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Uptake and bioreactivity of charged chitosan-coated superparamagnetic nanoparticles as promising contrast agents for magnetic resonance imaging

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Abstract

Bioreactivity of superparamagnetic iron oxide nanoparticles (SPION) coated with thin layers of either cationic or anionic chitosan derivatives and serving as contrast agents in magnetic resonance imaging (MRI) was studied *in vivo* using BALB/c mouse model. Synthesized dual-modal fluorescing SPION were tracked in time using both fluorescent imaging and MRI. Although SPION started to be excreted by kidneys relatively shortly after administration they were uptaken by liver enhancing MRI contrast even up to 7 days. Importantly, chitosan-coated SPION caused only mild activation of acute phase response not effecting biochemical parameters of blood. Liver histology indicated the presence of SPION and some increase in the number of Kupffer cells. The overall results indicated that SPION coated with ultrathin layers of chitosan ionic derivatives can serve as T_2 contrast agents for diagnosis of liver diseases or imaging of other organs assuming the dose is optimized according to the need.

Key words: Superparamagnetic iron oxide nanoparticles; Magnetic resonance imaging; Biodistribution; Chitosan; Fluorescence imaging

There is a growing interest in fabrication of superparamagnetic iron oxide nanoparticles (SPION) mostly due to their potential application in theranostics.[1-3] They can be used as contrast agents enhancing magnetic resonance imaging (MRI) signal thus allowing diagnosis and as drug carriers allowing its targeted delivery. There are two types of MRI contrast agents. So-called positive contrast agents shorten T_1 (longitudinal) relaxation time resulting in enhancing the signal while negative contrasts agents shorten T_2 (transversal) relaxation that results in darkening of MR images. SPION can be used as T_2 contrast agent due to their superparamagnetic properties[4] but proper adjustment of SPION size can also facilitate their application as T_1 contrast agents.[5,6] Various methods of SPION synthesis as well as their surface modification and functionalization have been developed to tailor them for specific biomedical applications, e.g., cellular therapy, tissue repair, drug delivery, hyperthermia, MRI.[7-9] While developing such novel biomaterials their biodistribution and bioreactivity, including potential toxicity, should be always taken into account.

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SPION typically consist of the iron oxide core and appropriate coatings that stabilize the nanoparticle and provide functional groups at their surface for further derivatization of application of other functional coatings.[10] While iron oxide can be metabolized by liver[11,12] selecting of proper biodegradable and/or biocompatible materials for coating is a prerequisite for biomedical application of SPION. Various materials have been used as such coatings,[13] including biodegradable polysaccharides like dextran and chitosan.[14] To assess toxicity of SPION, *in vitro* studies have been often performed.[15] However, toxicity of a material determined based on the *in vitro* tests is usually higher than that for the same material evaluated in *in vivo* experiments. That difference is caused by the fact that during *in vivo* experiments possible degradation products, often responsible for the toxicity, are continuously eliminated from the system while this is not the case in *in vitro* tests. Although there have been many attempts to improve methodology of the *in vitro* studies[16] it is still believed that the *in vivo* examination is the ultimate test for nanoparticles efficacy and toxicity.

Biodistribution and toxicity of SPION have been shown to depend on their size, charge and surface characteristics.[17-20] Opsonization process, by which foreign organisms or particles become better recognizable by phagocytic cells, occurs readily for hydrophobic and charged particles. To limit that process, thus to prolong the time of SPION circulation in a body, the stealth nanoparticles were prepared by coating iron oxide core with hydrophilic polymers such as poly(ethylene glycol) (PEG).[21] The effects of charge and dose of SPION on pregnant mice were also investigated to determine the risk of administration of SPION to pregnant women and to the developing fetus. It was shown that a single dose had no negative impact on pregnant mice and the developing fetus while multiple doses increased the number of fetal resorption, independently of the nanoparticles' charge, although higher bioaccumulation and toxicity were observed for SPION with positive surface charge.[22] The studies on the size effect on nanoparticle biodistribution were carried out showing that larger nanoparticles are eliminated from the bloodstream faster than the smaller ones.[23,24] Generally, nanoparticles with diameters greater than 200 nm are filtered by the spleen and the ones with diameters up to 100 nm are eliminated by liver. However, in case of particles smaller than 40 nm the coating material rather than the nanoparticles' size is the most important parameter determining their biodistribution.[12] Although the amount of accumulated data on the effect of nanoparticles on the living systems has recently considerably increased, it is still not sufficient to reach general conclusions. Thus, *in vivo* examination of the newly obtained nanoparticles with similar coatings differing only in surface charge may bring an important input to the ongoing discussion on bio-safety of nanomaterials.

