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TITLE: A Novel Approach to Monitoring Prostate Tumor Oxygenation: Proton MRI of the Reporter Molecule Hexamethyldisiloxane

PRINCIPAL INVESTIGATOR: Weina Cui, Ph.D.

CONTRACTING ORGANIZATION: University of Texas Southwestern Medical Center
Dallas, Texas 75390-9105

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A Novel Approach to Monitoring Prostate Tumor Oxygenation: Proton MRI of the Reporter Molecule Hexamethyldisiloxane

Weina Cui, Ph.D.

University of Texas Southwestern Medical Center
Dallas, Texas 75390-9105

E-Mail: weina.cui@utsouthwestern.edu

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

Growing evidence from experimental and clinical studies confirms that solid human tumors have foci of hypoxic cells, which have a profound influence on the therapeutic outcome of cancer chemotherapy and radiotherapy. A strong argument therefore exists for assessing the hypoxic fraction of tumors prior to patient treatment, and to tailor this treatment accordingly. It has been shown that there is a linear relationship between $R_1$ of hexamethyldisiloxane (HMDSO) and $pO_2$, and the $R_1$ of HMDSO is insensitive to various ions and minimally sensitive to temperature. The primary sequence for in vivo T1 measurement with water and fat suppression has been established, which can successfully monitor the global tumor oxygenation responding to respiratory intervention by measuring HMDSO spin-lattice relaxation time using spectroscopy. Although the imaging technique still needs to be optimized in tumor, $pO_2$ maps accompanying respiratory intervention has been obtained in rat muscle. So, Hexamethyldisiloxane shows promise as a reporter molecule to measure tumor oxygenation by $^1$H MRS and potentially by MRI. This opens new opportunities for MR tumor oximetry, particularly since HMDSO is used widely in biomedical materials and as an ingredient in consumer products; and HMDSO is reported to have minimal toxicity.
Introduction:

Growing evidence from experimental and clinical studies confirms that solid human tumors have foci of hypoxic cells, which have a profound influence on the therapeutic outcome of cancer chemotherapy and radiotherapy: the level and severity of hypoxia can be a strong prognostic factor of disease progression and survival. A strong argument therefore exists for assessing the hypoxic fraction of tumors prior to patient treatment, and to tailor this treatment accordingly.

Baseline pO2 and dynamic changes with respiratory intervention has been measured extensively in rat prostate tumors by \(^{19}\text{F} \text{ MRI} \) (FREDOM, Fluorocarbon Relaxometry using Echo planar imaging for Dynamic Oxygen Mapping) based on the spin-lattice relaxation of Hexafluorobenzene (HFB), which provides spatially resolved maps of tumor oxygenation at depth and allows monitoring of dynamic changes at specific locations. However, for clinical application \(^{19}\text{F} \text{ NMR} \) is not yet widely available. A proton MR analog of HFB could facilitate immediate widespread oximetry. We have identified hexamethyldisiloxane (HMDSO) as a potential reporter. HMDSO has extensive symmetry and a single proton resonance well removed from water.

We had three specific aims for this project:

**Task1.** Characterize the effects of external factors on T1 (spin-lattice relaxation time) of HMDSO in vitro and get the calibration curves, Month 1-6

**Task2.** Develop relevant MR pulse sequences with H\(_2\)O suppression for imaging. Establish experimental prostate tumor models and measure the retention time of HMDSO in tumors, Month 6-12

**Task3.** Make pO2 maps and measure prostate tumor oxygen dynamics with respect to growth rate and respiratory challenge, Month 12-24
Body:

Hexamethyldisiloxane (HMDSO) is used widely in biomedical materials and as an ingredient in consumer products, such as a thin polymeric coating on suture for cardiovascular surgery, or the thin layer onto the inner surface of plasma-modified small diameter tubing, etc. HMDSO is reported to have minimal toxicity. A previous test, in vitro indicated that the spin-lattice relaxation time of HMDSO was sensitive to oxygen tension. HMDSO is highly hydrophobic, and therefore can be injected intratumorally at specific sites and will not diffuse in the tumor. Moreover, the boiling point of HMDSO is 99-100°C. In addition, HMDSO has only one ¹H signal and the chemical shift difference between HMDSO and H₂O is about 4.7ppm. Thus, we believe HMDSO may be appropriate for measuring tumor pO₂ by ¹H MRS and MRI. In this proposal, I proposed to develop proton MR techniques using this new reporter molecule, HMDSO, to assess prostate tumor oxygenation in different prostate tumor sublines with several selected levels of histological differentiation.

