In vivo ^{19}F MRS and ^{19}F 2D-CSI investigation of a fluorine labeled 2-Nitroimidazole (TF-MISO). A potential functional reporter of hypoxia in solid tumors.

D. Procissi[1,2], F. Claus[1], J. Koziorowski[1], P. Burgman[1], C. Matei[1], S. Thakur[1], C. Ling[1], J. A. Koutcher[1]

[1]Medical Physics, Memorial Sloan Kettering Cancer Center, New York, NY, United States, [2]Brain Imaging Center, California Institute of Technology, Pasadena, CA, United States

INTRODUCTION

The ability to measure tumor hypoxia in real time and on a case to case basis is essential for hypoxia directed clinical trials. Several techniques, both invasive and non invasive, have undergone preclinical and in some cases clinical evaluation, yet none have entered widespread use [1]. 2-Nitroimidazoles represent a class of compounds that in the absence of an adequate supply of oxygen undergo further reduction and bind to cell components [2]. By monitoring the *in vivo* tumor uptake and retention of fluorine labeled nitroimidazoles using ^{19}F nuclear magnetic resonance (NMR) spectroscopy it is possible to assess the presence of hypoxic tissue. The advantage resides mainly in the possibility of obtaining anatomical ^{1}H MRI images and chemical shift distribution maps of the ^{19}F reporter molecules using the same exact setup. To this date the best example of a validated hypoxia tracer is the SR4554 molecule [3], which was designed specifically to this end and is now in a Phase I clinical trial. It has been suggested that to thoroughly investigate hypoxia, an intrinsically heterogeneous problem, it is necessary to identify and test other compounds with equivalent ability but with slightly different chemical characteristics.

We focused our attention on the magnetic resonance (MR) spectroscopy and multi-dimensional chemical shift imaging (CSI) investigation of trifluoro–nitroimidazole (TF-MISO) which has been proven to be hypoxia selective in cellular uptake experiments [4] . Our goal was to demonstrate the possibility of using TF-MISO as an in vivo ^{19}F NMR reporter of hypoxia in solid tumors and of functionally imaging the tumor distribution of the compound using ^{19}F chemical shift imaging.

EXPERIMENTAL METHODS

All experiments were performed on a small animal 7 Tesla Bruker Biospin spectrometer (30 cm horizontal bore), on C3H/He mice using a highly hypoxic MCa tumor, implanted on the right foot. TF-MISO (OHG Laboratories) was injected (i.v.) bolus tail-vein injection at a dosage of 75 mg/kg or 200 mg/kg . Approximately 30 minutes after the administration of the drug, the mice were anesthetized using a mixture of isofluorane and air (20% O_2) and placed in the animal holder. The leg with the foot tumor was placed inside a 3 turn home built coil tuned and matched at 282MHz and MR experiments were initiated. Throughout the experiment mice were kept at constant body temperature of ~36C°. MR spectroscopy acquisition parameters included a 60° pulse flip angle, a pulse repetition of 0.8 s, 1200 transients/spectrum (15 min/spectra). To quantify tumor tissue concentration of TF-MISO we used an external reference standard of 75 mM of NaF in D_2O. The two-dimensional ^{19}F CSI was performed using a k-space weighted 2D chemical shift imaging protocol (CSI_2D_PVM) with a voxel size of 3×3×3 mm^3 (field of view 2.5cm). Total time for each experiment was ~26 minutes following the 200mg/kg dose. The images were obtained using the CSI Visualization tool provided by ParaVision software as described in [4]. The intensity of each CSI voxel is proportional to the corresponding Fluorine spectral intensity. The anatomical ^{1}H reference images were acquired using the same setup (re-tuned to 300 MHz) and a rapid acquisition with relaxation enhancement (RARE) sequence (in plane resolution of ~ 100 μm).

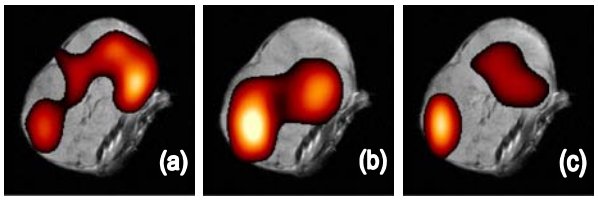

Fig. 2. *Functional chemical shift distribution maps of TF-MISO at (a) 1 hour (b) 3 hours and (c) 6 hours (200 mg/kg dose) are pixelated and overlayed on a ^{1}H Anatomical image (resolution 100μm) (d) whole tumor TF-MISO spectra as a function of time after injection.*

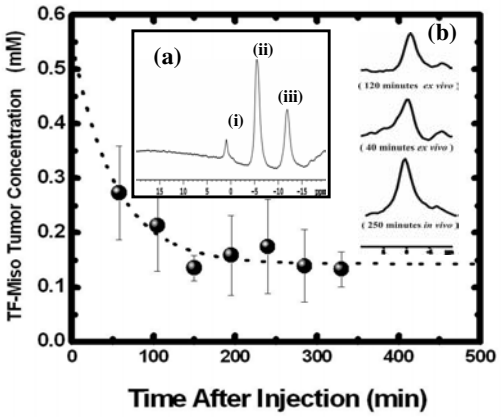

Fig. 1. *TF-MISO concentration vs Time (dose 75 mg/kg) (a) inset shows the in vivo resonance of (i) TF-MISO in respect to those of (ii) and (iii) of isofluorane. (b) in vivo spectra at 250 minutes as compared to ex vivo 40 and 120 minutes post*

RESULTS

Shown in Fig. 1 is the MCa tumor tissue concentration of TF-MISO as a function of time. Inset (a) of Fig.1 shows the MR resonances of (i) TF-MISO in respect to those of (ii) , (iii) isofluorane. One can note the rapid decrease from a concentration of 0.27 mM at 60 minutes to a nearly constant value of 0.14 mM after approximately 100 minutes. Signal was visible for greater than 12 hours (not shown). *In vivo* and *post mortem* spectra are also shown in Inset (b) of Fig. 1. Although there is a slight decrease in the intensity of the resonance before and after death, no drastic change was observed in the spectral characteristics of the signal. Finally in Fig 2 (a), (b) and (c) high resolution anatomical images of a single slice of the tumor are shown with the overlayed TF-MISO pixelated intensity distribution at 1, 3 and 6 hours. The corresponding whole tumor stacked spectra are shown in Fig 2(d).

DISCUSSION AND CONCLUSION

We have shown that the ^{19}F MRS *in vivo* signal from TF-MISO is readily detectable and can be used to monitor the tumor tissue concentration as a function of time. The pharmacokinetic results show retention in highly hypoxic tumors for lengths of time longer than those expected for unbound TF-MISO. The retention of the compound is consistent with the presence of hypoxia in these types of tumor [5]. In addition experiments performed post mortem under physiological condition (constant body temperature) showed no drastic change in the spectral characteristics of the signal, which suggests that the signal is indeed observable even when in its bound and reduced form. This differs from the results with EF5 (6). Finally we demonstrated how it is possible to image tumor tissue distribution of TF-MISO as a function of time for up to 6 hours which will allow the tracer to wash out from blood and other organs, providing a potentially significant advantage over comparable PET studies. In addition these data demonstrate that it is feasible to obtain high resolution anatomical details for image registration without moving the animal. These preliminary results suggest that TF-MISO is a valid candidate to be used as an in vivo MR reporter of tumor hypoxia. Presently several studies, both using MR and other analytical techniques, are being undertaken to provide a more comprehensive validation of the compound as an hypoxia tracer.

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