In this paper, we report the results of *in vivo* studies on novel type SPION coated with either cationic or anionic chitosan derivatives that we have recently developed.[25-27] Application of ionic derivatives of chitosan in the process of fabrication of such SPION resulted in formation of electrostatically stabilized aqueous dispersions of the nanoparticles that are very stable even in body fluid environment and exhibit exceptional magnetic properties desired for MRI applications. Moreover, such chitosan-based coatings were shown to have anticoagulant properties[28] that are particularly important for the considered applications of developed here coated nanoparticles. The biodistribution was studied here up to 7 days after the nanoparticles' injection using dual-modal SPION formed by attaching fluorescent probe to chitosan coating of iron oxide nanoparticle cores. The fluorescent signal was tracked for 2 h after injection. For longer time experiments, T_2 relaxation time measurements for liver were performed. The effect of SPION coated with cationic and anionic chitosan derivatives on blood count and alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine, glucose as well as acute phase proteins' activity was evaluated. The histology of the liver specimens was also performed to observe the pathological changes induced by SPION.

Methods

Nanoparticles synthesis

Superparamagnetic iron oxide nanoparticles coated with charged chitosan derivatives were obtained using the method, which we developed and described earlier.[25] Briefly, iron salts (puriss. p.a., Sigma-Aldrich) at the molar ratio Fe(III):Fe(II) = 2:1 (0.1622 g $FeCl_3 \cdot 6H_2O$ and 0.0596 g $FeCl_2 \cdot 4H_2O$) were dissolved in 50 mL of 0.1 M sodium chloride solution of the cationic chitosan derivative (CCh) at the concentration of 3 g L^{-1}. The degree of substitution of low molecular weight chitosan (Sigma-Aldrich) with quaternary ammonium groups was ca. 57% in the synthesized CCh. The solution was deoxygenated by purging with argon and sonicated (Sonic-6, Polsonic, 480 W, 1 s pulse per 5 s break) for 10 min in a thermostatic bath at 20 °C. Then 5 mL of 5 M $NH_{3(aq)}$ was added dropwise and the solution was further deoxygenated and sonicated for 30 min at 20 °C. Finally, the obtained SPION-CCh were purified by magnetic chromatography and their suspension was filtered with a syringe filter (0.2 μm pore size).

In order to obtain SPION with negative surface charge the fraction of SPION-CCh was coated with an anionic chitosan derivative, carboxymethyl chitosan substituted with sulfonate groups (ACh), using electrostatically-driven layer-by-layer deposition technique.[29] The degree of substitution of carboxymethyl chitosan (AK Scientific, Inc.) with sulfonate groups was ca. 66%. The SPION-CCh suspension was mixed with 0.2 M sodium chloride solution of ACh at the concentration of 4 g L^{-1} in the volume ratio 1:1 and sonicated continuously for 10 min. The obtained SPION-ACh were purified using magnetic chromatography and filtered with a syringe filter (0.2 μm pore size).

The SPION-CCh nanoparticles were also modified with Alexa Fluor® 647 (AF) fluorescent probe. Briefly, Alexa Fluor® 647 NHS Ester (Life Technologies) was dissolved in anhydrous *N,N*-dimethylformamide (DMF) at the concentration of 10 mg mL^{-1}. 100 μL of the dye solution was slowly added to 2 mL of the SPION-CCh aqueous suspension (c = 5 mg mL^{-1}, neutral pH) under stirring. The mixture was incubated for 1 h at room temperature with continuous stirring. After that time, the SPION-AF were purified by dialysis against water at room temperature in dark.