A NOVEL SERIES OF NITROMIDAZOLE PROBES FOR OXYGEN TENSION (pO_2) MEASUREMENTS IN VITRO BY ^{1}H MAGENTIC RESONANCE SPECTROSCOPY

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Introduction: Hypoxia is known to be an important physiological parameter determining tumour progression and metastatic behavior (1). Consequently, a variety of methods have been proposed to measure tumour oxygenation(2). In particular, several nitroimidazole derivatives (SR-4554, EF5) have been used in combination with either optical methods or nuclear medicine approaches (3). Nitroimidazoles undergo a one-electron reduction catalysed by intracellular reductases, resulting in reactive intermediates which form adducts with cellular components under anaerobic conditions(4). The presence of molecular oxygen at physiological levels hampers the reduction (5). Several enzymatic systems are involved in this process(6) some of which have been used to characterize the reduction mechanism both in vitro and ex vivo (7). In this study we report the use of a novel series of nitroimidazoles for the measurement of oxygen tension in preparations of C6 astrocytoma cells and the enzymatic reduction of these derivatives under normoxic and hypoxic conditions.

Materials and Methods: Nitroimidazolyl derivatives **1** and **2** were synthesized by Michael addition of the corresponding nitromidazol to the appropriate acceptor (Figure 1a). C6 cells were grown to confluence in DMEM medium. At this stage the medium was changed to Krebs Ringer Bicarbonate buffer containing 2.5 mM of the imidazolic for 3 and 6 hours. At the end of the incubation, the cells were harvested and a High Resolution Magic Spinning (HRMAS) ^{1}H NMR spectrum (Figure 1b) taken from the cell pellet. (500.13 MHz, 4^0C, 4000 rpm). The Xantine/Xantine oxidase and NADPH:cytochrome P450 reductase enzymatic systems were used to investigate the *in vitro* reduction of derivatives **1** and **2** under normoxic (21% O_2) or hypoxic (1% O_2) conditions in aqueous media. The UV absorbance peaks of of the nitroimidazolyl compounds (300 – 330nm) were used to follow the time course of reaction and quantify the reduction rate (Figure 1c).

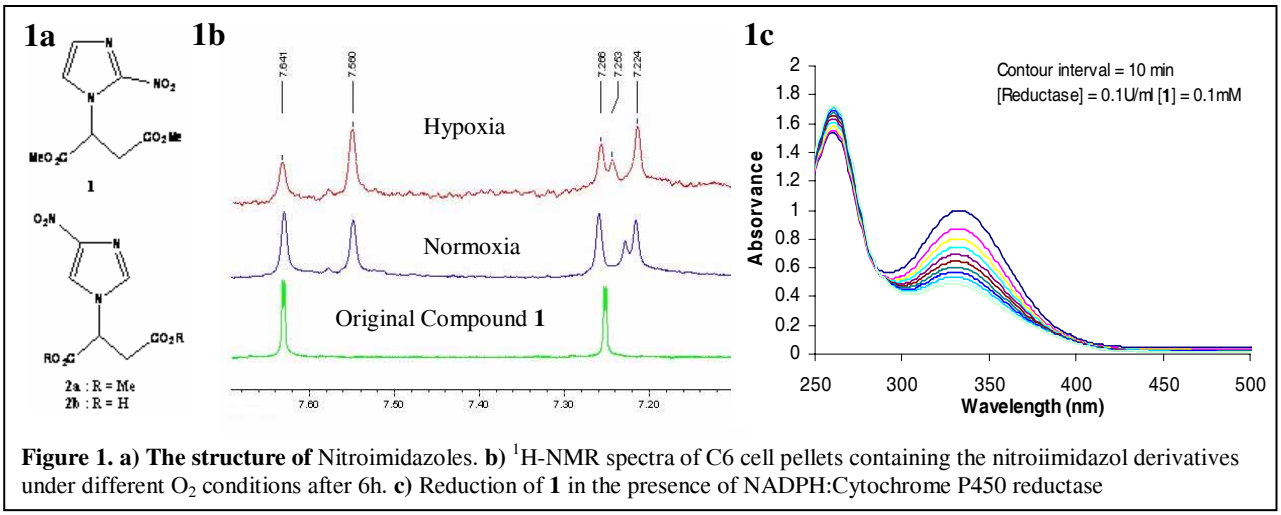

Figure 1. a) The structure of Nitroimidazoles. **b)** ^{1}H-NMR spectra of C6 cell pellets containing the nitroiimidazol derivatives under different O_2 conditions after 6h. **c)** Reduction of **1** in the presence of NADPH:Cytochrome P450 reductase

Results and Discussion: Fig. 1a shows the chemical structure of the nitroimidazol compounds investigated in this study. The ^{1}H HR MAS spectra of the cell pellets (Fig. 1b) shows, in the aromatic region, the H2 (7.64 ppm) and H5 (7.26 ppm) resonances from the diester **1**, as well as the H2 (7.56 ppm) and H5 (7.22 ppm) resonances from the corresponding hemiacid **,** produced by intracellular hydrolysis. Notably, the H2 resonance from the diester in hypoxic cells is significantly smaller than in normoxic cells, revealing its predominant disappearance during hypoxia. Concerning the in vitro red ox properties, we did not find reduction with Xantine/Xantine Oxidase nor with NADPH:cytochrome P450 reductase systems under aerobic conditions. However, significant reduction was observed with the NADPH:cytochrome P450 reductase system under anaerobic conditions (1% O_2). Figure 1c demonstrates this by showing the progressive disappearance of the 330 nm peak from **1** due to its reduction by cytochrome P450 reductase. In contrast, the Xanthine/Xanthine oxidase enzymatic system didn't show any reduction capacity with these probes, under hypoxic conditions.

Conclusions: We report the in vitro properties with C6 cells and the red ox properties of novel family of nitroimidazoles as pO_2 indicators for ^{1}H Magnetic Resonance Spectroscopic Imaging (^{1}H MRSI). The incubation of C6 cells with the diester derivative, resulted in the intracellular production of the corresponding hemiacid. However, the diester showed a faster reduction rate than the hemiacid under hypoxic conditions. Thus, hypoxia results in important spectral changes, suitable for detection by ^{1}H MRSI. Finally, cytochrome P450 reductase appears to be the major enzyme involved in reduction of these nitroimidazolyl derivatives under anaerobic conditions.

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Acknowledgement:

This research was supported in part by grant SAF 2004-03191 to PB and SC. JP received a fellowship from the Community of Madrid.