SUPERPARAMAGNETIC IRON OXIDE NANO-PARTICLE CONTRAST FOR TARGETED MAGNETIC RESONANCE IMAGING OF VASCULAR ENDOTHELIUM INFLAMATION

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Introduction

Cardiovascular diseases (CVD), such as atherosclerosis, myocardial infarction and stroke, are the leading cause of morbility and mortality worldwide [1]. Although the medical procedures were considerably improved in the last decades, their general therapeutic results are not satisfactory. That is mostly due to the fact that the therapy is implemented when the disease is already advanced, or quite often even after it has been manifested in cardiovascular events. Thus, there is a need for the sensitive and possibly noninvasive procedures allowing detection of these diseases at the early stage and for novel drugs/therapeutic methods. It is known that all of these diseases begin with the inflammation of the endothelium - the tissue lining the blood vessels [2]. There are studies suggesting that magnetic resonance imaging (MRI) might be useful for early detection of this pathological condition. MRI allows for non-invasive imaging of internal organs and distinguishing healthy from diseased structures at the molecular and cellular level. Unfortunately, it usually requires application of contrast agent. The superparamagnetic iron oxide particles are used for that purpose, but their performance is still not satisfactory. Currently, the main area of research on these systems is focused on the possible improvement of their magnetic properties by decreasing their size to nanoscale region, increasing their biocompatibility and selectivity [3-7].

Materials and Methods

The size and the size distribution of the nanoparticles were characterized by TEM [Tecnai G2 F20 (200 kV) with field emission gun, bright-field, high resolution images. Their hydrodynamic sizes and zeta potentials were measured using dynamic light scattering (DLS, Zeta Sizer Nano ZS). Determination of the magnetic properties was done with a Vibrating Sample Magnetometer, Quantum Design Physical Property Measurement System equipped with a superconducting 9 Tesla magnet. ^{57}Fe Moessbauer measurements were carried out in the transmission mode at a constant acceleration spectrometer. MRI measurements *in vitro* were performed using 9.4 T Bruker BioSpec 94/20 MR imaging system (Bruker, Germany). The content of iron in SPIONs was determined using a classical colorimetric method based on absorbance measurements of the complex of Fe(II) with phenanthroline. The specific interaction of the SPION-VCAM-1 and SPION-P-selectin nanoparticles with the endothelium in the state of early inflammation was studied using the 10 μm- thick cross-section slides of the aorta of diabetic db/db mice at the age of 24 weeks with endothelial dysfunction.

Results and Discussion

The paper presents the results of our studies on development of the superparamagnetic iron oxide nanoparticles (SPIONs) targeted to the areas of vascular endothelium changed in the initial inflammation process, a first step of numerous cardiovascular diseases. The iron oxide nanoparticles (round shape, diameter about 50 nm) coated with cationic derivative of chitosan (CCh) were prepared via co-precipitation of the iron salts in molar ratio Fe(III) : Fe(II) = 2:1 in alkaline deoxygenated aqueous solution of cationicaly modified chitosan. The monoclonal antibodies - anti VCAM-1 and anti P-selectin (0.016 mg per 1 mg of iron), were attached to nanoparticles' surface via tosylation. The permanent attachment of these primary antibodies to the SPIONs surface was confirmed by the immunostaining with IgG-TR (Texas Red) antibodies. Due to the electrostatic stabilization the nanoparticles form a stable colloidal dispersion in aqueous media. The SPION-CCh-anti-VCAM-1 maghemite nanoparticles obtained were superparamagnetic. The *in vitro* studies confirmed the specific interaction of anti-VCAM-1 antibodies bound to the surface of SPIONs with endothelial cells of aorta of db/db mice, known to display endothelial inflammation associated with diabetes. The obtained nanoparticles have also been visualized in aortic arch of the mice with endothelial dysfunction using MRI technique.

Conclusion

Novel, biocompatible, superparamagnetic iron oxide nanoparticles formed stable dispersion in aqueous media and recognizing the area of endothelium changed by early inflammation were obtained and shown to have a potential to serve as a MRI contrast agent.

Acknowledgments

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