Nitrosyl-iron Complexes as Potent Smart Nitric Oxide Biosensors: MRI imaging Techniques in Progress

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ABSTRACT

Presently, EPR and fluorescent biosensors are sole choice of in vivo bioimaging of NO and accurate measurement still remains a problem. We propose different NO biosensors as potent imaging contrast agents using multimodal bioimaging techniques of MRI, EPR and fluorescent imaging with their limitations and quantitative limits. Our focus in this paper is on different characteristics of known biosensors and nitrosyl-iron complexes as MRI signal intensity enhancers and NO visualization by magnetic resonance imaging (MRI) technique. The paramagnetic NO-Fe-DTC metal complex used as contrast agents in MRI enhances the relaxation of neighboring protons that visualize the NO generated in living animals. Other natural contrast effect is imparted by NO exposure to hemoglobin during MRI signal recording and it serves as source of in vitro MRI and in vivo functional MRI. The functional MRI signal intensity of venous blood in T1-, T2-, and T2*-weighted images proportionately changes with NO. Different approaches of blood hemoglobin and NO interaction appear to monitor fMRI signal. Mainly, metHb and NO-Hb enhanced the signal intensity. These observations suggest a blood flow-independent effect. Other approaches are emerging to get biosensors for multimodal imaging. These new approaches open a perspective on the bioimaging of NO and the in vivo elucidation of NO effects by magnetic resonance techniques.

Key words: *nitric oxide, MRI, NO-iron complex, biosensor, EPR, MRI, multimodal NO imaging*

1. INTRODUCTION

In vivo imaging of NO as biosensor is an emerging art by EPR, fluoroscopy and MRI. The success basically depends on visualizing free radical distribution of in vivo spin-trapped NO. The bioimaging of nitric oxide (NO) imaging techniques utilize mainly magnetic resonance (MR), electron paramagnetic resonance (EPR) spectrometry and fluorometry. NO is a stable free radical containing one unpaired electron derived from L-arginine. The in situ visualization of NO using bioimaging techniques speculates the production and diffusion processes of NO. Other bioimaging techniques are chemiluminescence, fluorometry, EPR spectrometry, and electrochemical methods feasible to detect NO *in vivo* or *in situ*. Recently, real-time bioimaging techniques emerged as EPR, fluorescent indicators, chemiluminescence. Bioimaging of NO is done by electrochemical, Electron Paramagnetic Resonance (EPR) Spectrometry and fluorometry methods using nitroyl-complexes as shown in Table 1.

1.1 EPR Imaging of NO: EPR spectrometers operating at S-band (1.6-4 GHz) and L-band (0.4-1.6 GHz) microwave frequency and at radio frequency (0.2-0.4 GHz) are utilized for in vivo measurements of the whole body of small animals. The electronic configuration of NO, with 11 valence electrons as exemplified in Figure 1, is $(K^2K^2)(2s\sigma^b)^2(2s\sigma^*)^2(2p\pi^b)^4(2p\sigma^b)^2(2p\pi^*)^1$. Thus, NO is a free radical with one unpaired electron in the antibonding πorbital. Therefore, EPR is considered to be the most appropriate tool for its detection. The electronic ground state of NO is expressed by the term symbol, $^2\Pi_{1/2,3/2}$. The NO imaging is pewrformed by spin-trapping technique.

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Table 1: Various complexes as nitric oxide biosensors are shown used as spin trap, chemosensitizer or fluorescent compounds with potential as EPR, MRI or fluorometry.

