In vivo $^{19}$F Magnetic Resonance Spectroscopy and Chemical Shift Imaging of Tri-Fluoro-Nitroimidazole as a Potential Hypoxia Reporter in Solid Tumors

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**Abstract**

**Purpose:** 2-Nitro-$\alpha$-[(2,2,2-trifluoroethoxy)methyl]-imidazole-1-ethanol (TF-MISO) was investigated as a potential noninvasive marker of tissue oxygen levels in tumors using $^{19}$F magnetic resonance spectroscopy (MRS) and $^{19}$F chemical shift imaging.

**Experimental Designs:** In vitro data were obtained using high-performance liquid chromatography on tumor cells incubated under varying oxygen conditions to determine the oxygen-binding characteristics. In vivo data were obtained using a well-characterized hypoxic murine breast tumor (MCa), in addition to studies on a rat prostate tumor model (R3327-AT) implanted in nude mice. Detection of intratumor $^{19}$F signal from TF-MISO was done using MRS for up to 10 h following a 75 mg/kg i.v. injection. Localized distribution of the compound in the implanted MCa tumor has been imaged using slice-selective two-dimensional chemical shift imaging 6 h after injection.

**Results:** The in vitro results showed that TF-MISO preferentially accumulates in cells incubated under anoxic conditions. The in vivo $^{19}$F MR spectral features (line width and chemical shift) were recorded as a function of time after injection, and the results indicate that the fluorine atoms are indeed sensitive to changes in the local environment while still providing a detectable MR signal.

**Conclusions:** Based on the results presented herein, it is suggested that TF-MISO has the potential to be a valid magnetic resonance hypoxia imaging reporter for both preclinical hypoxia studies and hypoxia-directed clinical therapy.

Hypoxia is a condition common to many diseases, particularly malignant tumors. Poor blood supply and/or an abnormal vascular architecture can decrease the tissue oxygen tension and render tumors more resistant to radiotherapy and to many kinds of chemotherapy (1). Recent studies aimed at understanding the molecular mechanisms underlying hypoxia have revealed its correlation with genetic instability, poor prognosis, tumor progression, and angiogenesis and have shown how hypoxia is a widely heterogeneous problem (2–8). In many cases, tumors that seem identical by clinical and radiographic criteria can vary greatly in their extent of hypoxia. Therefore, measuring tumor hypoxia in vivo on an individual basis is not only important to further characterize lesions but also essential for developing hypoxia-directed therapeutic approaches. Several techniques have been suggested as diagnostic tools to detect and image hypoxia, both invasively and noninvasively. Some of these methods have undergone preclinical evaluation, although none have entered widespread clinical use (9).

Among the noninvasive hypoxia markers, 2-nitroimidazoles represent a class of compounds that are reduced intracellularly via several enzymatic pathways (10). In the absence of an adequate oxygen supply, these compounds undergo reduction to reactive products that bind to cellular components (11). By monitoring the in vivo uptake, tissue distribution, and retention of these compounds, it is potentially possible to assess the presence of hypoxic tissue. Nitroimidazoles have been used in a wide variety of applications, including immunohistochemical assays, fluorescence microscopy, positron emission tomography (PET; refs. 12, 13), and nuclear magnetic resonance (NMR; refs. 14–25). In the past, Chapman (12) has conducted a thorough biological characterization of several different nitroimidazole compounds. Among the
several compounds investigated, he has identified a fluorinated 2-nitro-α-[2,2,2-trifluoroethoxy)methyl]-imidazole-1-ethanol (TF-MISO), analogue of the more well-characterized fluoromisonidazole (MISO), with physiochemical properties suggestive of a good MR cellular hypoxia probe (26). In this perspective, the most relevant properties of TF-MISO as determined by Chapman et al. are the following. (a) The octanol/water partition coefficient of TF-MISO is larger than most other nitroimidazoles, including MISO (partition coefficient of TF-MISO is ~2.6, whereas partition coefficient of MISO is ~0.43). A higher partition coefficient is associated with higher lipophilicity, which is in turn connected to higher affinity to tissues, higher affinity for metabolizing enzymes and transporter proteins, decreased solubility, and thus more free drug available to target hypoxic tissues. (b) The in vitro nitro-reduction binding rate of TF-MISO to EMT-6 hypoxic cells, which relates to the rate of adduct formation, is similar to those of MISO and therefore has been established as related which relates to the rate of adduct formation, is similar to those of MISO and therefore has been established as related to hypoxia levels in tissue. (c) Finally, measurements of 14C activity recovered from tissue samples excised at different times after administration of labeled compound indicate that TF-MISO is characterized by a plasma half-life of ~23 min and was shown to be rapidly cleared from most tissues, such as blood, muscle, brain, spleen, and kidney (after 2 h, there is a 6-fold decrease of activity), and was retained for longer times in tissues that exhibit a certain degree of hypoxia, such as tumor and liver (at 6 h and later, these tissues exhibit the highest activity, 3-fold higher than other normal tissue; ref. 26).