![Chemical Structure of HMDSO](image)

I completed task 1 and 2 in the first year. For the second year, my statement of work is:

Task 3. Make pO₂ maps and measure prostate tumor oxygen dynamics with respect to growth rate and respiratory challenge, Month 12-24

a. Measure baseline pO₂ of tumors and make the pO₂ map
b. Measure tumor oxygen dynamics with respiratory intervention
c. Excise and process tumor tissue for histology
d. Prepare manuscripts and reports (month 18-24)
Key research accomplishments:

**Task 3a. Measure baseline pO2 of tumors and make the pO2 map (Dr. Vikram Kodibagkar, a MR scientist in the Department of Radiology at UTSW collaborated and assisted me in the task)**

It has been reported last year that the pulse sequence (Fig1) with fat and water suppression developed by Dr. Kodibagkar worked very well with phantom. When it was used in Copenhagen rat thigh, the water and fat suppression are very effective, and baseline pO2 map was obtained (Fig 2). However, when this pulse sequence was used in tumor, water and fat suppression was incomplete; therefore, HMDSO imaging was less satisfactory. This maybe can be ascribed to different physiological characteristics of tumor and muscle, and tumor has more fat.

![Fig 1. Pulse sequence for HMDSO relaxometry with fat and water CHESS suppression. For spectroscopy: both $\pi/2$ and $\pi$ pulses are frequency selective for the HMDSO resonance. For imaging: a frequency selective $\pi/2$ and a slice selective $\pi$ pulse with EPI detection are used.](image)

![Fig 2. (a) T2 weighted spin-echo image of rat thigh showing hyperintense HMDSO (H) (b) comparative proton density weighted EPI image with fat and water suppression. (c) Corresponding pO2 map showing a mean pO2 = 125 ± 20 torr](image)

**Task 3b. Measure tumor oxygen dynamics with respiratory intervention**

I. As a proof or principle, the global oxygen dynamics curve (Fig 3) and pO2 maps (Fig4) responding to respiratory challenge was successfully measured by the developed pulse sequence in rat thigh muscle. During the experiment, the rat was breathing air- oxygen- air.
Fig 3. Rat thigh muscle oxygen dynamics with respect to respiratory intervention (7 individual investigations in 2 rat strains)

Air(35±7torr) 15 min O2(77±8) 30 min O2(134±43) 15 min air(44±15) 30 min air(37±9)

Fig 4. Rat thigh muscle pO₂ map with respect to respiratory intervention

II: We measured the global tumor oxygen dynamics using frequency selective spectroscopy for the HMDSO resonance (Fig1). 120 μl HMDSO was injected intratumorly using 32 gauge Hamilton needle to distribute in the peripheral region of the AT1 tumor. MRI studies were performed on a Varian 4.7 T system. During the experiment, the rat breathed air (15min)-oxygen (30min)- air (20min) (tumor 62R2L2 is exceptional, 10min air-25min oxygen- 15min air). Fig 5 showed the pO₂ value for each tumor with respect to the breathing sequence. Table 1 and Fig 6 showed average pO₂ values for air-oxygen-air.

Fig 5. Tumor oxygen dynamics with respect to respiratory intervention for rat prostate AT1 tumor
Table 1. Global tumor oxygen dynamics with respiratory intervention

