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MR Relaxivity Measurement of Iron Oxide Nano-Particles for MR Lymphography Applications

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Abstract: The aim of this study was to assess the T_1, T_2 and T_2* relaxivity of Ultrasmall Super Paramagnetic Iron Oxide (USPIO) nano-particles *in vitro* and *in vivo* in rat models with magnetic resonance imaging at 1.5T. First, relaxation properties of USPIO nano-particles at different doses were measured using related SE and GRE MR imaging protocols. The relation between dose and relaxation were observed which is linear; Higher dose of the nano-particles means higher relaxivity. Based on this relation, an optimum protocol can be proposed for obtaining the best image contrast at each situation. Then detection ability of MRI protocols was studied for USPIO nano-particles with injection of the particles in the rat. The optimum MR protocols were used to observe the signal change of lymph nodes in rat.

Key words: MR relaxation, USPIO, nanotechnology, contrast agent

INTRODUCTION

Prognosis of malignant tumors could be possible with optimum display of metastasis at initial steps of the tumor progress. Metastasis may involve Liver and lymph nodes in some stages. New advances in MRI methods have increased its capability in cancer staging, following up the progress of disease and accurate detection of lymph nodes involvement in disease. New Ultrasmall Super Paramagnetic Iron Oxide (USPIO) nano-particles can be used as contrast agents to study these tissues with MRI (Kim, 2001; Pankhurst, 2003).

Super Paramagnetic materials create magnetic fields around themselves when placed within an external magnetic field. Therefore, they promote small field inhomogeneities within the external magnetic field and hence, T_2-relaxivity increases due to the rapid dephasing of the spins. Through this so-called susceptibility effect, the signal decreases dramatically (Rogers *et al*., 1998; Mikhailova, 2004; Klaber *et al*., 2005).

These particles, useful for MR imaging, consist of iron oxide particles that are in the order of 100 nm in diameter for Liver imaging and smaller than 20 nm for lymph imaging. The particles are coated with dextran and suspended in water, in order to form a stable compound. It is administered as a slow intravenous infusion. USPIO is biodegradable and the iron is eventually incorporated into normal iron metabolism of the body. Maximum increase in T_2 relaxivity (i.e., T_2 relaxation rate) of lymph nodes occurs approximately 6-12 h after intravenous administration. The blood half-life of the USPIO is approximately 2 hours. Liver signal normally appears after the particle infusion within 3-7 days (Bellin *et al*., 2000; Klaber *et al*., 2005; Bellin and Roy, 2007).

Biodistribution of these Particles and their efficient delivery to a specific tissue depends on their properties such as physical and chemical characteristics. Nano-particles can be described with their size, surface coating, surface charge, surface hydrophobicity, density and pH of their suspensions. These characteristics in turn affect physiochemical properties such as toxicity, protein adsorption capacity, stability and aggregation behavior in biological environment, plasma half-life period and more important their super paramagnetic response which is useful for efficient signal changes in MRI (Shen *et al*., 1993; Weissleder, 1990a, b). There is also correlation between USPIO concentration and Relaxivity parameters of R2 ($= 1/T_2$) and R1 ($= 1/T_1$) in MRI (Cho *et al*., 2006; Voit *et al*., 2001).

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Particular antibodies can be conjugated with USPIO nano-particles in order to deliver them to a specific tumor or its metastasis. The required dose of the labeled antibody is still too high to make an efficient imaging practice for tumor specific imaging (Wilhelm *et al*., 2003). Although, focusing on some specific over-expressing Biomarkers on the surface of cancer cells and/or increasing administration dose may overcome this deficiency, however, any attempt to increase the sensitivity of particle detection (using i.e., optimum MR protocol, proper size and type of nano-particle and imaging time after injection) is advantageous.

The aim of the current study is to optimize USPIO concentration for an efficient signal change in biological tissues (as similar to water content materials). Accordingly, relaxivity of a contrast agent material should be found in an optimum concentration level in order to be able to design a proper MR protocol with efficient signal changes.

MATERIALS AND METHODS

Nanomag®-D-spio nano-particles (Micromod Partikeltechnologie GmbH) was used for relaxivity measurements. The solid concentration of the material is 10 mg mL^{-1}. Fe concentration of the USPIO was measured using atomic absorption spectroscopy technique which showed a concentration of 2.2 mg mL^{-1} for Fe. Four concentrations of the nano-particles (0.0650, 0.0325, 0.0162 and 0.0081 mg mL^{-1} USPIO) were obtained by dilution with Sodium Chloride 0.9% serum.

