM8 software. 3D rendering of co-registered images was performed with MRICRON software.

Results

Figure 1 shows the histogram distribution of measured pH values from 3D EPR images of right hind leg for mouse 1 (mean pH=6.67) and mouse 2 (mean ph=7.17). Figure 2 shows a 3D rendering of EPR pH map of mouse 1 tumour leg overlaid on 3D MRI.

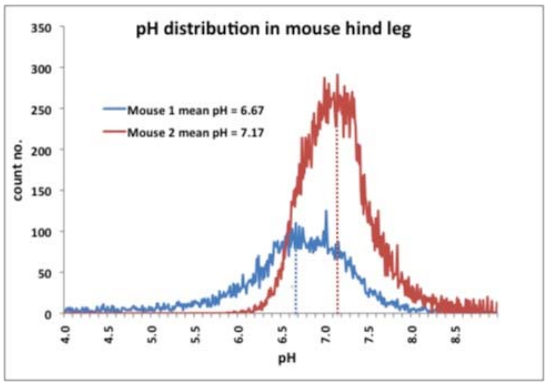

Figure 1. pH distribution from 3D volume measurement of right hind leg from mouse 1 (tumour) and mouse 2 (control).

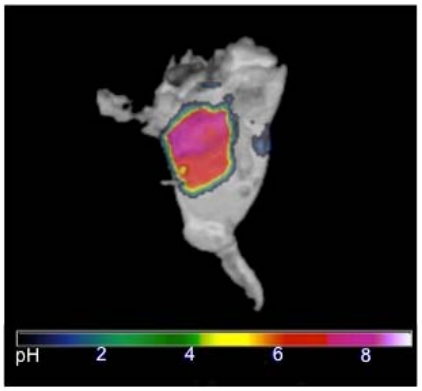

Figure 2. Co-registered 3D EPR pH map with MRI for mouse 1 hind leg.

Discussion

The hydrophilic structure and membrane-impermeable properties of the RSG probe ensured its extracellular localization[4] . Measured extracellular pH (pH_e) values of mouse 1 & 2 demonstrate a reduction of average pH_e and higher pH_e heterogeneity in tumor compared with normal tissue. This observation highlights the potential for the use of RSG nitroxide for *in-vivo* pH mapping using CW-EPR. Successful demonstration of the technique will allow further exploration of the relationship between pH_e in tumour, and how the relationship changes in response to radiotherapy with and without radio-sensitizing drugs. Obtaining a better understanding of the pH changes associated with radiotherapy in tumour may offer valuable information for successful treatment planning.

Acknowledgements: Work financially supported by JSPS (NEXT program LR002)

References

1. Zhang, X., Y. Lin, and R.J. Gillies, Tumor pH and its measurement. J Nucl Med, 2010. 51(8): p. 1167-70.
2. Khramtsov, V.V., et al., Biological applications of spin pH probes. Cellular and Molecular Biology, 2000. 46(8): p. 1361-1374.
3. Foster, M.A., et al., In vivo detection of a pH-sensitive nitroxide in the rat stomach by low-field ESR-based techniques. Magnetic Resonance in Medicine, 2003. 49(3): p. 558-567.
4. Bobko, A.A., et al., In Vivo Monitoring of pH, Redox Status, and Glutathione using L-Band EPR for Assessment of Therapeutic Effectiveness in Solid Tumors. Magnetic Resonance in Medicine, 2011. DOI 10.1002/mrm.23196
5. Sato-Akaba, H., et al., Half-Life Mapping of Nitroxyl Radicals with Three-Dimensional Electron Paramagnetic Resonance Imaging at an Interval of 3.6 Seconds. Analytical Chemistry, 2009. 81(17): p. 7501-7506.
6. Ohfuchi, M., et al., Three-Dimensional EPR/NMR Image Coregistration Using a MATLAB-Based Software. Concepts in Magnetic resonance Part B 2011. 39B(4): p. 180-190.