In Vivo Imaging of Spin-Tapped Nitric Oxide (NO) in Septic-Shock Rats: MRI Spin-Trapping Method

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Purpose

The purpose of this study was to demonstrate the first in vivo NMR images of the distribution of NO using MRI spin-trapping technique, MRI combined with EPR spin-trapping method.

Introduction

Nitric oxide (NO) was first appreciated as a biological mediator in its role as endothelial derived relaxing factor, which is responsible for regulation of blood vessel relaxation and its maintenance [1]. It also became evident that NO was produced in the brain, catalyzed by a nitric oxide synthase (NOS), where NO acts as a synaptic neuronal messenger [2]. NO is a gaseous and highly reactive, short lived free radical. Therefore, direct detection by stabilizing NO with suitable spin-trapping reagents is requisite to estimating the in vivo NO concentration. We have previously employed iron-dithiocarbamate spin-trapping reagents and have succeeded in direct detection of NO in septic-shock mice by whole body L-band EPR spectroscopy [3]. The in vivo EPR and EPR imaging results reveal that NO is produced mainly in the upper abdomen near the liver [3,4]. While we and other groups have demonstrated the feasibility of EPR imaging in visualizing free radical distribution in vivo, the spatial resolution of most EPR imaging system is not satisfactory enough to resolve much fine structure for specific organs by visualizing the distribution of free radicals, especially where the intrinsic linewidth of the radical is large, such as the spin-trapped NO [4]. In the present study, we employed MRI combined with NO spin-trapping reagent to evaluate the feasibility of mapping the distribution of this NO spin-trap complex in septic shock rats.

Methods

The spin-trap solution was prepared by mixing a deoxygenated solution of N-methyl-D-glucamine (MGD:100 mM) and $FeSO_4$ (20 mM) under nitrogen [3]. Septic-shock mice or rats were prepared by injection intraperitoneally with lipopolysaccharide (LPS) at dose of 50 mg/kg. 6 hour after LPS injection to mice or rats, NO spin-trap reagents, $(MGD)_2$-Fe(II), were injected to mice or rats intraperitoneally. EPR spectra of spin-trapped NO in mice were measured by L-band in vivo EPR spectrometer [5], and MR images of rats were obtained with a Signa Horizon 1.5 T scanner, GE medical Systems.

Results

At 6 h after LPS administration to mice, the mice were subcutaneously injected with spin trap, $(MGD)_2$-Fe(II) followed by L-band EPR measurement. The maximum EPR signal was detected near the upper abdomen due to $(MGD)_2$-Fe(II)-NO with the hyperfine coupling constant, a_N=12.5 gauss. In vitro X-band EPR spectra showed that the NO complex levels in the liver and kidney were 89 ± 5.9 and 17.3 ± 3.9 nmol/g, respectively. The NO molecules detected by our method were inhibited by NOS inhibitor, N-monomethyl L-arginine (L-NMMA), suggesting that NO observed in this study was generated enzymatically by NOS.

Next, we injected rats with LPS and carried out subsequent MRI detection and visualization of NO complex in septic-shock rats. Figure 1 A showed MR image of septic-shock rat after administration of spin trap, showing that the liver was clearly distinct from other organs. Figure 1 B showed EPR image obtained from the liver of septic-shock mice.

A similar MRI experiment was carried out in the presence of NOS inhibitor, L-NMMA. The obtained MR image showed no image contrast enhancement as a result of inhibiting NO generation by L-NMMA. This confirms tha MRI contrast enhancement originates from proton relaxation mechanism between water and $(MGD)_2$-Fe(II)-NO.

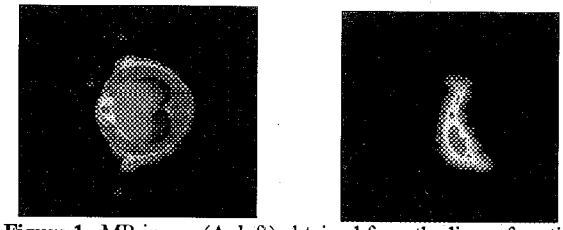

Figure 1. MR image (A: left) obtained from the liver of septic-shock rats and EPR image (B: right) from the liver of septic-shock mice.

Discussion

In this study, we found that the spin-trapped adduct, $(MGD)_2$-Fe(II)-NO, functioned amazingly well as a NMR contrast agent, allowing one to accurately map NO distribution in vivo. MR images of the liver obtained from the septic-shock rats clearly demonstrated the site of NO generation, especially where the vascular structure of the liver was enhanced remarkably.

In conclusion, employing MRI spin-trapping method, we have successfully visualized and mapped the site of NO generation in septic-shock rats by MRI.

References

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