Based on the above considerations and results reported, we investigated TF-MISO with the aim of (a) examining the in vivo kinetics of TF-MISO by 19F magnetic resonance spectroscopy (MRS) in solid tumors with known levels of hypoxia, (b) showing that the signal is visible for extended periods and therefore associated with intracellular bound and reduced adducts of TF-MISO, and (c) investigating the feasibility of imaging the spatial distribution of TF-MISO inside the tumor within a reasonable time frame following the administration of nontoxic doses.

Materials and Methods

TF-MISO. TF-MISO was obtained from SynChem OHG Laborato-
ries (CAS no. 21787-91-7). The chemical structure is shown in Fig. 2A. The chemical formula is C30H30F3N6O8 and the molecular weight is 629,178. The compound was dissolved in sterile saline solution (5 mg/mL).

In vitro uptake of TF-MISO as a function of concentration and oxygenation status. HCT8 cells (2 × 105) were seeded as monolayers in glass culture vessels (Leighton type culture tubes, Bellco Glass, Inc.) 1 day before the uptake experiments. For the hypoxia experiments, cells were incubated in 0.5 mL HEPES-buffered DMEM/F12 (without sodium bicarbonate, supplemented with 10% fetal bovine serum and penicillin/streptomycin; pH 7.4). Anoxia was induced as described previously (27). Briefly, the tubes were sealed with an open-top screw cap and a butyl rubber septum stopper through which 19-gauge stainless steel needles were inserted for equilibration with humidified sodium bicarbonate, supplemented with 10% fetal bovine serum and penicillin/streptomycin; pH 7.4). Anoxia was induced as described previously (27). Briefly, the tubes were sealed with an open-top screw cap and a butyl rubber septum stopper through which 19-gauge stainless steel needles were inserted for equilibration with humidified

Centrifugation and the TF-MISO content of the supernatant was analyzed by high-performance liquid chromatography using a 19C 3-m Rocket column (7 × 33 mm; Alltech Alltima) eluted with 40:60 acetonitrile + 0.1% trifluoroacetic acid-water + 0.1% trifluoroacetic acid. Detection of the nitroimidazole ring was at 315 nm. From the cell number (determined for each individual sample after collection of the supernatant) and the decrease in the amount of TF-MISO in the medium, the cellular uptake of TF-MISO was calculated and expressed as nmol/10^5 cells.

Cell lines and animal models. All animal studies were done according to institutionally approved protocols. For studies with the MC38 tumor model, the tumors were removed aseptically from tumor-bearing animals by previously described methods (28). Briefly, a single-cell suspension was prepared from a solid tumor by teasing and abrasion against a stainless steel mesh immersed in MEM containing 2% heparin. Cell suspensions were additionally disrupted by aspiration through an 18-gauge needle, and the final suspension was agitated constantly by a magnetic spin bar. A tumor inoculum of 0.025 to 0.04 mL (10^5 cells) was injected s.c. into the dorsum of the foot of male C57/He mice (The Jackson Laboratory) using a 26-gauge needle. Mice bearing MCA tumors were scanned when the tumor size ranged between 200 mm^3 and 400 mm^3. R3327-AT cells were maintained as monolayer cultures in DMEM supplemented with 10% FCS and antibiotics at 37°C in a humidified CO2 incubator. On reaching 75% to 80% confluence, the cells were harvested and 0.1 mL cell suspension (2 × 10^6 cells/mL) was injected in the right hind leg of 6-week-old male athymic nude mice (Charles River). The size of the R3327-AT tumors used for scanning ranged from 80 mm^3 to 300 mm^3. For the 19F MR experiments, two different dose levels of TF-MISO were investigated, 75 and 200 mg/kg, both injected in the tail vein of the mice.

