Functional Imaging using Nitroxides - a feasibility study at 11.7T

K. Shet[1], G. He[2], and J. L. Zweier[2]

[1]Biomedical Engineering, Ohio State University, Columbus, OH, United States, [2]Internal Medicine, Davis Heart and Lung Research Institute, Columbus, OH, United States

Abstract

Contrast enhanced MRI has proven to be an indispensable imaging technique for obtaining anatomical and functional information *in vivo.* High field MRI in the recent years is gaining increased importance in animal research. Presented here is a study conducted at 11.7T to determine the feasibility of using stable nitroxide free radicals to study cardiovascular disease in small animal models; also shown are in-vitro studies aimed at comparison of the contrasts achieved from gadolinium (Gd-DTPA) and two stable nitroxides namely 3- carbamoyl proxyl (3-CP) and AM-proxyl (AMP). It has been shown that 3-CP is less toxic and more stable among the nitroxides and is the probe of interest in this study.

Introduction

3-CP is a nitroxide complex, with a single unpaired electron. It is a commonly used probe in electron paramagnetic resonance imaging (EPRI) and MR based free radical imaging techniques namely proton electron double resonance imaging (PEDRI). It has a single unpaired electron when compared to seven unpaired electrons in case of gadolinium, and therefore the maximum contrast achieved from 3-CP is less than that observed in case of gadolinium. However it has been shown here, that at high field, the increase in signal provided by the nitroxide due to T1 and T2 relaxation effects is significant and this contrast can be utilized in differentiating between normal and diseased tissue.

Materials and methods

In-vitro experiments - Varying concentrations of gadolinium, 3CP and AM proxyl (AMP) were prepared in phosphate buffered saline (PBS). The images of the different probes used are indicated in figure 1(a). The different concentrations used were 1, 2, 3, 4, 5 and 10 mM (moving from left to right in the fig 1(a)). Also shown in fig 1(a) is a test tube containing ordinary PBS for comparison of the NMR signal between saline and the contrast agents. The pulse sequence used was a variable repetition time fast spin echo sequence with an echo time of 12.8ms and a flip angle of 30˚.

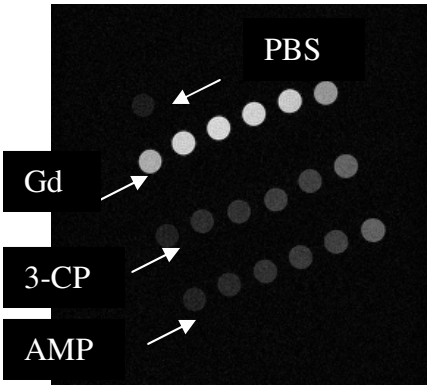

Fig 1(a) Phantom

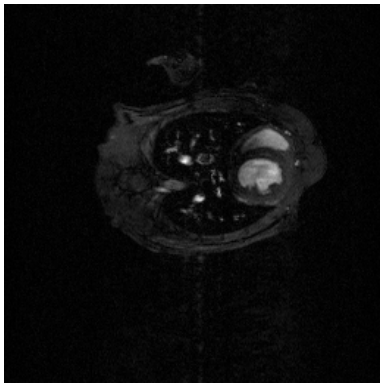

Fig. 1(b) Pre-injection

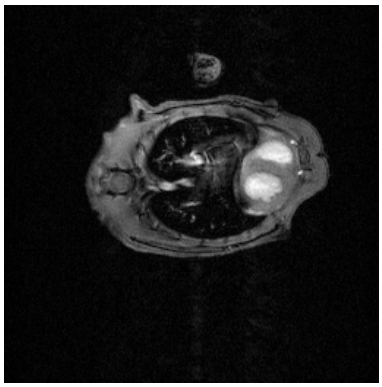

Fig. 1(c) Post injection

In vivo experiment – 3-CP the more stable of the two nitroxides was chosen for study in the mice experiments. The fast spin echo images of an anesthetized mouse, which was treated as a control is shown in fig.1 (b). This was followed by an intraperitoneal injection of 0.5ml of 200mM 3-CP in the mouse. Images were acquired at various intervals after administration of 3-CP; the image acquired approximately 30 min post injection is shown in fig 1 (c). Both *in vivo* images were acquired using ECG gated fast spin echo pulse sequence with an echo time of 1.4 ms, a repetition time of 350ms and a 30˚ flip angle. The recorded signal intensity in different regions of interest (ROI) in the control and the post injection image is shown in table 1.

ROI	Pre-injection	Post –injection
Background	616	853
Blood	17618	27497
Muscle	6122	13936

Table 1. Signal intensities in various ROI's

Discussion

The in-vitro studies clearly show that the contrast provided by gadolinium based compounds is much higher than the nitroxides. However it is also observed that nitroxides at concentrations of 4mM and higher also provide a sufficient T1 and T2 shortening and hence higher NMR signal when compared to PBS. A similar difference in NMR signal level is observed between the pre- and post-injection images in the *in vivo* experiment and the resulting signal intensities in specific ROI's are shown in table 1. A significant increase in signal intensity is observed after injection of 3-CP and this could be attributed to the T1 and T2 shortening effects produced by the nitroxides. In fig 1(b) and 1(c) the artifact that is observed maybe attributed to motion of the subject.

Conclusion

The above-mentioned contrast can be used to distinguish tissues that retain the nitroxide after administration from those that reduce the nitroxides into an undetectable form. A specific application for the use of nitroxides is their use in studying cardiovascular disease, namely cardiac ischemia and myocardial infarction. Reactive oxygen species (ROS) are induced in the cardiac muscle that has been subjected to short period of ischemia. The ROS thus produced oxidize the injected nitroxides to oxoammonium cations [1], which unlike the nitroxides do not provide any T1 or T2 relaxation effects. The use of nitroxides in such a study will help to localize and assess the ischemic region non-invasively. Therefore nitroxide based imaging provides a functional imaging tool to study cardiac disease in small animal models.

References

1. Goldstein S. *et al.* Chem. Res. Toxicol. 2004, 17, 250-257.