In vivo detection of the metabolism of novel hypoxia probes in models of glioma by ^{1}H NMR

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Introduction: Hypoxia is known to play a central role in many oncologic processes providing the common link among tumor aggressiveness, poor outcome and resistance to different therapies (1,2). Consequently, a variety of methods have been proposed to measure tumour oxygenation *in vivo* and *in vitro* (3). In particular, several nitroimidazole derivatives (SR-4554, EF5) have been used in combination with either optical microscopy methods (EF5, Pimonidazole) or nuclear medicine approaches (SR-4554, 18F-MISO) (4). The procedure is based on the selective reduction and trapping of these derivatives in hypoxic regions of the tumor *in vivo* (5). These approaches require the use of either tissue biopsies for histological analysis or PET scanners and cyclotron associated synthetic facilities. It would become then useful to develop an in vivo NMR protocol, compatible with currently available scanners, to observe in situ the metabolism of nitroimidazoles in tumors under different oxygenation conditions. The present work reports, for the first time to our knowledge, the in vivo ^{1}H NMR detection of some commercially available nitromidazole derivatives under normoxic and hypoxic conditions.

Subjects and Methods: Nude mice were implanted with C6 glioblastoma cells in the flanks. Tumors were allowed to grow up to 1 cm diameter. Animals were then anesthetized using Isoflurane (1.5%) and either medical air or 100% oxygen, receiving a single intratumoral injection (multisite) of misonidazole 1 M (300 mg/kg (6)) + TSP 2 M. TSP was added to the injection to monitor the wash out of a non reducible compound. The hypoxia sensitive probe misonidazole was expected to be both, selectively reduced in hypoxic zones, as well as cleared from the tumor (as TSP) by circulation, disappearing then at a faster rate than the non reducible reference The time course of the probes inside the tumor after the injection was followed using single voxel spectroscopy (125 µL) using a PRESS sequence (7T Bruker PharmaScan, number of scan =128, TR= 3000 ms, TE = 20 ms, acquisition time = 7 min). During the experiments, animals breathed air or pure oxygen. The area of TSP and Misonidazole resonances were normalized to the mobile lipid methylene resonances (1,3 ppm), which did not change appreciably during the time course of these experiments. Normalized decays of TSP and Misonidazole resonances with time, were fitted non lineasrly to a single exponential decay ($y=(y_0$ - plateau)*exp(-k*x) + plateau, where: y_0 and pleateau represent the y values at zero and infinite time, and k is the rate constant of disappearance (min^{-1}), respectively

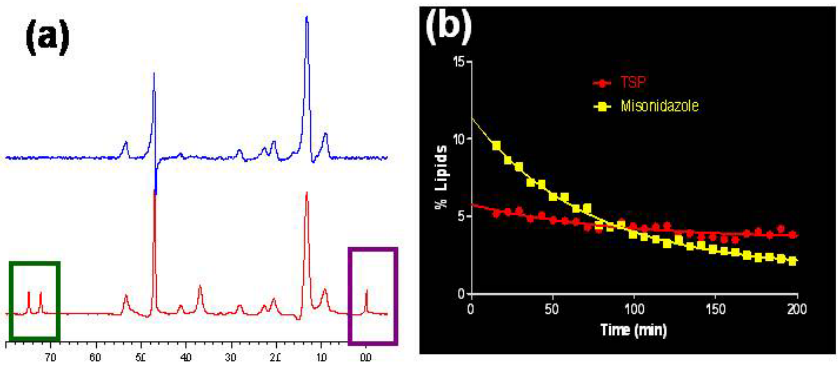

Figure 1. a) PRESS spectra of the C6 implanted tumor obtained before (blue) or after (red) the injection (multisite) of Misonidazole (green square) and TSP (purple square). b) Monoexponential decays of the Misonidazol and TSP resonances with time after the injection.

Results: Figure **1a** shows the ^{1}H PRESS spectrum of the tumor obtained before and after the injection of the Misonidazole + TSP mixture. The resonances of the H4 and H5 protons of the imidazole ring are easily observed in the aromatic region (between 7 and 8 ppm, green square). Simultaneously, the TSP resonance (at 0 ppm) becomes clearly detectable (purple square). These resonances disappear from the tumor with time. The process appears to follow first order kinetics, as shown in the representative study depicted in Figure **1b**. The decay is characterized by a rate constant k, which is different for the misonizadole or TSP compounds.

The degree of tumor hypoxia can be modified if the animal breathes either normal medical air (21% oxygen) or 100% oxygen. Comparing the rate constants fitted when the animals were breathing air or oxygen, clearly significant differences were found with misonidazole, with slower reduction rates in those animals breathing oxygen as expected (Figure **2a**). However, the TSP probe disappeared at very similar rates in the two different oxygenation states, as expected for a hypoxia insensitive molecule (Figure **2b**).

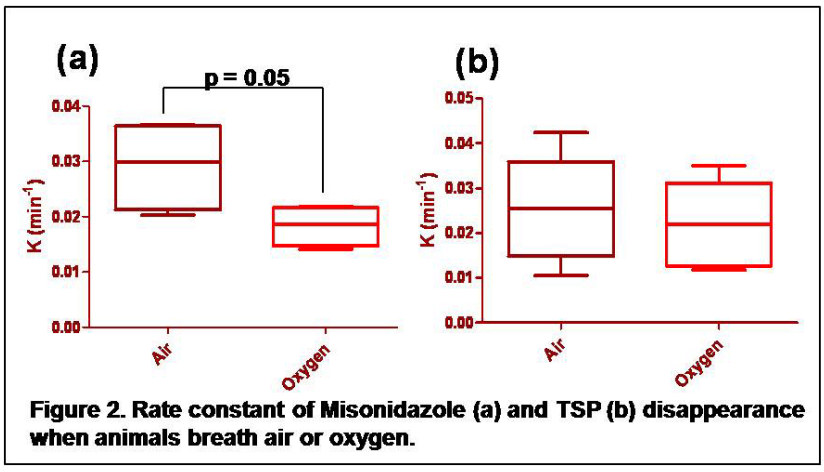

Figure 2. Rate constant of Misonidazole (a) and TSP (b) disappearance when animals breath air or oxygen.

Conclusions: We have monitored by in vivo ^{1}H-MRS the in vivo kinetics of disappearance of the pO_2 sensitive misonidazole probe from a tumor in vivo. We demonstrate that the rate of disappearance of the misonidazole is dependent of the oxygen tension in the tumor, while the time course of the non reducible TSP reference is not. We propose that the methodology implemented here in may be used to evaluate the oxygen tension of tumors in vivo by monitoring the rate of reduction of the oxygen-sensitive nitroimidazolil probes. This approach offers in addition the possibility to combine the pO_2 measurement with other MRI (DCE, ADC, Perfusion…) or MRSI (pHe) imaging methodologies.

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