Nanoencapsulation of perfluorinated trityl radicals and evaluation as sensors for EPR oximetry.

N. Charlier[1], B. Driesschaert[2], J. Marchand[2], V. Préat[3], and B. Gallez[1]

[1]Biomedical Magnetic Resonance Unit, Université catholique de Louvain, Brussels, Belgium, [2]Organic and Medicinal Chemistry Unit, Université catholique de Louvain, Brussels, Belgium, [3]Pharmaceutical Technology Unit, Université catholique de Louvain, Brussels, Belgium

Introduction

Triarylmethyl radicals (trityls) EPR oxygen sensors are characterized by a narrow EPR Line Width (LW) and high signal to noise ratio for EPR spectra or images. Trityls are also characterized by a good *in vivo* half-life compared to other soluble probes (nitroxides). However, one limitation of these sensors is their very low LW variation (ΔLW) compared to particular probes.

Oxygen is by far more soluble in a lipophilic medium compared to water (1). Thereby, for one specific pO_2, a lipophilic medium will have a larger oxygen concentration ($[O_2]$) than hydrophilic ones. Therefore, for one variation in the pO_2 (ΔpO_2), there will be a greater $\Delta[O_2]$ in a lipophilic medium, corresponding to a greater ΔLW, and so an increase in sensitivity.

This concept was first explored by Liu and co-workers (2) using a nitroxide probe in an organic solvent for preparation of microspheres. More recently, Sostaric and co-workers have used a trityl probe with the same approach (3). However, microspheres based on polymerised albumin are potentially immunogenic.

Material & Methods

In the present study, we used perfluorocarbon solvent which are characterized by very high oxygen solubility. These solvents present a good biocompatibility. We first synthesized a perfluorinated trityl, BD0122 (Fig.1.) that was soluble in HexaFluoroBenzene (HFB). We used a concentration of 1 mM. To make the "team" BD0122/HFB administrable *in vivo*, we performed a nanoemulsion stabilized by the use of lecithin. A crude premixing was handled with an UltraTurax device. In order to obtain an emulsion with optimal characteristics, it was further homogenized using a High Pressure Homogenizer (20 passes, 20 000 psi). Size measurements were carried out using a Zeta Sizer device.

EPR measurements were performed using an EMX spectrometer (9 GHz) in order to check the LW at 21% and 0% oxygen for BD122 emulsion. Same experiment was carried out using a hydrophilic trityl with a structure without fluorinated amide chain (BD040).

Using an L-band spectrometer, *in vivo* experiments were done by injecting 150 µl in the gastrocnemius muscle in NMRI male mice. Line width measurements were done at normoxia and after ligation of the leg.

Results

The size of the emulsion is about 150 nm and is stable for at least 2 weeks.

EPR measurements with X-band spectrometer were performed at 310K in order to check the LW at 21% and 0% oxygen. As can be seen on Fig.2., LW variation is by far more important with the emulsion than with the hydrophilic trityl solution (~ 2.3G for the emulsion compared to 0.1G for hydrophilic trityl). Kinetics experiment showed that 6 to 8 minutes was sufficient to allow equilibrium with the gas external content (data not shown).

In vivo experiments showed that the injected nanoemulsion is well sensitive to pO_2 changes. This is illustrate in Fig.3..

Discussion

A nanoemulsion containing a newly synthesized perfluorinated trityl was developped. The emulsion presents good characteristics to be used *in vivo*. The LW variation is by far increased compared to hydrophilic trityls.

References

(1) Linke W. F. Solubilities of inorganic and metal-organic compounds. American chemical society. 1958.

(2) Liu K. J, et al. In vivo measurement of oxygen concentration using sonochemically synthesized microspheres. Biophysical J., (67):896-901. 1994.

(3) Sostaric J. Z. et al. Encapsulation of a high sensitive EPR active oxygen probe into sonochemically prepared microspheres. J. Physical Chemistry, 111(12):3298-303. 2007.

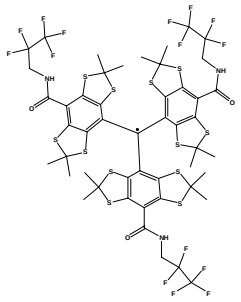

Fig.1.: Structure of BD122, a perfluorinated trityl radical.

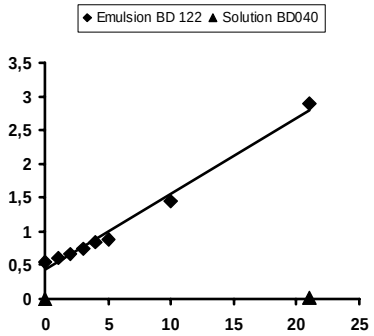

Fig.2.: Comparison of the sensitivity of BD122 emulsion and hydrophilic trityls (BD040) solution to changes in the oxygen environment.

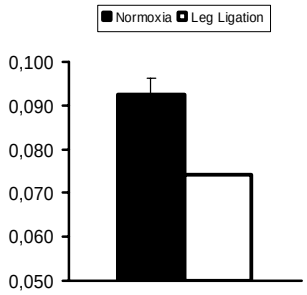

Fig.3.: *In vivo* comparison of the LW of BD122 emulsion in normoxia (black column) and after leg ligation (white column).