<table>
<thead>
<tr>
<th>Tumor size(cm3)</th>
<th>Air</th>
<th>Oxygen</th>
<th>air</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5x1.5x1.0</td>
<td>R1 0.1825</td>
<td>0.1963</td>
<td>0.1879</td>
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<tr>
<td></td>
<td>pO2 36</td>
<td>44</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>SD 10.9</td>
<td>8.8</td>
<td>13.0</td>
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<tr>
<td>1.4x1.7x2.0</td>
<td>R1 0.175</td>
<td>0.2146</td>
<td>0.1814</td>
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<tr>
<td></td>
<td>pO2 32</td>
<td>55</td>
<td>35</td>
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<tr>
<td></td>
<td>SD 17.5</td>
<td>16.7</td>
<td>7.7</td>
</tr>
<tr>
<td>1.5x1.3x1.0</td>
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<tr>
<td></td>
<td>pO2 43</td>
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<tr>
<td></td>
<td>SD 3.7</td>
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<td>34.2</td>
</tr>
<tr>
<td>1.6x1.4x1.3</td>
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<td>0.1889</td>
</tr>
<tr>
<td></td>
<td>pO2 25</td>
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<td></td>
<td>SD 1.2</td>
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<td>4.2</td>
</tr>
<tr>
<td>1.4x1.2x0.8</td>
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<td></td>
<td>pO2 71</td>
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<td>87</td>
</tr>
<tr>
<td></td>
<td>SD 36</td>
<td>29</td>
<td>49</td>
</tr>
</tbody>
</table>

Fig 6. The average pO2 value during respiratory intervention

III. Oximetry based on HMDSO imaging in tumor is still under development. It is more difficult to implement than in muscle. While continuing to tackle this problems, I have explored application of BOLD (Blood Oxygen Level Dependent) imaging in Dunning prostate rat tumor. During the experiment, the rat was breathing 15min air-25min oxygen-20min air. The data were normalized using the initial intensity. Fig 7 shows the signal intensity changed with gas challenge for individual data point. Table 2 and Fig 8 shows average values for air-oxygen-air.
Table 2. Normalized BOLD imaging intensity with respiratory intervention

<table>
<thead>
<tr>
<th>Tumor number</th>
<th>60R1</th>
<th>61R2</th>
<th>60R2L2</th>
<th>60R1L1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor size (cm³)</td>
<td>1.7x1.8x1.6</td>
<td>2.4x2.2x1.8</td>
<td>2.1x1.9x1.6</td>
<td>2.3x2.1x1.9</td>
</tr>
<tr>
<td>Respiratory intervention</td>
<td>The normalized signal intensity average</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Air</th>
<th>Oxygen</th>
<th>Air</th>
<th>Oxygen</th>
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<tr>
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<td>0.994902</td>
<td>1.063712</td>
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</tbody>
</table>

Fig 7. BOLD imaging signal intensity for AT1 tumor

Fig 8. BOLD imaging averaged signal intensity for AT1 tumor

I am continuing analysis particularly to examine original heterogeneity.

Task 3c. Excise and process tumor tissue for histology
The tumor tissues for above experiments were frozen and stored. However, the histology has not been done yet. This task will be finished in 2005 during the approved 1 year no cost extension.
Task 3d. Prepare manuscripts and reports

A manuscript describing measurement of tumor oxygenation with HMDSO as a reporter molecule is under preparation.

Reportable outcomes:
One abstract describing work supported by this grant has been presented as poster at Society for Molecular Imaging, Sep 2004.

A New Frontier for Proton MRI: Quantitative Tissue Oximetry, Vikram D. Kodibagkar, Weina Cui, Ralph P. Mason. SMI, St. Louis, Sep 2004

A second abstract describing work supported by this grant will be presented as poster at the International Society of Magnetic Resonance in Medicine on May 2005.


Conclusions:
The above experiments showed that: 1) As a imaging technique, it has been proved that the chemical shift select imaging of HMDSO with water and fat suppression can successfully measure the oxygen tension in rat muscle and tumor; 2) the developed pulse sequence can successfully monitor the rat thigh muscle oxygen dynamics; 3) HMDSO spin-lattice relaxation time (T1) in tumor responds to respiratory intervention (air-oxygen-air); 4) BOLD imaging signal intensity in tumor also responds to respiratory intervention (air-oxygen-air). So, Hexamethyldisiloxane shows promise as a reporter molecule to measure tumor oxygenation by $^1$H MRS and potentially by MRI. This opens new opportunities for MR tumor oximetry, particularly since HMDSO is used widely in biomedical materials and as an ingredient in consumer products; and HMDSO is reported to have minimal toxicity.
Quantitative Tissue Oximetry using Proton MR of Hexamethyldisiloxane

