# Heparin-polynitroxide derivatives: first application as site specific MRT imaging contrast media for vascular wall

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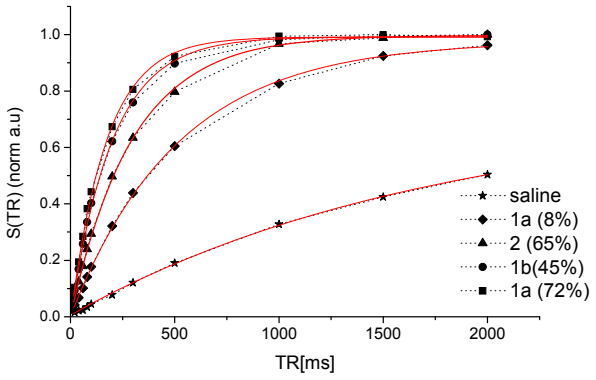
## Introduction

Cyclic nitroxides are stable free radicals with potentially multiple biomedical applications in MRI. Specialized nitroxides e.g. TEMPO derivatives, are actively used in development of new contrast media for $^{13}C$ MRI using DNP hyperpolarization. A common limitation for cyclic nitroxides is their short in vivo half-life time which is mainly due to the rapid intracellular bioreduction into corresponding hydroxylamines. Recently, the synthesis of three different heparin-polynitroxide (HNR) derivatives in which the nitroxide moiety (TEMPO) is linked either to uronic acid or glycosaminne residues of the heparin macromolecule via amide bound was reported [1]. Heparin is known to have a high affinity for the variety of vascular extracellular structures. We propose that the cyclic nitroxides could be specifically delivered to the strategically important sites e.g. the vascular wall by means of HNR derivatives. The paramagnetic properties of TEMPO are expected to introduce essential NMR relaxivity effect (both $R_1$ and $R_2$) and, therefore, provide the enhancement of contrast of $T_1$-weighted MRT images at these specific locations. The particular motivation of this work were a proof of principal experiment for using HNR complexes as principally new biomarker with simultaneous contrast media and protective antioxidant activity effect.

## Materials and method

The $R_1$ and $R_2$ relaxivity of heparin-polynitroxide was preliminary investigated. The $T_1$ measurements were performed with saturation recovery using a SGRE sequence: TE=4ms, FA=$90^0$, varying TR from 20 to 2000ms to acquire the relaxation recovery curve (Fig 1a). In total, 4 samples with different concentration of different degrees and types of heparin labeling and saline buffer reference sample were tested. The samples **1** have the binding of heparin and TEMPO via COO-group, with additional $H_2N(CH_2)_5C(O)NH$ linker for **1b**. In the case of sample **2** the NH-groups bond are used. Fractions of labeled disaccharides in the samples of 1a,b and 2 vary in the range of up to 70% (see Fig 1a legend). The $T_2$ measurements were done using multiple spin-echo sequence (CPMG) sequence with the same spatial resolution parameters, TR0200ms and 2 averages. The echo train consisted of 15 echoes with echo time TE varied from 45 ms with the step 22.5ms. For ex-vivo blood vessels experiments, the isolated porcine vascular segments were pre-treated with heparin-polynitroxide, wash-out, then placed in 0.7% agar. Measurements have been performed using Tim Trio System 3T system (Siemens, Erlangen, Germany) using the standard extremity coil. A spin-echo sequence with TR/TE=200/16ms, slice thickness 1mm and image resolution 226μm$^2$ was used for imaging.

## Results



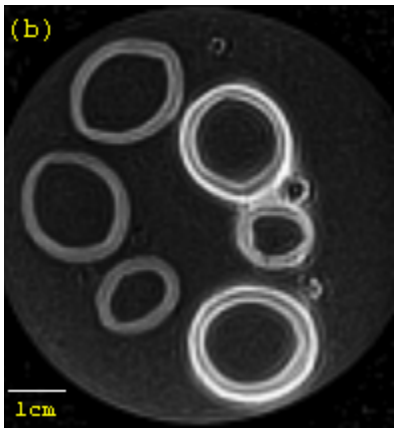




***Fig 1.*** (a) Relaxation curves for different heparin-NR complexes, the fraction of labeled disaccharides shown in brackets. (b): $T_1$-weighted MR image of isolated porcine vascular segments (aorta, carotid and coronary arteries). Control (let) and heparin-polynitroxide (**2** or **1a**) labeled vessels (right).

The X-band EPR in-vivo measurements shows that the EPR-signals of nitroxides persists during several hours demonstrating stability of HNR complexes [1]. As expected, the maximal relaxation time shortening was observed for 72% spin labelled heparin (1 order of magnitude in comparison with saline buffer). Interestingly, that derivatization of heparin amino groups demonstrated a relatively small efficiency (in terms of relaxivity) when compared with derivatization of carboxyl groups. At the same time, the use of a linker apparently increases the relaxivity of heparin-polynitroxide. For the ex-vivo treated blood vessels the $T_1$-weighted contrast enhancement (by factor of 3.5) in the strategically important inner layer of the vascular wall, as well as in adventitia, but not in the media was observed (Fig 1b). Another key result was a large spin echo time frame in which the $T_2$-weighted contrast could be seen in labelled vessels (up to TE=50 ms)

## Conclusion

In conclusion, the newly synthesized HNR exhibit superoxide scavenging activity, have long-lasting in vivo half-life time and bind to the inner layer of vascular wall, where they can be visualized both by EPR and MR techniques. The HNR can be considered as first dual-purpose molecular platform for the advanced cardiovascular EPR and MR imaging, as well as for topical antioxidative protection of the vascular extracellular space. These proof-concept results may stimulate further efforts to synthesize functionalized polynitroxide derivatives targeted to the specific structures of the vascular wall for diagnostic and therapeutic purposes.

## Reference

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