All imaging protocols were done on the 1.5T GE MRI (Signa) system of the Emam Khomeini imaging center during last 6 months. Relaxation rates of USPIO contrast agents were measured for different concentrations of the USPIO samples, using different Spin Echo (SE) and Gradient Echo (GE) protocols. For T_2 measurement, two multi-echo SE protocols was used with TE of 12, 24, 36, 48 ms and TE of 46, 92, 138, 184 (TR = 1000 ms) (Eq. 1). T_2* measurements was done using multi-echo GE images with increasing TE of 5.4, 6.3, 8.7, 17.2, 17.6 ms and TR = 800 ms (Eq. 2).

$$\text{Signal}_{SE} = S_0 e^{-R_2 \cdot TE} \quad (1)$$

$$\text{Signal}_{GE} = S_0 e^{-R_2^* \cdot TE} \quad (2)$$

TR-variable SE imaging (with the same TE value) was used for T_1 measurement. The Signal Intensity Equation used for T_1 measurement is as follows:

$$\text{Signal}_{SE} = S_0 (1 - e^{-TR/T_1}) \quad (3)$$

Then, detection ability of MRI protocols for nano-sized Iron Oxide Particles was studied using a phantom, to find the optimum protocol for performing this task. The essential study of USPIO contrast agent found to be consisted of 2D or 3D GE pulse sequences; T_1 weighted and T_2* weighted GE, T_2 weighted SE and FSPGR protocol with fat suppressions using a small surface coil.

Also, Relaxation properties of these USPIO nano-particles in biological tissues were evaluated with injection of the nano-particles to Rats. By applying an optimum MR protocol, Signal change of the lymph nodes in rats were observed. Relaxation rates were measured using signal intensities obtained from MR images. For MR imaging, the animals were anesthetized and fixed to be placed in MR system.

RESULTS

Multi-echo SE and GE images of Micromod nano-particles (20 nm) in 4 different concentrations showed increment of signal intensity by decreasing Iron concentration (Fig. 1). In a same image at a given TE, signal loss for more concentrated samples is higher. The images show that the higher Iron concentration corresponds to the higher signal loss due to shortening T_2 and T_2*.

Also due to T_2 and T_2* effects, signal curve of one sample over time is decreasing. Figure 2a and b shows change of ln(signal) over time (at different TEs) for one sample in Multi-echo SE and GE images, which is linear. Negative slope of this line is the measured R2 of the sample Eq. 1 and 2. Signal change of the samples in multiple SE images with the same TE and varying TR, can give T_1 quantities based on Eq. 3. Figure 2c depicts the signal change with increasing TR for the same sample.

The results of MR relaxation measurements of Micromod samples at different concentrations are shown in Table 1. Increasing T_2 and T_2* is consequence of decreasing concentration.

The relation between R_1, R_2 and R_2* vs. USPIO concentration (mg mL^{-1}) are as follows (Fig. 3):

$$1/T_1 = 37.94*C\ (\text{mg mL}^{-1}) + 0.212$$
$$1/T_2 = 260.49*C\ (\text{mg mL}^{-1}) + 10.813$$
$$1/T_2^* = 361.95*C\ (\text{mg mL}^{-1}) + 56.922$$

Accumulation of USPIO nano-particles in Rat lymph nodes was observed both in T_1 and T_2 (as well as T_2*) images 4 h after S.C injection of USPIO in one paw. Dramatic decrease of the signal intensity in the lymph node of rat in Fig. 3c confirms the existence of magnetic susceptibility effects in a T_2*-weighted gradient-echo