Complexes bound with NO	NO biomaging contrast applications and limitations
(N-methyl-Dglucamine)2-Fe(II)-NO complex	EPR low contrast and MRI high contrast
5-diethoxyphosphoryl-5-methyl-1-pyrroline-N-oxide	NMR imaging with good possibility
5,5-dimethyl-1-pyrroline N-oxide (DMPO),	EPR spin trap with possibility of EPR imaging
5-diethoxyphosphoryl-5-methyl-1-pyrroline-N-oxide	EPR spin trap with possibility of EPR imaging
α-phenyl-N-tert-butylnitrone (PBN),	EPR spin trap with possibility of EPR imaging
α-(4-pyridyl-1-oxide)-N-tert-butylnitrone	EPR spin trap with possibility of EPR imaging
nitroso traps: 2-methyl-2-nitrosopropane (MNP)	EPR spin trap with possibility of EPR imaging
3,5-dibromo-4-nitrosobenzenesulfonic acid	EPR spin trap with possibility of EPR imaging
3-carbamoyl-2,2,5,5-tetramethylpyrrolidine-1-yloxyl	EPR spin trap with possibility of EPR imaging
dithiocarbamate derivatives (Fe-DTCs)	EPR spin trap with possibility of EPR imaging
pyrrolidine dithiocarbamate (PDTC);	EPR spin trap with possibility of EPR & MRI
N-methyl-D-glucamine dithiocarbamate (MGD);	EPR spin trap with possibility of EPR imaging
N-(dithiocarboxy)sarcosine (DTCS);	EPR spin trap with possibility of EPR imaging
N-methyl-L-serine dithiocarbamate (MSD);	EPR spin trap with possibility of EPR imaging
L-proline dithiocarbamate (ProDTC);	EPR bioimaging
disulfiram (disulfide of DETC);	EPR bioimaging
diglutathionyl dinitrosyl iron complex, [DNIC-$(GS)_2$].	EPR bioimaging
Fe(III)$(DTCS)_3$ and NO-Fe(II)$(MGD)_2$.	MRI, EPR, chemilumeniscence bioimaging
Fe-DETC trap	EPR imaging of cultured alveolar cell
[^{14}N]ISDN or [^{15}N]ISDN	EPR-CT bioimaging
Dinitrosyl Dithiolato Iron Complex	EPR-CT bioimaging
NO-Fe$(DTC)_2$	EPR-CT bioimaging
(MGD)2-Fe(II)-NO complex	MRI, NMR, EPR bioimaging
Diaminonaphthalene: DAN	Fluorescent biosensor
Dichlorofluorescin: DCFH	Fluorescent biosensor
iron(II)-quinoline pendant cyclam	Heme Fluorescent Reporter biosensor
Co Complex: [Co$(NO)_2$(^{R}DATI)]	Fluorescent biosensor
Cheletropic Traps: FNOCTs	ESR, Fluorophobic bioimaging
Diaminofluoresceins: DAFs	Fluorometry
Diaminorhodamines: DARs	Fluorometry

1.3 In Vivo EPR Detection and Imaging of Free Radicals: EPR spectrometers operating at lower frequency are now applied to in vivo measurements of the whole body of small animals. In vivo EPR imaging experiments using TEMPOL, 3-carbamoyl-2,2,5,5-tetramethylpyrrolidine-1-yloxyl (carbamoyl-PROXYL), the hydroxyethyl radical is produced by ionizing radiation (3000 Gy) in the tumor of a living mouse. In vivo detection of hydroxyl radical using DEPMPO spin trap in mice, iron complexes with dithiocarbamate derivatives (Fe-DTCs) were used as spin traps NO adduct [NO-Fe$(DTC)_2$]

2.NO-SPECIFIC TRAPPING REAGENTS

NO radical more than EPR detection limit (0.1-0.01 μM) can be detected by *nitrone traps*: 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO), 5-diethoxyphosphoryl-5-methyl-1-pyrroline-*N*-oxide (DEPMPO), α-phenyl-*N*-*tert*-butylnitrone (PBN), and α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (POBN); and *nitroso traps*: 2-methyl-2-nitrosopropane (MNP) and 3,5-dibromo-4-nitrosobenzenesulfonic acid (DBNBS). Conventional nitrone (DMPO) and nitroso (MNP, DBNBS) spin traps; NO cheletropic trap (NOCT); *o*-quinodimethane; 2-Phenyl-4,4,5,5-tetramethylimidazoline-1-yloxyl-3-oxide (PTIO); Ferrous iron complexes such as Hbs and Fe-DTC complexes; Fe(III) hemoproteins and porphyrin complexes. Cytochromes *c′* bioprobes for NO. Pyrrolidine dithiocarbamate (PDTC); *N*-methyl-D-glucamine dithiocarbamate (MGD); *N*-(dithiocarboxy)sarcosine (DTCS); *N*-methyl-L-serine dithiocarbamate (MSD); L-proline dithiocarbamate (ProDTC); disulfiram (disulfide of DETC); *N*,*N*-diethyldithiocarbamate (DETC); diglutathionyl dinitrosyl iron complex, [DNIC-$(GS)_2$].

