First in-vivo application of heparin-polynitroxide derivatives for labeling of vascular wall

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Introduction

Cyclic nitroxides are the stable free radicals with potential applications in MRI as redox-sensitive contrast agents. 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 (HPN) derivatives in which the nitroxide moiety (TEMPO) is linked either to the uronic acid or glycosaminne residues of the heparin macromolecule via amide bound. The novel HPNs demonstrated the long lasting in vivo half-life and ability of binding to a vascular endothelium and extracellular matrix [1]. The high affinity of heparin to the variety of vascular extracellular structures could be used to deliver HNR specifically to the vascular wall. The paramagnetic properties of TEMPO are expected to introduce essential enhancement of contrast of T_1-weighted MRT images at these specific locations. In previous studies the high effectiveness of the HPN labeling of vascular wall was proven in isolated blood vessels [1]. In this work were performed the proof of principal **in-vivo** measurements for using HPN complexes as principally new biomarker with contrast media effect.

Materials and method

All measurements have been performed using Magnetom Tim Trio System 3T system (Siemens, Erlangen) using standard extremity (knee) 8 channel coil. The in-vivo measurement was done with permission of local Animal Care Committee. The white New Zeeland rabbits (4-5 kg, 1 year age) were anesthetized with ketamin-xylazine mixture. Two animals received the intravenous injection of 10 µmol/kg of low or high molecular weight HPN (l-HNR and h-HNR respectively) and scanned during first 6 hours and then after 48 hours post injection. Another 2 animals were measured as controls. Measurements were performed using Turbo Spin-Echo with fat suppression, TR/TE=500/19ms, turbo factor=3, GRAPPA iPAT=2, slice thickness = 1.5mm, in-plane resolution 0.2mm^2

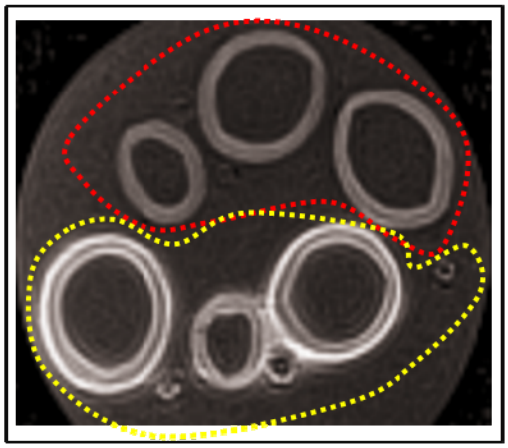

Fig 1 T_1-weighted MR image of isolated porcine vascular segments (aorta, and coronary arteries). Control (up) and heparin-polynitroxide labeled vessels (down) Spin-echo sequence with TR/TE=200/16ms, slice thickness 1mm and image resolution 226µm^2

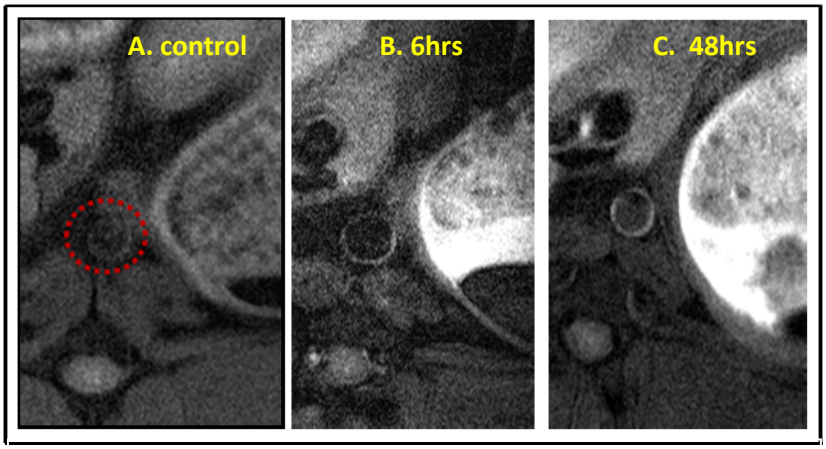

Fig 2(a,b,c) In-vivo T_1-weighted MR images of rabbit abdomen (fragment). A – control animal. B after 4 and C after 48 hours after injection of high molecular heparin-nitroxide.TSE sequence with TR/TE=500/19ms, TF=3, GRAPPA iPAT=2, slice thickness = 1.5mm, in-plane resolution 0.2mm^2 were used, acquisition time = 21 min/10 slices. The enhancement of the signal on aorta (marked my dotted line) wall could be clearly observed in comparison with the control animal. The further increase of contrast enhancement was observed after 48 hours post injection (C) that allows hypothesizing that the cumulative effect takes place.

Results and Discussion

Fig 2 shows the MR images of the near-aorta area in the abdominal part of rabbits. The control image (A) shows no contrast enhancement (CE) of the aortic wall (red marker) in comparison with surrounding tissues. The images obtained at 4-6 hours after injection of h-HPN labeling agent shows the enhancements of the signal in abdomen, especially and particularly, in the aortic wall, which could be clearly seen on at the surrounding muscles background. The further increase of CE was detected in experiment repeat after 48 hours. The increase of the visible aorta wall thickness allows hypothesizing that accumulating and penetrating of hHPN complex inside the aorta vascular wall took place. The l-HNR labelling has shown no obvious enhancement effect on aorta wall. However substantial CE was observed in the abdominal organs (stomach, kidney, intestine) both after hHPN and lHPN injections.

The in-vivo study shows that high molecular HPN exhibits the long-lasting in-vivo life time and bind irreversibly to the inner layer of vascular wall, where they can be visualized both by EPR and MR techniques. These proof-of-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. Further experiments using in-house built dedicated 16 channel phased array optimized for rabbit abdomen, various doses and types of HPNs complexes for the evaluation of oxidative stress and endothelial dysfunction in animal models of atherosclerosis are under way.

Reference: [1] M. V. Terekhov, V. Sen', V. Golubev, S. Weber, A. W. Scholz, T. Munzel, A. L. Kleschyov, and L. M. Schreiber, Heparin-polynitroxide derivatives: first application as site specific MRT imaging contrast media for vascular wall, Proceedings ISMRM (2010)

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