Molecular NMR and EPR in vivo detection of inflammation using specific E-selectin targeted iron oxides

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Introduction:

The aim of the study was to evaluate a molecular marker for non invasive diagnosis and monitoring in the early phases of inflammation. At the beginning of the inflammatory process, endothelial permeability increases and cell adhesion molecules such as E-selectin are expressed on the endothelial cell surface (1). Leukocytes are slowed down in blood flow by adhesion on E-selectin *in vitro* and *in vivo* (2). A mimetic of the sialyl Lewis X molecule, which is the E-selectin's natural ligand, was coupled to the coating of pegylated ultrasmall iron oxide nanoparticles (USPIO). USPIO possess superparamagnetic properties and are used as negative MRI contrast agent due to strong T_2 and T_2* effects. Besides this method, EPR offers the unique capability of a sensitive and quantitative determination of these particles (3).

Materials and Methods:

An inflammation was induced in the right gastrocnemius muscle of male NMRI mice (20-25g) by intramuscular injection of complete Freund's adjuvant. The left muscle serves as internal control. After intravenous injection of either the grafted or ungrafted USPIO (7.7mg Fe/kg BW), we evaluated the USPIO concentration *ex vivo* by a EPR X-band (Bruker, EMX®, 9.4 GHz) and *in vivo* by an EPR L-band (Magnettech®, 1.2 GHz), as well as by a MRI T_2-weighted fast spin echo sequence (Bruker Biospec® 4.7T).

Results:

After establishing a calibration curve by EPR X-band, we determined the mean iron oxide concentration in the inflamed muscles after intravenous injection of grafted or ungrafted UPSIO, which was 0.8% and 0.4% of the initially injected dose respectively (data not shown). By L-band EPR, we observed that the grafted USPIO concentration in inflamed muscles was twice higher than for the ungrafted particles (Fig. 1). Using MRI experiments, a higher signal loss was clearly observed in the inflamed muscle when grafted USPIO were injected in comparison with the ungrafted USPIO (Fig. 2). All these differences were statistically significant.

Discussion:

Using these three methods, we demonstrated that the specific targeting of grafted iron oxides was twice higher than for the ungrafted ones. Interestingly, ungrafted USPIO were also more concentrated in inflamed muscles than in control muscles. This is likely the result of an accumulation of a large number of macrophages in inflamed tissues which can take up USPIO in a non specific manner. Furthermore inflammation causes a vasodilation: the resulting improvement in blood flow carries more iron particles to the inflamed sites. Even taking into account this non specific accumulation of iron oxides, the targeting of USPIO with E-selectin ligands significantly improved the sensitivity of detection of inflamed tissues.

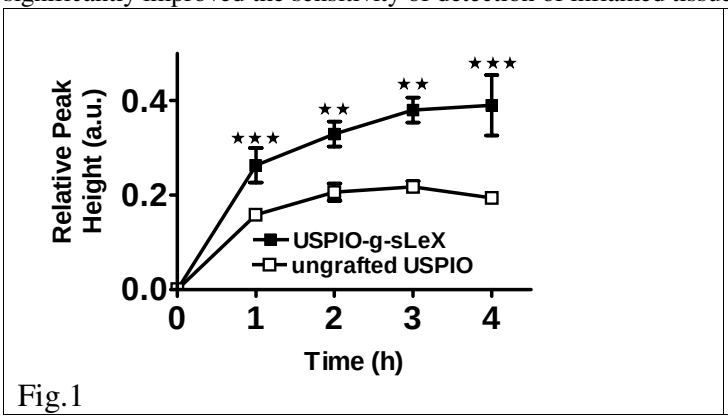

Fig.1

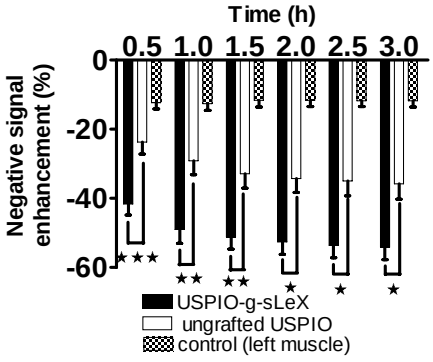

Fig.2

Fig.1 Time course of USPIO concentration in inflamed muscle as measured by in vivo EPR.
Fig.2 Time course of negative contrast enhancement produced by USPIO.

References:

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