Magnetic resonance studies. All MR experiments were done on a wide horizontal bore (31 cm) small animal imaging 7 T magnet (Bruker). Pulsing and acquisition of the 19F MR signal was achieved using a standard home-built series-parallel radio frequency circuit with a three-turn solenoid coil tuned to 282 MHz and matched to 50 Ω for maximum power transmission and reception. The loaded coil quality factor Q was approximately 80 to 100 during all in vivo experiments. A ParaVision PVM SINGLEPULSE sequence (Bruker) was used for MRS acquisition in vitro, in vivo, and ex vivo. Approximately 30 min after the administration of the drug, the mice were anesthetized with a mixture of isofluorane and compressed air and inserted into a 60 cm^3 syringe barrel used as an animal holder and connected to the animal. This animal holder was covered with copper tape, thus providing a partial Faraday shield and reducing the possibility of MR signals coming from tissues outside the coil. Isofluorane mixed with air (21% O2) was chosen as the anesthetic for this hypoxia investigation (a) to preserve normal respiration conditions (i.e., to maintain the same rate of oxygen intake for the anesthetized animal as the animal awake), allowing measurement of hypoxia at different times after injection of the compound, and (b) based on results of a recent study (29) that showed how tissue partial oxygen pressure measured using polarographic oxygen electrodes (OxyLite System) remains stable in animals anesthetized with isofluorane/air, whereas it fluctuates in animals anesthetized with other types of anesthetic agents, such as ketamine combined with xylazine, phenoperibol, fentanyl, citrate/fluamisone, and midazolam. The leg with the tumor was anesthetized with isoflurane/air, whereas it fluctuates in animals anesthetized with other types of anesthetic agents, such as ketamine combined with xylazine, phenoperibol, fentanyl, citrate/fluamisone, and midazolam. The leg with the tumor was anesthetized with isoflurane/air, whereas it fluctuates in animals anesthetized with other types of anesthetic agents, such as ketamine combined with xylazine, phenoperibol, fentanyl, citrate/fluamisone, and midazolam.
removed from the holder and allowed to rest for 2 to 3 h before being restudied.

In all cases, hydration of the mice was provided prior and after each imaging session by s.c. injection of sterile saline solution (–0.1–0.2 mL). To preserve the core temperature of the mouse throughout the experiments, thus preventing hypothermia and associated effects, temperature-controlled warm air was blown softly on the animal (carefully padded and shielded in the holder) and a rectal temperature sensor was used to assess body temperature and provide feedback to the animal monitoring and temperature control system (model 1025 from SA Instruments, Inc.). Using this system, the temperature was maintained at ~37°C, the normal rectum physiologic temperature. Respiration was monitored via a pressure pillow placed under the body of the mouse also connected to the animal monitoring system. When euthanasia of the animal during the experiment was deemed necessary, an overdose of inhaled anesthetic was used (isofluorane) as recommended by animal protocols and death was ascertained by cessation of respiratory signal.

A glass sphere (18 μL) filled with 75 mmol/L sodium fluoride was used as an external reference for 19F MRS quantification purposes. Gadopentetate dimeglumine (15 mmol/L; Magnevist) was added to the reference solution to enhance relaxation effects (nuclear spin-lattice relaxation time T1 < 0.3 s) and to enable a faster acquisition of the reference signal [i.e., shorter repetition times (TR)].

$$\frac{M(\text{TR})}{M_0} = 1 - \exp(-\text{TR}/T_1)$$  \hspace{1cm} (A)

where M(\text{TR}) is the longitudinal nuclear magnetization at time t = TR, M_0 is the equilibrium nuclear magnetization, and T_1 is the nuclear spin-lattice relaxation time. By doing experiments with different TR values, the longitudinal 19F nuclear magnetization recovery curves of TF-MISO both in vivo and in vitro at room temperature were measured. The T_1 values were then obtained from the data using Eq. A.