V. Kodibagkar, W. Cui, M. Merritt and R. P. Mason

Synopsis
There is increasing evidence for the importance of tissue oxygenation in development, progression, and response to cancer therapy. Thus, the opportunity to measure tissue oxygen tension (pO2) non-invasively may be significant in understanding mechanisms of tissue function and in clinical prognosis. The linear dependence of the spin lattice relaxation rate, RI, of the 19F NMR resonances of fluorocarbons on pO2 is well known and has been studied extensively. We recently presented hexamethyldisiloxane (HMDSO) as a potential analogous 1H NMR pO2 reporter molecule. HMDSO has a single proton NMR resonance, which is ideal for imaging. 1H MRI of reporter molecules is subject to potential interference from the large water and fat resonances, but the chemical shift of ~5 ppm relative to water allows chemical shift selective imaging. We now demonstrate application with bulk pO2 measurements and pO2 maps obtained in vivo in rat thighs and tumors following a direct intra tissue injection of 100 μl HMDSO. Dynamic changes in pO2 were assessed with respect to respiratory challenge. Given the minimal toxicity and ready availability of HMDSO, we believe it has great potential for ultimate application as a pO2 reporter molecule in the clinic.
Introduction: There is increasing evidence for the importance of tissue oxygenation in development, progression, and response to cancer therapy. Oxygen is required for efficient function by most tissues and hypoxia leads to rapid cellular dysfunction and damage. In addition, hypoxic tumor cells are refractory to radiotherapy. Thus, the opportunity to measure tissue oxygen tension (pO2) non-invasively may be significant in understanding mechanisms of tissue function and in clinical prognosis. The linear dependence of $R_1$ of fluorocarbon $^{19}$F resonances on pO2 is well known and has been studied extensively. We have previously studied the potential of HMDSO as a $^1$H based pO2 reporter molecule and found the linear dependence of $R_1$ of HMDSO on pO2 ($R_1 = 0.12 + 0.00173*pO2\text{[torr]}$ at 37°C). Here, we study the modulation of tissue oxygenation in response to oxygen challenge in order to further validate the use of HMDSO as a pO2 reporter molecule.

Materials and Methods: A spin-echo EPI based pulse sequence was used for imaging and measuring $T_1$ values using a Varian 4.7 T scanner. The sequence consisted of a) 20 non-selective saturation pulses followed by a delay $\tau$ for magnetization recovery, b) 3 CHESS pulses for selective saturation of water and fat immediately followed by c) spin-echo EPI detection with a slice selective 90° pulse and a frequency selective 180° pulse. $T_1$ maps were obtained using this sequence with the ARDVARC (Alternating Relaxation Delays with Variable Acquisitions for Reduction of Clearance effects) protocol, by varying $\tau$ (3 and half min. per $T_1$ measurement). For comparison, reference images were obtained using a spin echo sequence. $T_1$ and pO2 maps were computed using homebuilt software based on the Matlab programming language.

Results and Discussion:

Modulation of tissue oxygenation in response to oxygen challenge was successfully monitored by bulk spectroscopy and imaging. The short total acquisition time reveals dynamic response to therapeutic interventions. Minimal toxicity and wide availability add to the promise of HMDSO as a pO2 reporter molecule. We believe it has great potential for application in the clinic especially as all the techniques used can be implemented on clinical scanners.

Acknowledgements: This work was supported by DOD DAMD17-03-1-0101, Cancer Imaging Program P20 CA086354 and BTRP P41 RR02584