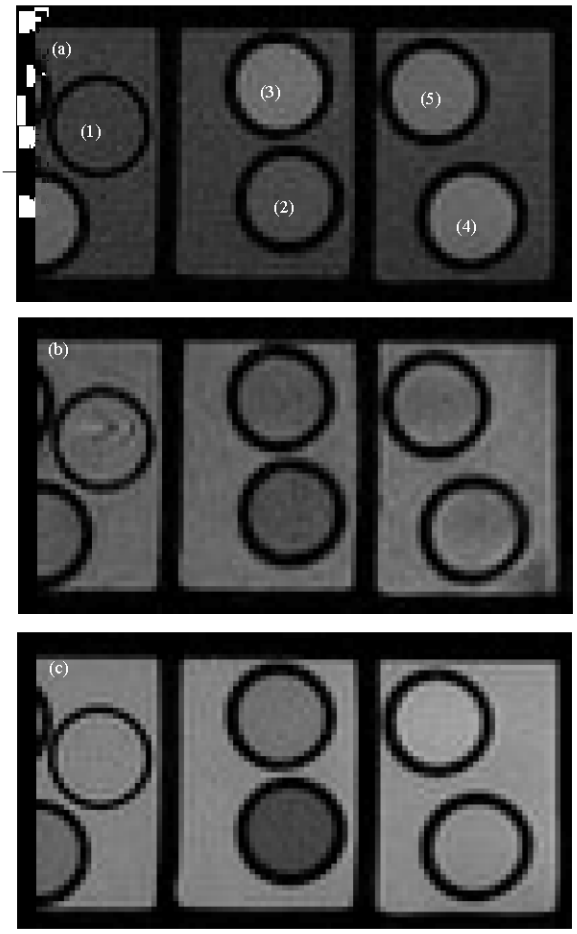

Fig. 1: (a) T_2-weighted Spin Echo image of the USPIO material (Fe_3O_4 20 nm Micromod) samples in different vials 2 to 5 corresponding to four concentrations of the USPIO (respectively 0.065 to 0.0081 mg mL^{-1}) and a water vial 1, at the minimum TE = 12 ms of a 4-echo spin echo image series for T_2 relaxivity measurement (TR = 1000 ms). All the vials were placed in a water phantom. (b) T_2 Gradient Echo image of the vials at TE = 5.4 ms for T_2* relaxivity measurement (TR = 800 ms) and (c) T_1-weighted Spin Echo image of the vials at TR = 5000 ms for T_1 relaxivity measurement (TE = 11 ms). Increment of the signal intensity by decreasing the USPIO concentration can be observed in vial 2 to vial 5

Table 1: Measured T_1, T_2 and T_2* of Fe_3O_4 20 nm (Micromod)

Solution	Iron concentration (mg mL^{-1})	T_1 (ms)	T_2 (ms)	T_2* (ms)
Water	0	3425.90	172.41	144.93
Micromod	0.008125	1850.48	91.74	22.94
Micromod	0.01625	1118.32	69.44	11.61
Micromod	0.0325	762.19	46.08	16.08
Micromod	0.065	366.84	37.59	12.52

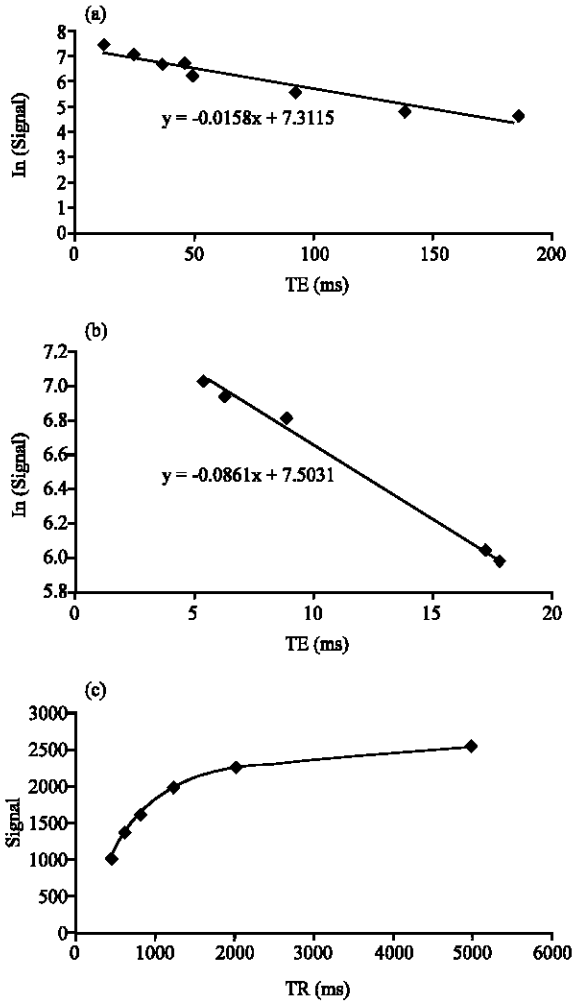

Fig. 2: (a, b) ln (Signal) vs. TE for Micromod 0.01625 mg mL^{-1} USPIO in (a) Multiple-Echo SE protocols that shows linear relation between ln (Signal) vs. TE. (y = -0.0158x + 7.3115, R2 = 0.0144, therefore T_2 = 69.44 ms) and (b) In GE protocol and (c) Signal vs. TR for Micromod 0.01625 mg mL^{-1} Fe shows exponential relation between Signal vs. TR. (T_1 = 1118.32 ms)

