^{1}H MRSI maps of tumor hypoxia using an extrinsic hypoxia probe

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Introduction: Several studies indicate a relationship between tumor hypoxia and tumor aggressiveness, poor outcome (1) and resistance to therapies (2). Due to the chaotic neoangiogenic development, neoplastic tissues often show hypoxic areas heterogeneously distributed in the tumor mass. There are several methods to measure hypoxia, but none of them have reached routine clinical application (3). 2-Nitroimidazole derivatives (EF5, Pimonidzole, ^{18}F-MISO) have been extensively used as molecular markers of hypoxia in combination with different techniques as immunohistochemistry, PET or ^{19}F MRI (3). Here, we aimed to test whether nitroimidazole derivatives can be used as hypoxia marker in vivo in combination with widely applicable ^{1}H MRSI methodologies.

Materials and Methods: Nude mice (NU/NU, 20-25 g), were implanted subcutaneously with glioblastoma cells (C6) in flanks. Tumors were allowed to grow up to 1 cm diameter. MR evaluations were performed in vivo in a 7.0 T Bruker Pharmascan ® system using a ^{1}H rat brain receive-only surface coil in combination with the actively detuned transmit-only resonator. Animals were anesthetized by inhalation with 2-3 % of isofluorane in an induction chamber (1 L min^{-1}) and maintained with 2 % of isofluorane in different gases: 100 % oxygen (n=2) and air (n=2). MRSI was performed in two spatial dimensions, exciting a PRESS selected volume of 8 × 8 × 4 mm. Acquisition parameters were: FOV = 32 × 32 mm, matrix = 8 × 8 zero-filled to 32 × 32, TR = 1500 ms, TE = 19.3 ms and 500 transients acquired during 12.6 min. Water suppression was performed with a VAPOR sequence. After basal spectrum, the animal was removed from the magnet and injected intratumoraly with 50 µL of an aqueous solution of the oxygen reporter MISO (1M) and TSP (2M) using a gas tight syringe with a custom-made fine sharp needle. The animal was then introduced again in the magnet and the MRSI process repeated. The evolution of the injected reporters was monitored during at least two hours after injection. MRSI images were reconstructed and quantified by using 3DiCSI 1.9.9 software (Hatch Center for MR Research, Columbia University, New York, NY). The integral of MISO, TSP and lipids signals were measured at different times after drug administration. The percentage of TSP and hypoxia marker relative to lipid signal was calculated relative to the lipid signal. Only those voxels with SNR equal or bigger than 2 were taken into account. Statistical calculations were performed by using GraphPad Prism (GraphPad Software, Inc. La Jolla, USA). Parametric color-based maps were generated by using Matlab based home made software.

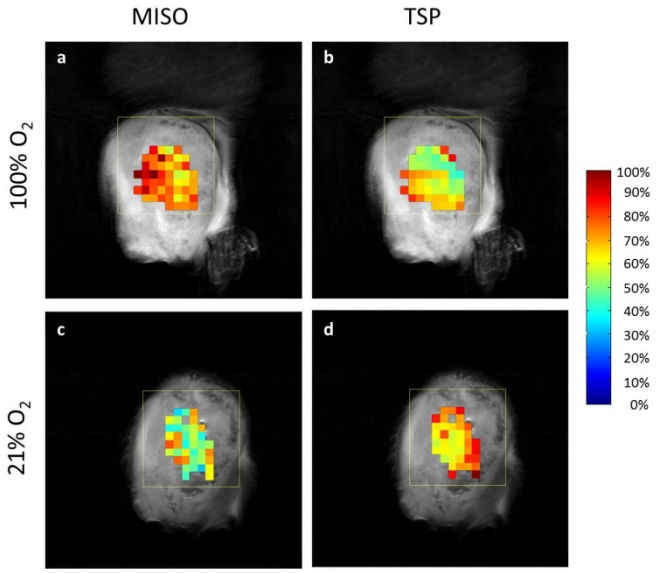

Fig 1. Percentage of MISO and TSP remaining in the tumor after 70 minutes of injection relative to the initial amount detected 30 minutes after injection. a) % of MISO in a representative animal breathing pure oxygen. b) % of TSP in a representative animal breathing pure oxygen. c) % of MISO in representative animal breathing air and d) % of TSP in representative animal breathing air. Note the lower MISO concentration remaining in tumors from animals breathing lower atmospheric oxygen.

Results: Fig 1 shows maps of the remaining MISO and TSP percentage 70 min after injection compared to that of 30 min after injection for hyperoxigenated (breathing 100% O_2) and normoxic animals (breathing 21% O_2). Tumors depicted statistically significant differences in the percentage of MISO signal remaining in animal breathing pure oxygen and air ($P < 0.0001$), whereas it was not the case for the percentage of TSP ($P > 0.05$), used as a marker for clearance (Fig 2). It is also important to notice that there is no spatial correlation for the values obtained for MISO and TSP in a voxel by voxel way.

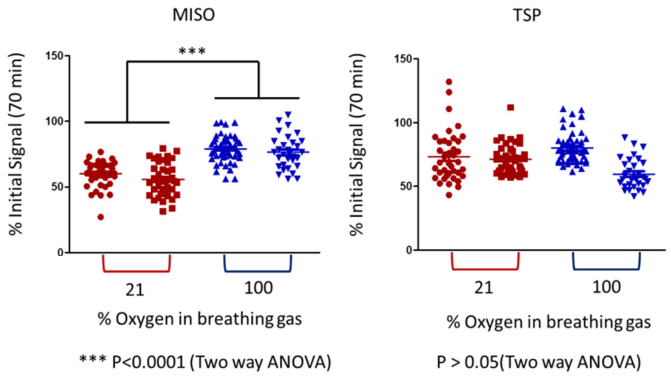

Fig 2. MISO and TSP disappearance in vivo as detected by ^{1}H MRSI. Scatter plot of the amount of MISO (left) and TSP (right) remaining in the tumor at 70 min relative to 30 min after the injection of the compounds in hyperoxygenated (blue) and normoxic (red) tumors. Note the significantly larger disappearance of MISO in the 21% oxygen breathing.

Conclusions: We showed that imaging the real time distribution of MISO and TSP is possible using ^{1}H MRSI. Maps of the percentage of MISO and TSP remaining revealed high intratumor variability for both MISO (SD ≈ 10) and TSP (SD ≈ 12) in all the animals tested (Fig 1 & 2). However, hyperoxygenated animals showed a significantly higher percentage of remaining MISO (77%) than normoxic animals (58%), revealing higher MISO disappearance for the lower oxygen tensions breathed. There is no such a difference in TSP, an indicator for neovascular clearance, which showed 72% of remaining compound for hyperoxic and normoxic tumors respectively. The use of ^{1}H MRSI to infer tumor hypoxia complements and extends earlier ^{1}H MRSI methods implemented to measure extracellular pH (pH_e) maps (4), allowing for the first time to our knowledge, an integral ^{1}H MRSI approach to resolve spatially pH_e and pO_2 through the tumor microenvironment. The proposed methodology is direct and easily adaptable to clinic, especially in superficially located tumors, as lymph nodes from head and neck tumors, where hypoxia has been demonstrated to be particularly relevant (5). Together, present results favor the use of nitroimidazole derivatives as ^{1}H MRSI hypoxia markers.

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