The spectral area of the TF-MISO resonance was obtained by integration of the corresponding 1H peak after line broadening (line broadening, 50 Hz) and appropriate baseline correction. For quantification purposes, the resulting value was rescaled to that of the 19F external reference standard of sodium fluoride (resonance at approximately -46 ppm compared with TF-MISO). The intratumor concentration of TF-MISO was estimated from 19F M1 MR area ratios using the measured in vivo longitudinal relaxation rate of TF-MISO (T_1 = 0.37 s) and of the sodium fluoride reference (T_1 = 0.2 s). The method was validated using three 110 μL phantom spheres (Wilmed Glass) filled with 0.5, 1, and 5 mmol/L concentrations of TF-MISO, respectively. It is to be noted that the measurement of the relaxation time T_1 was done in all cases at least 2 h after injection (i.e., when the signal reached a value of relative stability) and did not exceed 30 min. Hence, the contribution from changes in the 19F signal due to changes in local TF-MISO concentration was neglected (estimated maximal contribution of this variable to T_1 values is within experimental error <5%).

Spatial distribution of TF-MISO by combined 1H MRI and 19F chemical shift imaging. The 1H anatomic imaging experiments and the two-dimensional 19F chemical shift imaging (CSI) studies were done using the same animal holder and radiofrequency circuit as used for spectroscopy. When acquiring proton images, the resonant frequency of the circuit was changed from 282 to 300 MHz. 1H anatomic imaging was done using the ParaVision PVM Method Rapid Acquisition with Relaxation Enhancement. The following variables were used: effective echo time, –40 ms (Rapid Acquisition with Relaxation Enhancement factor, 8); TR, 3,000 ms; number of averages, 8; and matrix size, 256 × 256, with a field of view of 2.5 cm × 2.5 cm (spatial resolution, ~100 μm). Slice-selective two-dimensional 19F CSI was implemented using the ParaVision PVM method two-dimensional CSI using a Hanning function to do a k-space weighted acquisition (31, 32). This weighting scheme was used to improve the shape of the spatial response function and the signal to noise ratio of the in vivo data. The following variables were used for the CSI acquisition: a single 60° pulse with TR = 0.8 s and the same geometry as for anatomic imaging was used (i.e., a field of view of 2.5 cm × 2.5 cm and 3-mm slice thickness). The effective number of voxels was chosen to be 8 × 8, yielding an effective voxel size of approximately 3 × 3 × 3 mm³. After applying the Hanning filtering and spatial zero filling up to 16 × 16, we obtain a digital resolution of approximately 1.5 × 1.5 mm². The number of total acquisitions was different for each experiment depending on the dose of TF-MISO and the resulting averaging requirements (~26 min at 200 mg/kg and ~60 min at 75 mg/kg). Chemical shift data processing was done using the CSI Visualization Tool included in the ParaVision software. The relative spectral intensity of TF-MISO over the defined interval was calculated and color encoded for each CSI voxel. Care was taken when analyzing the data to discriminate between the TF-MISO and the isofluorane peak (also visible in the CSI data). The resulting TF-MISO map was then overlaid on top of the anatomic reference image. After spatial zero filling and Fourier transformation, resizing of the CSI voxels was also done via quadratic interpolation using the resolution of the morphologic image (~100 μm). The intensity of the metabolite map was adjusted according to chosen settings with the purpose of overlaying on the morphologic reference only those pixels that have intensities greater than a chosen threshold. The criterion for choosing the threshold values was to obtain a color TF-MISO map with no signal in those voxels that did not include the tumor (i.e., we set the threshold values to completely remove background noise). For the case of the 200 mg/kg data, the signal to noise ratio from the voxels included in the metabolite map was ~2, whereas for the case of the 75 mg/kg the signal to noise ratio was ~1.5. No additional software post-processing of the images or of the metabolite map was involved in the coregistration procedure.

Experimental graphs and data processing. All data were plotted and processed using Origin 7.0 (OriginLab Corp.). Curve fitting was done using the nonlinear least squares fitting capability of the software. Mean quantities are reported with experimental error given by the SD of the data or using standard propagation of error theory when using equations with experimentally derived quantities (33).