images. Figure 2a and b also show lymph node of Rat after USPIO administration in related T_1 and T_2 contrast images (by SE pulse sequence) as appropriate images for determination of anatomic regions of lymph nodes. The T_1 SE sequence can also help to detect and discriminate fats in abnormal nodes. T_2 weighted SE pulse sequence is always required for differentiation of abnormal tissues from normal tissues. In addition we found that FSPGR

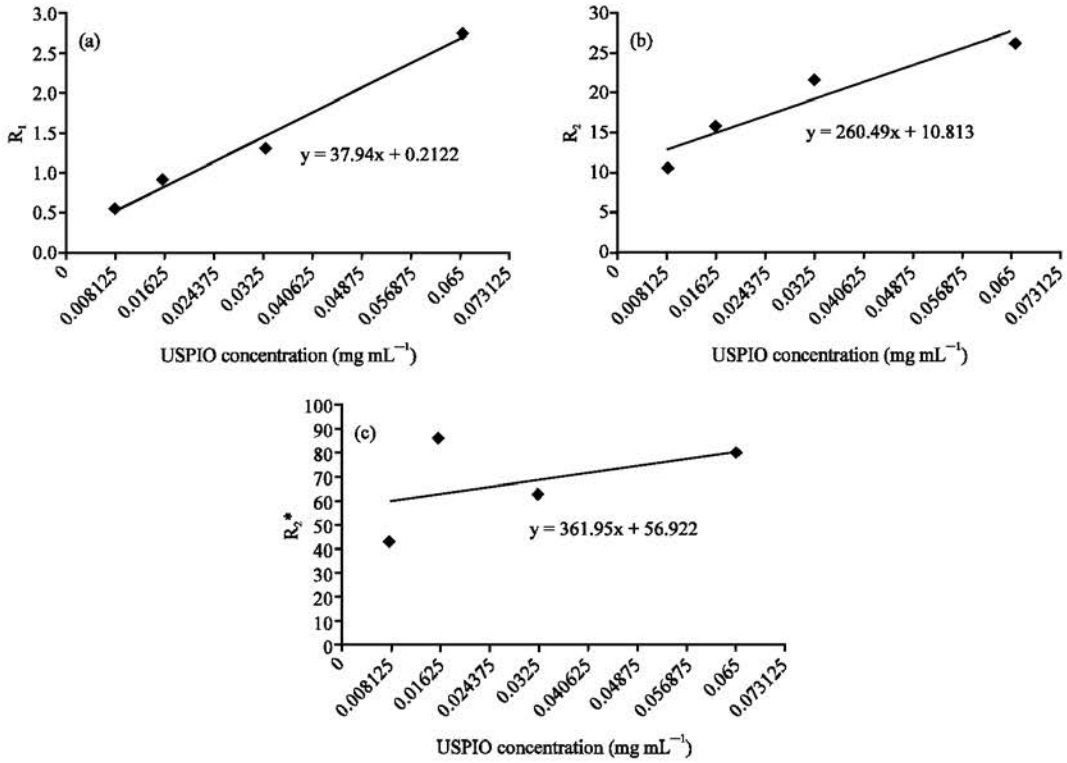

Fig. 3: Correlation between relaxation rate and USPIO concentration for Micromod USPIO contrast agents. (a), (b) and (c): A linear positive correlation can be seen between USPIO concentration and $1/T_1$, $1/T_2$ and $1/T_2$*