References
A new frontier for proton MRI: quantitative tissue oximetry

V. Kodibagkar, W. Cui, and R. P. Mason

There is increasing evidence for the importance of tissue oxygenation in development, progression, and response to cancer therapy. Thus, the opportunity to measure tissue oxygen tension (pO₂) non-invasively may be significant in understanding mechanisms of tissue function and in clinical prognosis. The linear dependence of the spin lattice relaxation rate, R₁, of the ¹⁹F NMR resonances of fluorocarbons on pO₂ is well known and has been studied extensively. However, proton MR is far more readily available. We now present hexamethyldisiloxane (HMDSO) as a ¹H NMR based pO₂ reporter molecule. HMDSO has a single proton NMR resonance, which is ideal for imaging. It is highly hydrophobic interacting with dissolved gases, but not metal ions. We have found a linear response: R₁ = 0.13 + 0.00175*pO₂ [torr] at 37 °C and 4.7 T. ¹H MRI of reporter molecules is subject to potential interference from the large water and fat resonances, but the chemical shift of -5 ppm relative to water allows chemical shift selective imaging. We have implemented a spin-echo EPI based pulse sequence for imaging and measuring T₁ values. The sequence comprises a train of 20 non-selective 90° pulses for saturation of signal followed by a delay τ for magnetization recovery. Three CHESS pulses provide frequency selective saturation of water and fat immediately followed by spin-echo EPI detection with a slice selective 90° pulse and a frequency selective 180° pulse. pO₂ maps were obtained in vivo in rat tumors in 21/2 mins following direct intra tumoral injection of 100 µl. Most significantly dynamic changes in pO₂ were assessed with respect to interventions (e.g., respiratory challenge). Given the minimal toxicity and ready availability of HMDSO, we believe it has great potential for ultimate application as a pO₂ reporter molecule in the clinic.

This work was supported by NCI Pre-ICMIC P20 CA086354 and DOD DAMD17-03-1-0101
A New Frontier for Proton MRI: Quantitative Tissue Oximetry

Vikram D. Kodibagkar, Weina Cui, Ralph P. Mason.
Cancer Imaging Program, Department of Radiology.
The University of Texas Southwestern Medical Center at Dallas, TX

Introduction

- There is increasing evidence for the importance of tumor oxygenation in development, progression, and response to therapy.
- Oxygen is required for efficient function by most tissues and hypoxic tumors exhibit rapid cellular dysfunction and damage.
- In addition, hypoxic tumor cells are refractory to radiation therapy.
- Thus, the opportunity to measure tissue oxygen tension (\(pO_2\)) non-invasively may be significant in understanding mechanisms of tissue function and in clinical practice.

MRI relaxometry to study oxygen tension in tissue

- The linear dependence of R1 of fluorocarbon F-NMR resonances on \(pO_2\) is well-known and has been studied extensively (1,2).
- Previous studies in our lab used intracellular injection of the reporter molecule 6-aminofluorene (AFB) (1-3). However, this method uses a spin-echo technique and relies on EPI for detection.
- A Y 3 MRI pO2 reporter molecule would greatly help translation of the MRI oximetry method to clinical studies.

We have now studied the potential of HMDSO as a Y based pO2 reporter molecule.

1H MRI pO2 reporter
Hexamethyldisiloxane (HMDSO)

\[
\text{Si-CH}_3
\]

Figure 1

- Symmetric molecule with a single proton resonance.
- Chemical shift close to TMS (0 ppm) well separated from the water resonance.
- Negligible toxicity.

Linear dependence of R1 of HMDSO on pO2

\[
\text{R1 (sec)} = \frac{10.3}{pO_2 (torr)} + 0.0173
\]

Figure 2

This relationship was derived from T1 weighted spin-echo images of a second phantom containing 50% HMDSO and water in the temperature range 20-40 °C.

In vivo relaxometry by imaging/spectroscopy: pulse sequence

- The spin-echo pulse sequence was modified to inject \(\Delta T\) between the pulse and the refocusing pulse.
- Change in T1 was measured using real-time T1 mapping.
- The entire T1 map was obtained with 14 refocusing pulses.

Results

- Spectroscopy:
  - The spin-echo pulse sequence was modified to inject \(\Delta T\) between the pulse and the refocusing pulse.
  - Change in T1 was measured using real-time T1 mapping.
  - The entire T1 map was obtained with 14 refocusing pulses.

Conclusions

- Hexamethyldisiloxane (HMDSO) is shown to be a promising probe for Y MRI.
- Relaxometry of HMDSO was demonstrated with efficient fat and water suppression using frequency selective saturation and suppression.
- This approach is tailored towards future applications and the signal-to-noise ratio and contrast-to-noise ratio will allow us to monitor dynamic responses to interventions.
- Modulation of Y availability and its potential promise of HMDSO as a Y reporter molecule.

References