Results

In vitro uptake of TF-MISO as a function of concentration and oxygenation status. Figure 1A and B show the amount of cell-associated TF-MISO for cells exposed to different concentrations of TF-MISO as a function of time under normoxic or anoxic conditions. During the first 3 h of incubation, uptake of TF-MISO was observed under all experimental conditions. After this initial (oxygen independent) uptake, TF-MISO levels remained constant or declined slightly in cells incubated under...
normoxic conditions, whereas TF-MISO levels continued to increase in cells incubated under anoxic conditions for up to 24 h (end of experiment). The uptake is higher in cells incubated with 200 μmol/L TF-MISO compared with the cells incubated with the lower concentration (50 μmol/L) of TF-MISO, although the difference between anoxic and normoxic conditions decreases as the concentration of TF-MISO increases. The data clearly indicate that the uptake of TF-MISO is oxygen sensitive, the higher uptake occurring in cells incubated under anoxic conditions.

19F MRS in vivo T1 measurements and in vivo tumor pharmacokinetics of TF-MISO. Shown in Fig. 2A is the in vivo 19F spectrum of TF-MISO for a R3327-AT tumor 3 h following the i.v. administration of 200 mg/kg TF-MISO. The resonance peak in vivo is shown together with the two resonances of the isoflurane anesthesia, CF3 and CF2, respectively, shifted by -6 ppm and -12.5 ppm in respect to the TF-MISO peak. The resonance signal from the sodium fluoride external reference is not shown (shift, approximately -46 ppm). The in vitro spectrum of TF-MISO (data not shown) resonates at the same frequency as in vivo and is characterized by a line width of ~80 Hz (full width at half maximum height). The in vivo 19F TF-MISO resonance was monitored for several hours (>10 h), and its spectral characteristics (full width at half maximum height and chemical shift) were monitored and recorded for each mouse (n = 5) at specific time points. Time point averaging of the mouse data was not done on the spectral data, which is reported without error bars in Fig. 2B. However, for all subjects, we observed a consistent time-dependent (after injection) broadening of the signal with the full width at half maximum height slightly increasing from ~100 Hz to 250 to 300 Hz at later times. This observation suggests that a real-time in vivo binding of the compound is being observed. No detectable change in the chemical shift value was observed throughout the experiment, indicating that the fluorine atoms are sensitive to changes in the biological microenvironment while still approximately preserving the spectral characteristics detectable by MRS.

Figure 3 shows the in vivo and postmortem spectra for the same mouse maintained under physiologic conditions (body temperature at ~37°C) throughout the whole experiment. Spectra were obtained before the mouse being sacrificed (250 min after injection) and after death (40 and 120 min after sacrifice) with the animal kept in the magnet and in the same position. One can observe a decrease in the intensity of the height of the TF-MISO resonance postmortem compared with the in vivo resonance but no significant spectral change is noticed 40 and 120 min after death of the animal. A modest broadening of the peak is observed.

The inset of Fig. 4A shows the nuclear magnetic longitudinal recovery curve plotted as a function of time for TF-MISO.
and for TF-MISO in a phantom (10 mmol/L of parent compound). The in vivo data following a 75 mg/kg i.v. injection were obtained using different mice, for which each data set was fitted using Eq. A. The resulting values for $T_1$ were averaged, yielding $T_1 = 0.37 \pm 0.02$ s ($n = 3$). For comparison purposes, we also obtained the value from a 10 mmol/L TF-MISO phantom: $T_1 \approx 2.5$ s. The clear difference between the two relaxation rates, also evident from the recovery curves, indicates that TF-MISO molecules are located in a restricted tissue environment where the relaxation mechanisms are more efficient than those in a free solution. Because these relaxation mechanisms have many potential sources (i.e., different chemical environments for different TF-MISO by product molecules), one cannot ascribe the differences between the two $T_1$ values entirely to TF-MISO bound to hypoxic tissues, although the measurement is done at a latter time when most free unbound TF-MISO has been washed out of the system.

