Development and evaluation of injectable spin traps for the detection of nitric oxide

N. Charlier[1], V. Préat[2], B. Gallez[1]

[1]Biomedical Magnetic Resonance Unit, Catholic University of Louvain, Brussels, B, Belgium, [2]Pharmaceutical Technology Unit, Catholic University of Louvain, Brussels, B, Belgium

Introduction

Nitric Oxide (NO) is known to be a very important messenger and regulator in many physiological pathways as well as pathological states. NO can be detected and quantified *in vivo* by EPR using the so called "spin-trapping" technique. As a spin trap, DETC-Fe(II) is commonly used for *in vitro* experiments on cell cultures or isolated vessels (1). It can also be used *in vivo* but, it has to be administered in two different sites (DETC by IP and iron by SC) because of the insolubility of the complex (2). Therefore, the achievement of a reproducible concentration of spin trap at a given time in a given tissue is questionable. The aim of this study is to develop new systems carrying this spin trap that is compatible with an IV injection. Two different systems were investigated: a nano-dispersed system including the DETC-Fe(II) that is stabilized by lecithin, and a solution containing β-cyclodextrin and DETC-Fe(II).

Material and methods

Two formulations were developed. The first one is a nano-emulsion using L-α-phosphatidylcholine (lecithin) as surfactant. This system was obtained by mixing lecithin (18 % W/V), DETC (62.5 mMol) and Fe(II) (12.5 mMol), witch was followed by ultra-sonication. The second system is composed by β-cyclodextrin (35 % W/V), DETC and Fe(II) at 62.5 mMol and 12.5 mMol, respectively. The efficiency of spin trapping was compared for both systems with the aqueous soluble spin trap MGD-Fe(II). *In vitro* spin trapping experiments were carried out at 310 K using DEA NONOate as a NO donor. The amount of NO was measured in a model of septic shock after administration of LPS to NMRI male mice. Blood samples and liver samples were put in liquid nitrogen and the EPR signal was recorded using a 9 GHz EPR spectrometer.

Results

In vitro spin trapping experiment showed that the DETC-Fe(II) complex with lecithin or cyclodextrin has a greater ability than MGD-Fe(II) to trap nitric oxide in an aqueous medium (Fig. 1). Blood kinetics showed that C^{max} in trapped NO is around 30 minutes post-injection for both formulations (Fig. 2). We found that the nano-dispersed system stabilized with lecithin had a higher ability to detect NO in the liver than MGD-Fe(II) and the solution containing β-cyclodextrin (Fig. 3.), although the detection in blood was more sensitive using β-cyclodextrin.

Discussion

New formulations based on DETC-Fe(II) were developed for *in vivo* spin trapping of nitric oxide. *In vitro* spin trapping experiments showed us the ability of the DETC-Fe(II) complex with lecithin or β-cyclodextrin to realize spin trapping in an aqueous medium. Experiments carried out *ex vivo* indicate different efficiency in spin trapping depending on the chemical form used or the organ investigated. Investigations are in progress to assess the optimal time-window for detecting NO in different organ for both systems.

References

1. Spin trapping of vascular nitric oxide using colloid Fe(II)-diethyldithiocarbamate. Kleshyov A. L., Mollnau H., Oelze M., Meinertz T., Huang Y., Harrison D. G., Munzel T. *Biochem Biophys Res Commun*. 2000 Aug 28 ; 275(2) : 672-677

2. Simultaneous measurement of NO and oxygen from tissue by *in vivo* EPR. James P. E., Miyake M., Swartz H. M. *Nitric oxide*. 1999 Aug ; 3 (4) : 292-301

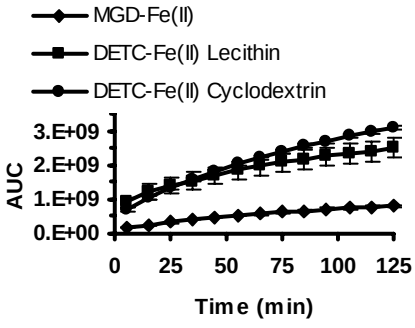

Fig. 1. In vitro spin trapping of NO produced by DEA NONOate. AUC were obtained from DETC-Fe(II) complexes with lecithin (■) or β-cyclodextrin (●) and from MGD-Fe(II) complexes (♦).

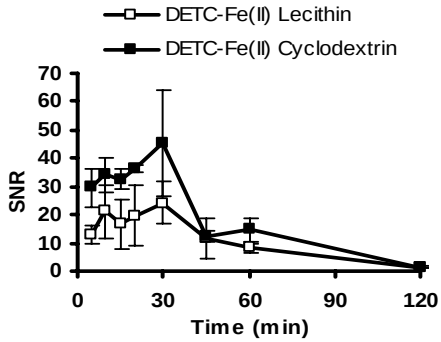

Fig. 2. Blood spin trapping kinetics of NO during septic shock. SNR were measured in liquid nitrogen for different times after injection of DETC-Fe(II) with lecithin (□) or β-cyclodextrin (■).

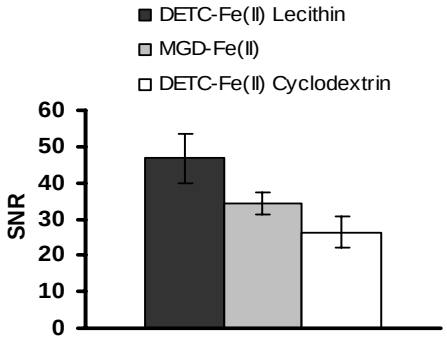

Fig.3. Ex vivo spin trapping of NO produced in livers of mice 30 minutes after LPS administration.