2.1 Multimodal EPR-CT imaging by Nitrosyl Iron-Dithiocarbamate Complexes: Instrumentation and Imaging Techniques for *In Vivo* EPR Measurements: The three-dimensional EPR image (i.e., EPR-CT) is constructed by three-dimensional zeugmatography along the *X*-, *Y*-, and *Z*-axes produced by magnetic field gradient coils. A pair of magnetic field gradient coils for the *X*-, *Y*-, and *Z*-axes attached to the surface of the main magnet to obtain one set of EPR-CT images.

2.2 *In Vivo* EPR Detection of Endogenous NO: In vivo real-time detection of NO Fe-MGD trap, Fe-$(DETC)_2$ trap with an L-band (1.14 GHz) EPR spectrometer in ischemia-hypoxia. EPR images from the frozen resected brain were obtained by employing an Fe-DETC trap and an EPR imaging system with a microwave frequency of 1.2 GHz. [^{14}N]ISDN or [^{15}N]ISDN for EPR-CT images in the *z-x* plane in abdomen and liver. Other examples are Fe-MGD,RSNO,NO-Fe$(DTC)_2$.

3. APPROACHES TO NO EVALUATION BY MAGNETIC RESONANCE IMAGING (MRI) TECHNIQUES:

Recently, EPR- NMR techniques of proton-electron-double-resonance-imaging (PEDRI) showed enhancement of proton NMR signal intensity in the presence of radicals through the Overhauser effect or relaxation of neighboring protons such as nitrosyl iron complex may be potentially useful as a functional MRI contrast agent specific for NO in living organisms. Other use of NO exposure to hemoglobin is capture fMRI BOLD signal perhaps hyperintensities on T1-, T2-, and T2*-weighted images due to addition of aqueous NO, nitrite, or dithionite and nitrite to the blood i.e.met Hb and NO-Hb. L-arginine increased the cerebral blood volume in hypertensive rats while ISDN increased both tumor blood flow on the NO images by magnetic resonance techniques.

3.1 Multimodal in Vivo NO spin-trapping MRI –EPR experiments: In Vivo MRI imaging of Fe(II)-chelate spin-trapped nitric oxide by N-methyl-D-glucamine dithiocarbamate (MGD)-NO maping reveals radical distribution to localize nitric oxide in liver. Synthase (iNOS) is main the source of NO. At optimal concentration of (MGD)2-Fe(II) [MGD:100 mM, Fe:20 mM], MR images on a GE 2-T CSI and IBM PC20 MiniSpect measured millimolar relaxivity of (MGD)2-Fe(II)-NO at parameters of TR 500 msec, TE 10 msec, NEX 2, 4-mm slice thickness, 1-mm slice gap, field of view 12 x 3 x 12 cm, and matrix, 256 x 256. For EPR imaging, 20 MHz Jeol JES-FG2XG EPR spectrometer: microwave frequency, 9.4 GHz; incident microwave power, 20 mW; 100-kHz modulation amplitude, 2 G; sweep width, 100 G; scan time, 2 min. The NO complex acts a very effective ‘‘intrinsic contrast agent,’’ enhancing contrast in the images of several organs. The MRI demonstrates NO complex as a potentially useful *NO-specific* contrast agent. Why NO is so important in bioimaging? NO is a signal transmitter in the vascular endothelium, central and peripheral neurons in vivo. Mapping the site of NO generation is possible by L-band EPR, combined with MRI spin trapping, for the direct detection of NO radicals in vivo. Here we propose multimodal MRI-EPR-flurometry approach to map NO radicals within tissues and organs at much higher spatial resolution. The spin-trapped adduct,(MGD)2-Fe(II)-NO, a NMR contrast agent to give much higher spatial resolution than with EPR. NO is known to bind to iron compounds to form a generally stable complex such as (MGD)2-Fe(II)-NO. In vivo, hemoglobin is normally the natural NO spin-trap such that NO tends to bind to or oxidize hemoglobin, followed by conversion to nitrosyl-hemoglobin or methemoglobin, both of which are paramagnetic.The nitrosyl-hemoglobin and methemoglobin are effective functional MRI (fMRI) means of spin-trapped NO using 5-diethoxyphosphoryl-5-methyl-1-pyrroline-*N*-oxide (DEPMPO).