Figure 4A shows the in vivo kinetic data reported as TF-MISO concentration in tumor tissue at different times after a dose of 75 mg/kg ($n = 11$). Although the signal in the tumor was already detectable after 15 min, we decided to use a temporal resolution of 45 min to obtain a kinetic profile with less scatter (see stacked plot in Fig. 4B). The $^{19}$F MR signal was followed in some cases for up to 10 to 12 h (data not shown; animals were removed from the magnet at various times to limit a single study to $\leq 5$ h), after which the intensity of the resonance decreased in some cases and disappeared in others. One can observe an initial rapid decrease during the first 100 min (the concentration at 60 min is $0.27 \pm 0.17$ mmol/L $\sim 73 \mu$g/mL) followed by a slow decreasing trend, which seems to be nearly constant in the range 100 to 300 min, to a concentration of $0.14 \pm 0.10$ mmol/L ($\sim 40$ $\mu$g/mL).

To analyze the effect of tumor volume on TF-MISO retention, we studied both the MCa foot tumors and a s.c. tumor model R3327-AT implanted on the thigh. The data (MCa and R3327-AT) revealed a correlation between the tumor size and the tumor tissue retention index (see Fig. 5A) defined by Aboagye et al. (18) as the ratio of TF-MISO concentration at 6 h to the TF-MISO concentration at 1 h

$$^{19} \text{FRI}(6h) = \frac{n(6h)}{n(1h)}$$

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It is interesting to note that whereas in general the spectra obtained at early times (1 and 2 h) after injection are characterized by peaks with approximately the same intensity, area, and spectral width regardless of the size of the tumor (see Fig. 5A and C), the later time spectra are significantly different. The larger R3327-AT tumors show retention of TF-MISO, whereas small tumors lose the signal completely.

Spatial tumor distribution of TF-MISO by $^1$H anatomic imaging and slice-selective two-dimensional $^{19}$F CSI. Figure 6A-C show the expanded $^1$H $T_2$-weighted anatomic images for the MCa foot tumor with the corresponding metabolic maps at 1, 3, and 6 h, respectively, following a 200 mg/kg dose of TF-MISO. The overlaid parametric plot was obtained from data acquired using two-dimensional $^{19}$F CSI of Tri-Fluoro-Nitroimidazole (TF-MISO) from R3327-AT tumor of volume $\sim 80$ mm$^3$ (B) and from R3327-AT tumor of volume $\sim 280$ mm$^3$ (C). One notes the disappearance of the signal from the small tumor (B) at $\sim 7$ h.

Fig. 5. A, the retention index $^{19}$FRI is plotted as a function of tumor volume. Black and white circle, R3327-AT ($n = 2$); black circle, MCa ($n = 11$). Stacked $^{19}$F spectra of TF-MISO at different times after i.v. injection of 200 mg/kg of TF-MISO: from R3327-AT tumor of volume $\sim 80$ mm$^3$ (B) and from R3327-AT tumor of volume $\sim 280$ mm$^3$ (C). One notes the disappearance of the signal from the small tumor (B) at $\sim 7$ h.

Discussion

The role of hypoxia in the development and progression of cancer and its importance in the response to therapy is well studied. The advantages of using $^{19}$F MRS and imaging of fluorinated compounds to trace hypoxia in vivo can be associated with the following reasons: (a) $^{19}$F is a NMR "friendly" nucleus (i.e., 100% natural abundance, high NMR sensitivity, and large $\gamma$ value) and therefore is an efficient reporter molecule for in vivo studies; (b) the technique is intrinsically noninvasive; (c) there is no radioactivity involved and therefore the compounds are inherently stable with a long shelf life, allowing studies at later time points after injection when the tumor/blood background is diminished; (d) there is virtually no endogenous background because fluorine is present only in low levels in the bones, which is not readily MR visible; (e) it is possible to obtain high-resolution anatomic $^1$H MR images together with the $^{19}$F images, which provide context...
in terms of organs and tissue heterogeneity, with no need for image after processing and coregistration procedures; and, finally, (f) one can resort to several other MR modalities to investigate tumor microenvironment, such as dynamic contrast-enhanced MRI (to map tumoral perfusion) and diffusion weighted MRI (to analyze microperfusion and diffusion variables), obtaining highly significant multiparametric data as shown recently by Oregioni et al. (34).

