In vivo stable spin probes for EPR and DNP Imaging

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Introduction

Electron Paramagnetic Resonance (EPR) spectroscopy is the most powerful technique for the study of free radicals because of its high sensitivity that permits detection of very low concentrations of free radicals. In vivo EPR experiments are usually performed in the 300 MHz - 3.5 GHz range, that allows detection of free radicals in biological samples and in small animals such as rats. For human studies, it is necessary to decrease the radiofrequency fields under 100 MHz for the penetration of electromagnetic waves, making difficult EPR experiments. Dynamic Nuclear Polarisation (DNP) is an NMR technique that allows detection of free radical. DNP relies on the irradiation by a radiofrequency field of an electronic paramagnetic transition of a free radical dissolved in solution. This EPR irradiation enhances the 1H NMR signal of the solvent proton. EPR and DNP imaging can be performed in vivo, but cannot detect in a direct way endogenous free radicals, such as hydroxyl or superoxide free radicals. This is due to the very short half time of endogenous free radicals. But, by introducing into the system to be studied, some stable spin probes and by detecting modification in their EPR or DNP signal, it is possible to measure free radical distribution. Because of their chemical stability, nitroxides are widely used as spin probes and labels. To date, the nitroxide used in in vivo experiment have a half time of about 1/2 hour. The aim of this communication is to present some new spin probes that have a structure derived from isoindoline and show a stability in biological media higher than conventional spin probes.

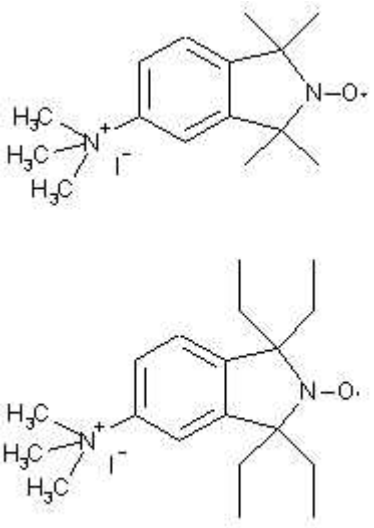

Figure 1 : structure of the isoindoline nitroxides TMIOS (top) and TEIOS (bottom)

Material and Methods

EPR studies were performed on a X band Bruker spectrometer operating at 9.7 GHz. Isoindoline nitroxide were synthesized following the procedure described in [1]. Human blood was collected in tubes containing EDTA and then centrifuged during 30 min at 4°C to separate red blood cells solution from plasma solution. Free radicals were dissolved (1 mM) into the solution just before ESR measurements. An ESR spectrum was obtained every 3 mn for the 10 first spectra, then every 10 min for the 20 next spectra and finally every 30 min for the last spectra. EPR and DNP experiments were performed at room temperature. DNP experiments were performed on a conventional NMR spectrometer (Bruker CXP 100) with a static magnetic field of 7.0 mT. The use of Field Cycled Dynamic Nuclear Polarization (FCDNP) allowed to switch the magnetic field before the EPR irradiation at different magnetic field strenghs [2]. The used double tuned probes was tuned to 300 kHz for the NMR signal detection and to 200 MHz for the EPR irradiation.

Results and Discussion

EPR spectra present three lines due to the hyperfine coupling between the unpaired electron and the nitrogen 14N nucleus. Figure 2 show the decay of the EPR signal of the isoindoline nitroxide in whole blood. Calculation of the half-life time in biological media shows that the TEIOS nitroxide is more stable than TMIOS nitroxide (more than 100 hours for TEIOS and 20 hours for TMIOS). This great difference between may be attributed to a larger steric hindrance introduced by the ethyl groups. Rather than absolute values of reaction rates, these results are to be taken as relatives values. The rate of reduction of the TEIOS nitroxide is an order of magnitude smaller than those of the most resistant nitroxide. The study of the EPR spectra shows that there is no change neither in the line width or in the pattern indicating that there is no complex formation between nitroxide radical and blood protein. DNP spectra were obtained for both isoindoline nitroxide. The measured DNP factor, that represents how the NMR signal is enhanced was about 10. Theoretically, the DNP factor should be about 110. This weak factor is explained by the line width of the free radicals that are too large to allow a complete saturation of the EPR transition. The synthesis of a TEIOS nitroxide with a 15 nitrogen nucleus instead of a 14 nitrogen and the substitution of protons by deuteriums in the ethyl groups should results in an increase of the DNP signal.

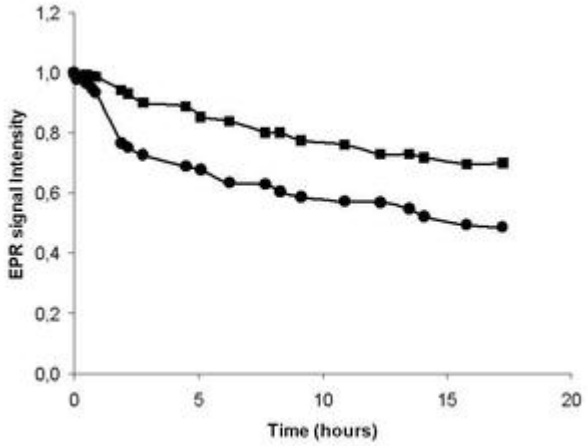

Figure 2 : kinetic curves of reduction of TEIOS (square) and TMIOS (circle) in blood

Conclusion

We have presented in this communication some new water soluble nitroxides that should be used in in vivo experiment especially for EPR or DNP imaging. The great stability of this probes allows their use for functional analysis such as measurement of local pH or oximetry. This can be done by added some functional groups on the aromatic cycle of the free radicals

References

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