Trifluoromisonidazole (TFMISO) as Imaging Agent of Hypoxia in Solid Tumors - An *In Vivo* ^{19}F MR Study

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Introduction: Tumor hypoxia has been indicated as a prognostic factor in treatment response and outcome [1]. Therefore, it has been attempted to measure tumor hypoxia [2-4]. Here, we investigate in two cancer animal models the ability of Trifluoromisonidazole (TFMISO) to measure (i) the extent and distribution of tumor hypoxia and (ii) changes in tumor hypoxia induced by changes in oxygenation.

Material and Methods: Rat prostate cancer cells, R3327-AT, implanted in the right hind leg of athymic nu/nu mice and the mouse mammary cancer MCa implanted in the right hind foot of C3H/He mice were studied [5]. When tumor volumes, as determined by caliper measurement, reached approximately 250 mm^3, 500 mm^3, and 700 mm^3 for R3327-AT tumors, and 150 mm^3, 250 mm^3, 500 mm^3 for MCa foot tumors, *in vivo* MRI and MRS was performed. The MR experiments were performed on a Bruker 7T Biospec MR spectrometer using home-built MR coils adjusted to the different tumor sizes. Following i.v. injection with 75 mg/kg TFMISO, the mouse was placed into the magnet and the body temperature maintained at 37ºC as described previously [5]. The water signal was shimmed to a line-width of 40-70 Hz and ^{1}H MR images acquired to assess the anatomy of the tumor. After reaching steady state levels of TFMISO in the tumor, global and localized ^{19}F MR spectra were acquired. After applying a line-broadening factor of 20 Hz to the FID, the TFMISO signal was fitted in the time domain using XsOsNMR. TFMISO was quantified as described previously by Procissi et al. [5]. Each animal was subjugated to three MR studies followed by injection of the hypoxia marker Pimonidazole (60 mg/kg) and tumor excision. Fresh-frozen tumors were sectioned and stained for Pimonidazole and DAPI.

Results: The plasma half-life of TFMISO is about 0.5 h and after about 2 h the TFMISO concentration in the blood is below MR-detectable levels (Fig. 1). In both tumor models, the TFMISO concentration reaches a steady state after about 1.5 h and stays constant for up-to 5 h (Fig. 2). In the MCa foot tumors, the intratumoral TFMISO concentration increases with tumor size irrespective of the animal breathing air or 100% O_2 (Fig. 3 (left)). In R3327-AT leg tumors, the intratumoral TFMISO concentration increases only slightly with tumor size (Fig. 3 (right)). The intratumoral TFMISO concentration for R3327-AT tumors of below 600 mm^3 volume tends to be lower in animals breathing 100% O_2 than in animals breathing 21% O_2 (Fig. 3 (right)). Fig. 4 displays the distribution of TFMISO in a R3327-AT tumor. In both tumor models, tumors above 200 mm^3 revealed stronger overall staining of Pimonidazole and different-sized necrotic regions (Fig. 5).

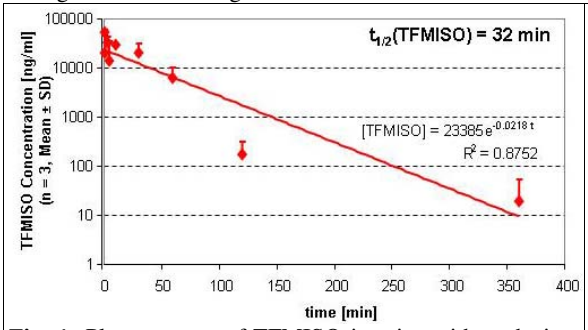

Fig. 1: Plasma curve of TFMISO in mice with each time point averaged over 3 mice (mean ± SD). The plasma half-life of TFMISO is 32 min.

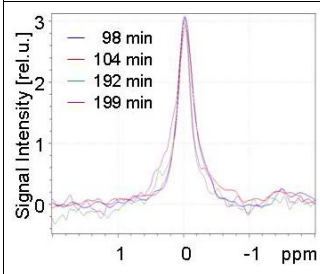

Fig. 2: Representative global ^{19}F MR spectra. Intratumoral TFMISO levels reach steady state about 1.5 h after injection.

Conclusion: Our results suggest that TFMISO may be useful for tumor hypoxia imaging by MR, as it permits the noninvasive quantification of extent and localization of hypoxia in solid tumors. Currently, we are working further on the quantification to include B_1 variations across the MR coil and to increase the cohort size in each group.

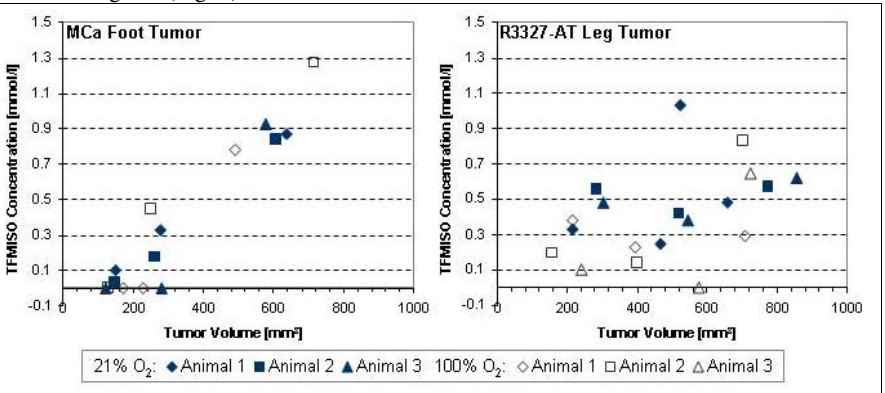

Fig. 3: TFMISO concentrations in dependence of tumor size at about 2 h after i.v. administration of 75 mg/kg TFMISO. Open signs represent anesthetized animals breathing 100% O_2 and closed signs 21% O_2.

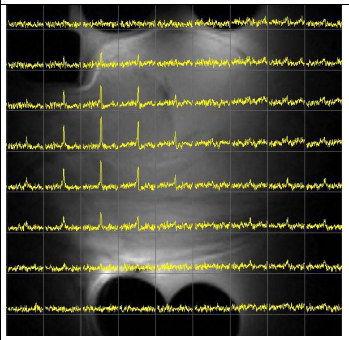

Fig. 4: Spectral region of TFMISO in 2D ^{19}F MRSI spectra (1.56 x 1.56 x 3 mm^3) overlaid on ^{1}H MR image (3 mm slice) depicting the tumor region.

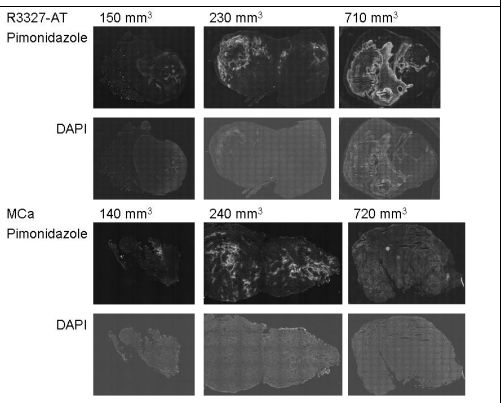

Fig. 5: Representative tissue sections stained for DAPI and Pimonidazole.

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