Many investigators have extensively reported on the use of fluorine-labeled 2-nitroimidazoles for the study of hypoxia with MRS (14–22), and from this extensive work, some very specific characteristics emerge as most relevant for an ideal 19F MR hypoxia probe: (a) an appropriate redox potential for selective reduction and binding in hypoxic tumor cells, (b) a suitable hydrophilic/hydrogen bonding character in the side chain to limit nervous tissue penetration and prevent neurotoxicity (35–39), and (c) a chemical structure where the fluorine atom (or atoms) is (are) in a metabolically stable location (i.e., a location where any change in the local chemical environment arising from intracellular binding does not influence the 19F MR signal visibility). Among the various nitroimidazoles reported to measure hypoxia with MRS, SR4554 fits this profile very well (18, 19). This molecule was rationally designed to measure hypoxia specifically by magnetic resonance and is currently undergoing phase 1 clinical trials (38). Although the preliminary data with this agent have been promising, a recent study showed no 19F MRS in vivo signal was detected after injection of SR4554 in mice bearing wild-type mutated gliomas with a well-known hypoxic fraction (39). This suggests the need to continue exploring other agents with slightly different characteristics (such as lipophilicity, protein binding, or plasma clearance) to optimize hypoxia detection via MRS in a broad range of tumors. For these reasons and based on previous work by Chapman et al. (26), we selected TF-MISO for this preliminary 19F MRS and CSI in vivo study aimed at establishing its potential as a MR reporter of cellular hypoxia in solid tumors.

Our focus has mainly been concentrated on characterizing the in vivo kinetics of TF-MISO by 19F MRS and on investigating the possibility of imaging the spatial distribution of TF-MISO by CSI. A major issue when addressing the utility of 19F MRS as a hypoxia marker is the nature of the adducts formed by the fluorinated bioreductive probes (40). Previously reported data (26) suggest that the TF-MISO adducts at latter times are present at significantly higher amounts in hypoxic (liver and tumor) versus nonhypoxic...
In our case, one can exclude the contribution to the signal of diffused TF-MISO within necrotic areas. However, we note that the possibility of late time retention of the signal arising from tumor (tumor size) and its degree of hypoxia is also important and will need to be addressed to assess the tumor. The result is highly consistent with the data reported by Chapman et al. (28). The connection between tumor development of conditions for intracellular reduction and binding of TF-MISO and tumor models that are currently being investigated with different techniques to assess and characterize hypoxia (R3327-AT), thus allowing cross-comparison and validation through other established techniques.

The second step was to characterize the spectral features of the TF-MISO resonance by recording the line width of the TF-MISO resonance as a function of time and showing that in all cases one observes a progressive broadening that levels to a maximum of ~300 Hz 2 h after i.v. injection. Because 19F nuclei are an ideal probe of the local chemical environment, we infer by these results that we are observing real-time binding of the compound. The third step was to attempt to observe the spectra of TF-MISO under extreme conditions of hypoxia. Therefore, we recorded postmortem spectra (while preserving the main body temperature at ~37°C) and compared them with the in vivo data. The spectral characteristics and the visibility of the 19F TF-MISO signal even under conditions of extreme hypoxia suggest that we are observing a bioreduced bound form of TF-MISO. One must note that, in similar studies conducted on EF5 (42), the MR 19F signal from bound reduced tracer was not detectable as a consequence of excessive broadening. This may have been due to differences in chemical structure, causing the fluorine atom to be more sensitive to changes in the local chemical environment (i.e., a more severe broadening following intracellular binding). The fourth step consisted in quantifying the in vivo kinetics of TF-MISO. We focused our attention on the time interval corresponding to 6 to 7 h after injection as was done in the case of SR4554, although the signal was usually detectable for longer periods. Loss of the MR signal after very long time intervals is usually explained by noting that the bioreductive adducts observed by MR are small molecules that can be lost from the tissue quite rapidly but still on a time scale much slower than the parent compound (40). Using a phenomenological biexponential model to describe the pharmacokinetic data, we were able to estimate the tumor retention index (54 ± 8%) for the MCA tumor. The result is highly consistent with the data reported by Chapman et al. (26) and with those reported by Mahnood et al. (28). The connection between the stage of development of the tumor (tumor size) and its degree of hypoxia is also important and will need to be addressed to assess the possibility of late time retention of the signal arising from diffused TF-MISO within necrotic areas. However, we note that in our case one cannot exclude the contribution to the signal of TF-MISO trapped in large necrotic areas by pointing out how such areas would be easily visible in the T2-weighted tumor image. This is similarly an important issue also when addressing the observation of nitroimidazoles using PET and pimonidazole staining (43).

