In Vivo Mitochondrial Labeling using Mito-Carboxy Proxyl (Mito-CP) Enhanced Magnetic Resonance Imaging

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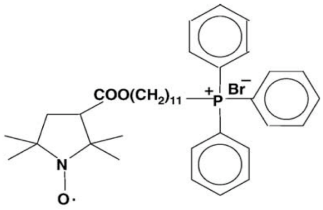
Introduction

Nitroxide radicals, due to a single unpaired electron, exhibit T_1-contrast enhancement. Nitroxides have also been shown to exhibit T_1 contrast enhancement *in vivo* [1]. Recent evidence suggests that Mito-Carboxy Proxyl (Mito-CP), depicted in Figure 1, preferentially targets mitochondria [2]. It is thought that the Mito-CP is taken up by the mitochondria for two reasons [3,4]. First, lipophilic cations, such as the triphenylphosphonium cation, distribute their charge over a large surface area allowing them to easily penetrate the lipid bilayers. Second, uptake of lipophilic ions through the lipid bilayers is increased 10-fold for every 61.5 mV difference in the membrane potential. This would explain the uptake of Mito-CP across the plasma membrane (30-60 mV) and across the mitochondria membrane (150-180 mV). We previously reported [5] Mito-CP relaxivities in solution and demonstrated the ability of Mito-CP to target mitochondria *in vitro*. The purpose of this study was demonstrate feasibility of Mito-CP as an *in vivo* contrast agent. A MR contrast agent specific to mitochondria would provide a marker of metabolic and/or mitotic activity. Pathologies, such as tumors, where metabolic activity is significantly increased would benefit from spatial identification *in vivo*.

Figure 1: Chemical structures of Mito-CP.



Mito-Carboxy Proxyl (Mito-CP)

Materials and Methods

All studies were performed on a Bruker Biospec 30 cm 9.4T using local gradient coils capable of achieving maximum gradient strengths of 40 G/cm per channel. *In Vivo Systemic Concetration*. A male Fisher rat weighing 345g was anesthetized with 1.2 mg/kg in a 20% urethane solution. Using PE50 tubing a femoral vein and arterial catheter was placed. To obtain high pressure the PE50 tubing was advanced as far as possible into the descending aorta. The PE50 tubing was then ran through the X-band electron paramagnetic resonance (EPR) resonator and connected to the venous catheter using a poly vinyl chloride (PVC) couple creating an arteriovenous (AV) shunt. This allowed for the continuous sampling of the *in vivo* Mito-CP blood concentration. Mito-CP was administered via a tail-vein catheter at in three doses, 1, 2, 4 mg/kg. Following each respective injection, EPR spectrums were collect every 86 seconds until the nitroxide could no longer be detected. Rectal temperature was monitored and maintained at 37°C ± 1°C.

In Vivo MRI. A female Fisher rat weighing 162g was anesthetized with 75mg/kg ketamine, 10mg/kg xylazine and 2.5mg/kg acepromazine and inoculated with the Mat B III cell line (ATCC #CRL-1666), a rapidly growing, well vascularized, rat mammary adenocarcinoma. At the time of imaging the rat was anesthetized with 1.2 mg/kg in a 20% urethane solution. Two minutes into a dynamic time series of T1-weighted SPGR images (TE = 3 ms, TR = 78.125, Effective TR = 10 seconds, Repetitions = 150, Slice thickness = 1 mm, flip angle = 30, 8-slices) 4 mg/kg Mito-CP with 1% DMSO vehicle was inject via the tail-vein in 0.25 mL of PBS. Also a pre and post-contrast T1-weighted images were obtained.

Results and Discussion

Depicted in Figure 2 are the concentration time curves obtained at X-band EPR. Fitting these curves to a monoexponential decay following the peak of the curve suggests that Mito-CP has an in vivo half-life of approximately 2 minutes. Results from a monoexponential fit of the dynamic T1-weighted time course revealed decay constants ranging from 100 to 500 seconds. Figure 3 depicts a representative dynamic T1-weighted time course. The duration of the tissue time curve compared to the blood concentration time curve suggests uptake of the contrast agent by the aggressive breast tumor. Further investigation into the distribution, absorption and elimination of Mito-CP in vivo will be pursued. Also, further *in vitro* cell and *in vivo* animal studies will be pursued to validate Mito-CP as a viable *in vivo* contrast agent. This is the first demonstration of the potential for a new MR contrast agent, such as Mito-CP, to specifically target mitochondria in vivo.

References:

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3. Murphy, M.P. et al. Federation of European Biochemical Societies Letters 2004;571:9-16.
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Figure 2: In vivo blood concentration vs time curves of Mito-Cp at various concentrations.

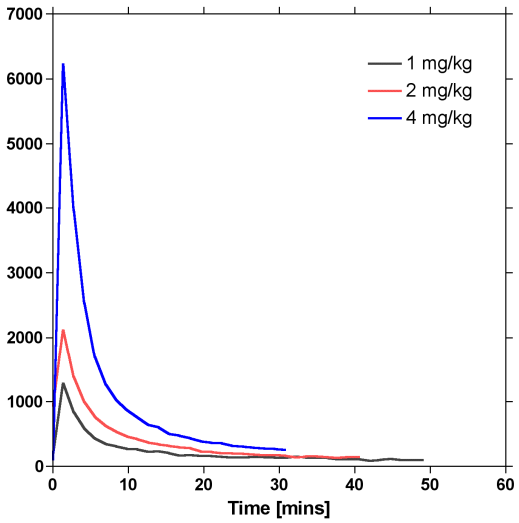

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Figure 3: A representative voxel from the dynamic time series of T1-weighted SPGR images obtained in regions of aggressive breast tumor.

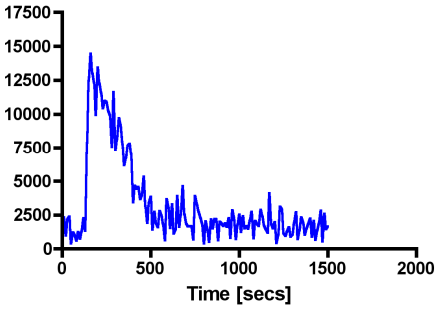

Figure 4: Mito-CP decay map (fit alpha = 0.05).