4. FLUOROMETRIC IMAGING OF NO

4.1 Fluorescent Probes for NO: Diaminonaphthalene (DAN) is good choice. NO readily decomposes to NO_2^- as a final product in the presence of O_2. The fluorometric assay for quantification of NO_2^- /NO_3^- up to 10 nM excited at 375 nm and emitted at 415 nm, is based upon the reaction of NO_2^- with 2,3-diaminonaphthalene (DAN) to form the fluorescent product 1-(*H*)-naphthotriazole (NAT). The method can serve as a tool for defining the role of NOS in both normal and pathophysiological processes. However, the method cannot be adapted for NO bioimaging due to irradiation serious damage to living cells. Dichlorofluorescin: DCFH 2,7-dichlorofluorescin (DCFH) for monitoring intracellular NO formed in neuronal cells but unsuitable for bioimaging. DCFH, a nonfluorescent species, is oxidized by NO to dichlorofluorescein. Iron Complexes: The iron(II)-quinoline pendant cyclam is a fluorescent probe for NO not convenient for NO detection in biological systems. 2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPO) labeled with acridine and Fe(II)$(DTCS)_2$ complex can be used for monitoring direct production of NO in biological systems not yet been applied for bioimaging. Heme Domain with Fluorescent Reporter Dye: Cytochrome *c′*

labeled with a fluorescent reporter dye with fluorescent microspheres serve as ratiometric sensor of intracellular macrophage NO levels in phagocytosis. NO-selective sensors with the heme domain guanylate cyclase (sGC) labeled with a fluorescent reporter dye. NO formation from NOS in endothelial cells needs detection limit of 8 μM NO. Co Complex: $[Co(NO)_2(^{R}DATI)]$: Aminotroponiminates ($H^{R}ATIs$) with a dansyl fluorophore serve as fluorescent NO biosensor. Paramagnetic Co^{2+} complexes quench the fluorescence. The $[Co(NO)_2(^{R}DATI)]$ increases fluorescence intensity, ideal for fluorescent NO sensing but not for bioimaging. Cheletropic Traps: FNOCTs:FNOCTs react with NO in a formal cheletropic reaction detect NO detection in alveolar macrophages.

4.2 Development of NO Bioimaging Probes: Diaminofluoresceins (DAFs) are as novel probes for NO. DAFs to triazole forms (DAF-Ts) with NO change of the absorbance maxima of fluorescence intensity due to the conversion of DAF-2 to DAF-2 T by NO in the presence of O_2. (DAF-4 M1, 4 M2, 5 M1 and 5 M2) fluorinated fluorescein derivatives. Diaminorhodamines (DARs): The fluorescent Rhodamine B fluorophore imaging with DAR-1 AM, DAR-1 EE, DAR-M, DAR-M AM, DAR-4M was little successful.

4.3 Biological Applications of DAFs and DARs: In Endothelial Cells, DAF-FM, endothelial NOS, DAR-4M are useful for bioimaging of samples that have strong autofluorescence. In Smooth Muscle Cells, DAF-2 DA and DAF-FM T enhance fluorescence intensity. In Brain, DAF-2 DA, DAF-FM DA was also applied to imaging of NO generated in rat hippocampal slices. Some ion Channels voltage-gated Na^{+} channels, NMDA receptor (NMDAR)-associated ion channel, DAF-2 DA are biosensor to hypoxia.

5. CONCLUSION: We propose bioimaging of NO by multimodal in vivo EPR/MRI/fluorometry and potential NO biosensors. Fluorescent indicators examine the production of intracellular NO such as DAFs and DARs. These indicators are a useful tool for visualizing the temporal and spatial distribution of intracellular NO. In vivo real-time imaging of NO is possible by EPR and NMR techniques and fMRI. DAFs and DARs are good for bioimaging of NO. Further studies on the development of novel ratiometric NO bioimaging probes are at large.

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