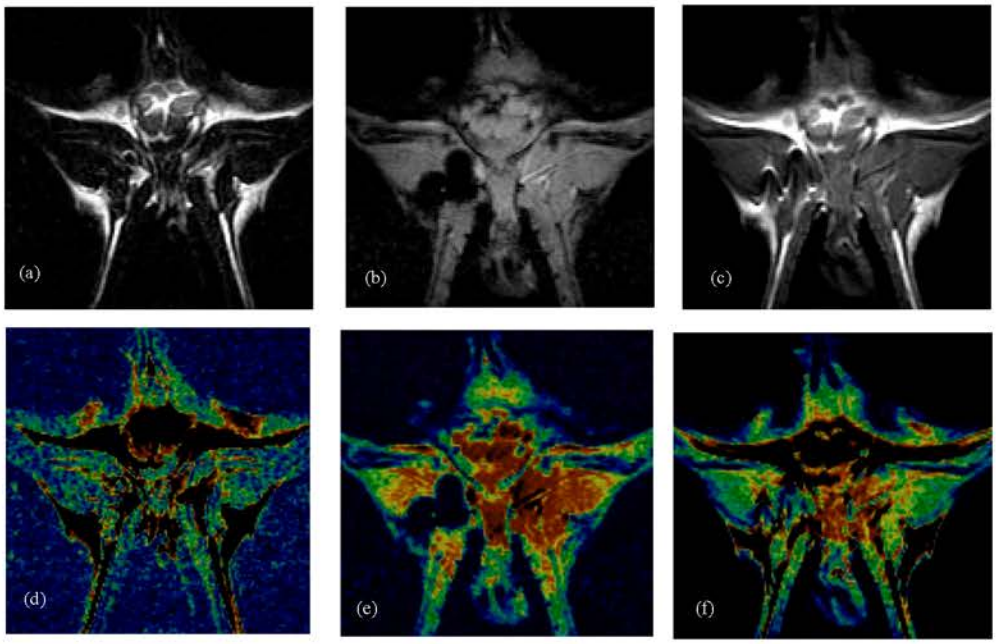

Fig. 4: (a) T_2-weighted image of USPIO in rat lymph node (right paw), (b) Susceptibility effects of USPIO with T_2*-weighted gradient echo image, (c) T_1-weighted image of USPIO in rat lymph node, (d) T_2 map of USPIO, (e) T_2* map of USPIO and (f) T_1 map of USPIO

technique provides enough sensitivity and gives a proper signal for determination of Rat lymph nodes, but care must be taken to ensure properly set T_2 and T_2* weighted protocols using optimum imaging parameters (Fig. 4).

DISCUSSION

Relaxation properties of different concentrations of USPIO nano-particles have been studied *in vitro* using MRI system to be compared with *in vivo* measurements. Then the effect of Iron concentration on obtaining an efficient signal change, after administration of USPIO MR contrast agent, was studied.

Adequate concentration of Iron, when using a long circulating particle in biological tissue, can be used in order to detect tumors and metastasis in lymph nodes. Required dose (concentration) to detect other types of tumors with different genetic characteristics is higher than the scope of a non risk imaging strategy. Targeted delivery of Iron particles is possible only after conjugation of these probes to a specific monoclonal antibody. However, the link of labeled antibody-USPIO to tumor cells is still too low to make an efficient imaging protocol for tumor specific imaging.

Focusing on some specific over-expressed receptors on the surface of cancer cells, increasing administration dose, modifying the type of particle or its surface and/or increasing relaxivity may help to overcome the deficiency of signal changes from labeled antibody-USPIO materials. Also, type of imaging sequence and MR imaging parameters and compartmentalization of the contrast agents are effective in increasing magnitude of susceptibility effect and enhancing signal changes.

Among the above techniques, an effective way is using suitable USPIO nano-particles with very high relaxivity properties (R_1 and R_2) in possible low concentration to increase the sensitivity of the particle detection. The hydrodynamic size of nano-particles is influenced by the number of crystals surrounded by coating Dextran and the thickness of the coating itself. Since this size properties affect the relaxivity and susceptibility of the particle and in turn the amount of MR signal change, using some USPIO nano-particles coated with small organic molecules, or designing new Iron Oxide particles with no overall coating (e.g., functionalized particles with non active core surface) is proposed for further assessment.

Since the amount of signal change due to Iron nano-particle depends on susceptibility effect and in turn relaxivity, a small effective dose can lead to a high enough signal changes for a suitable detection. The optimum MR protocols for obtaining such high negative signal still remain under investigation for the future.

CONCLUSION

The ongoing study aims to minimize particle concentration where obtaining an efficient signal change, after administration of USPIO MR contrast agent.

It was shown that an effective way to increase the sensitivity of the particle detection after using USPIO contrast agent is to increase concentration. Various concentrations affect through changing the relaxivity (e.g., R_1 and R_2) of tissues where they reside.

It was shown in some cases, where the susceptibility effect of a nano-particle and in turn its relaxivity is high, a small dose (concentration) can lead to a high enough signal change for a suitable detection.

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