After establishing the visibility and source of the 19F signal, this study also showed that real-time imaging of the tumor distribution of TF-MISO is possible using two-dimensional slice-selective CSI with a k-space filtering acquisition modality, which greatly improves the signal to noise ratio and thus the ability to visualize changes in TF-MISO distribution. To this end, it is noted that TF-MISO is a good candidate for real-time imaging studies of hypoxia because the 19F signal is characterized by a in vivo T1 (~0.37 s), which is very short compared with its in vitro value (2.5 s), and therefore the in vivo CSI imaging of the agent can be easily obtained at around 5 to 6 h after injection by averaging the signal with acceptable periods of data acquisition times (~30 min following a dose of 200 mg/kg or ~60 min following a dose of 75 mg/kg). Chapman et al. previously suggested that although this compound may not have advantages as a hypoxic cytotoxin over misonidazole, it had advantages as a NMR imaging agent. It is also noted that several studies have shown that the toxicity of these compounds is dose and intensity (i.e., interval of treatment time) related and that the doses administered to the mice (< 200 mg/kg) in this study are significantly less than those which are considered toxic in patients (cumulative dose of 12 g/m2 equivalent to 4 g/kg in mice; refs. 44, 45).

The feasibility of doing these studies clinically was also considered. If one considers, for example, lymph nodes from head and neck tumors where the issue of tumor hypoxia is under active investigation, the superficial location of these nodes would make it relatively easy to study with high MR sensitivity. Because hypoxia is a microscopic variable, all noninvasive imaging will be imperfect because the ideal goal is to map hypoxia at a resolution of the size of the cell. However, in noninvasive imaging studies, one may be trying to measure global hypoxia. If one assumes a target concentration of 140 μmol/L of TF-MISO (~500 μmol/L 19F), with the high sensitivity of 19F, one would expect that imaging of superficial nodes with a resolution of ~1 cm or less should be feasible. 1H CSI of the brain at 1.5 T has been done with 180 mm3 resolution (46) in 17 min. The 1H visible metabolites are often 1 to 3 mmol/L so one would expect that a resolution of approximately 800 to 900 mm3 in 17 min at 1.5 T is actually achievable because of the high sensitivity of the 19F nuclei. In addition, one would expect better sensitivity for the 19F hypoxia study because head and neck nodes are more superficial than those found inside the brain. A further gain in signal is expected if this were done at 3 T (or even higher field strength given the current availability of high field 7T clinical spectrometers in some institutions). Ultimately, a target resolution of ~500 mm3 (8 × 8 × 8 mm3 voxel) would seem to be feasible in a clinical setting for studying superficial nodes. One could also consider a 34-min acquisition as has been done with 31P MRSI tumor studies (46–48).

A major benefit to the study TF-MISO by 19F NMR is that it can be labeled with 19F also. This will allow direct comparisons between PET and NMR imaging of the same agent. Although PET-MR scanners for clinical use are under development (49), there are none currently being used in the clinics and therefore...
one would still need to do image coregistration for clinical studies; however, one would be assured that the origin of the signal would be identical for both modalities. In preclinical studies wherein one can do combined PET and MR studies either on the same instrument or on the same platform and simultaneously the issue of image registration would be simplified, providing assurance that both modalities were observing the same biological phenomenon.

Further investigation and validation of TF-MISO, which was out of the scope of this initial study, will require the following steps: (a) the in vivo validation of TF-MISO by recording the signal (intensity, line width, and chemical shift) in mice bearing tumors subjected to different levels of oxygen stress; (b) testing in a variety of tumor models (i.e., murine and human cell lines with known different levels of hypoxia), including wild-type gliomas (to establish MR visibility of TF-MISO in cells with known different levels of hypoxia), including testing in a variety of tumor models (i.e., murine and human

1. References

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Catenas G et al. Study of MR interference in a combined small animal PET/MR scanner and in vivo simultaneous imaging, submitted for publication.

References


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