Effect of iron oxide nanoparticles coating type on the relationship between nanoparticles concentration and signal intensity in inversion recovery T1-weighted MRI

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Abstract

Background: Ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles are used as blood pool contrast agent for magnetic resonance angiography and perfusion imaging. Our aim in this study was to investigate the effect of the two coating types of iron oxide nanoparticles on the relationship between nanoparticles concentration and signal intensity (SI) in T1-weighted MR images.

Methods: Different concentrations of the polyethylene glycol (PEG), and carboxydextran-coated iron oxide nanoparticles were imaged using inversion recovery Turbo-FLASH (Turbo fast low-angle shot) pulse sequence with inversion times (TIs) of 300-900 ms (interval of 100 ms). The maximum non-linear and linear relationship between the corrected SI (after non-uniformity correction) and the concentration of the two coated nanoparticles were calculated in T1-weighted images.

Results: The maximum non-linear relationship between the corrected SI and the concentration of the PEG, and carboxydextran-coated nanoparticles were obtained at concentrations of 400 and 200 µmol Fe/L at a TI of 900 ms, respectively. In addition, the maximum linear relationship between the corrected SI and the concentration of the PEG, and carboxydextran-coated nanoparticles (R²=0.99) appeared at 228.184 and 205.654 µmolFe/L with a TI of 300 ms, respectively.

Conclusion: The maximum non-linear corrected SI of the carboxydextran-coated nanoparticles was slightly higher than that of the PEG-coated nanoparticles at similar TIs. However, the PEG-coated nanoparticles were better than the carboxydextran-coated nanoparticles as a T1 contrast agent for perfusion measurements.

Keywords: MRI, T1-weighted image, Iron oxide nanoparticles, Coating, Inversion recovery.

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Introduction

Ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles are used as blood pool contrast agent (BPCA) for magnetic resonance angiography (MRA) and perfusion imaging. Their large magnetic moment and the intravascular distribution of the nanoparticles make them contrast agents for MR perfusion studies (1). The high r₁ relaxivity of the nanoparticles leads to a reduction of the blood T1 for a long period and produces high blood signal (2). The T1 effect of contrast agents is enhanced by the use of T1-weighted images. Moreover, among various types of MR sequences, the inversion recovery (IR) sequence is useful for MRA and perfusion imaging because of the excellent blood suppression and visualization of small amounts of contrast agent (3). Selecting an appropriate inversion time (TI) for the IR pulse sequence is important for providing significant diagnostic infor-
mation. The TI itself is affected by the dose of the contrast agent (4, 5).

The coating material surrounding the iron oxide core of the nanoparticles affects the relaxivity, chemical stability, biocompatibility, biodistribution, and blood half-life of the nanoparticles (1, 6, 7). So far, different types of coating materials, including dextran (1), carboxyldextran (8), carbohydrate-polyethylene glycol (PEG) (1), and citrate (9, 10), have been used for MRA and perfusion studies. Comparisons of citrate-coated iron oxide nanoparticles with gadopentetate dimeglumine for first-pass MRA of the aorta and renal arteries (9) and with gadofosveset trisodium for cardiac MRA in pigs (10) were performed and demonstrated the advantages of nanoparticles for the studies. Since the SI of MR images is affected by the concentration of the nanoparticles, some researchers have evaluated the relationship between iron oxide nanoparticles concentrations and SI using dextran (11, 12) and carboxyldextran-coated (8, 13) particles. They reported the nanoparticles’ concentration which led to maximum linearity between concentration and SI, or maximum signal enhancement. One study evaluated the effect of the coating material on the accumulation of iron oxide nanoparticles in the axillary lymph nodes for MR lymphography (14). However, it is not clear which coating type of USPIO nanoparticles is useful for MR perfusion measurement and which nanoparticles’ concentration leads to the maximum SI.

The present study was focused on comparing the relationship between SI and different concentrations of the two types of coated (carboxyldextran and PEG) USPIO nanoparticles in T1-weighted images by use of the IR Turbo-FLASH sequence with different TIs. Therefore, by determining which coated nanoparticles produce a higher SI with a lower concentration, it is possible to use a lower dose of contrast agent for MR studies.

**Methods**

**Contrast Agents**

Nanomag-D-spio nanoparticles (Micromod Partikeltechnologie GmbH, Rostoc, Germany) with a mean diameter of 20 nm, a core size of 7 nm and two different coating materials including carboxyldextran (COOH) and PEG were used as contrast agents. The concentration of Fe in ferrofluids was measured with an atomic absorption spectrometer (analytik jena, novAA® 400, Jena, Germany). The nanoparticles were diluted with distilled water, and different concentrations of both types of the coated particles were prepared. The concentrations were 0 to 100 µmol Fe/L (5 µmol Fe/L intervals) and 100 to 500 µmol Fe/L (100 µmol Fe/L intervals).

To obtain the real SI, the uniformity of the coil should be considered (15). The preparation of a constant concentration (50 µmol Fe/L) of the PEG-coated nanoparticles was also performed for measurement of the non-uniformity of the coil.

The glass vials were filled with different concentrations of each type of the nanoparticles and separately put into a perspex phantom with the dimensions of 13×13×13 cm³.

**Theory**

Standard IR sequence was used for SI calculation (16):

\[
S(t) = S_0 \left( 1 - 2 \exp \left( -TI \left( \frac{C(t)}{K} + \frac{1}{T1_{pr,c}} \right) \right) \right) + \exp \left( -TR \left( \frac{C(t)}{K} + \frac{1}{T1_{pr,c}} \right) \right),
\]

(1)

Where S(t) and C(t) are the SI after administration of the contrast agent and the concentration of the contrast agent at time t, respectively. \( S_0 \) is the observed SI in the absence of contrast agent, TI is inversion time, T1_{pr, c} is the longitudinal relaxation time before contrast application, and TR is the repetition time. K is a constant that depends on the contrast agent. The T2-shortening effect is negligible at low concentrations of the contrast agent while at
high concentrations of the nanoparticles; Eq. 1 should be multiplied by $\exp\left(-\frac{TE}{T_2}\right)$, where TE is echo time (16).

**Image Acquisition**

MR imaging was performed with a 1.5 T MRI system (Siemens MAGNETOM Avanto, Germany). A standard clinical head coil was used for MR imaging. The phantom containing vials filled with different concentrations of each contrast agent was carefully placed in the center of the coil.

An IR Turbo-FLASH pulse sequence was performed for T1-weighted MR imaging in a coronal orientation. The MR imaging parameters were: 920 ms/ 2.5 ms/ 15° (TR/TE/ flip angle); 10 mm slice thickness; 128×128 matrix size, and 2 signal averaging. The imaging parameters were applied for the phantom consists of different concentrations and then for the phantom containing constant concentration which was exactly placed at the same position of the vials with different concentrations.

Since for TIs less than 300 ms, the T1 recovery curve did not cross the horizontal axis, TI values were chosen between 300 and 900 ms (with 100 ms intervals) for this study.

**Image Analysis**

The image data in DICOM format was analyzed using the Interactive Data Language (IDL, Research Systems, Inc. http://www.rsinc.com) software. SI of each vial was determined in a region of interest (ROI) consists of 9 pixels in the center of the vial. Then calculation of the correction factors for the coil non-uniformity was performed by in-house IDL programs and the corrected SI was calculated with multiplying these factors by the SI of the vials with different concentrations. Plotting the curves and estimating the maximum linear relationship between SI and TI with squared correlation coefficient ($R^2$) of 0.99 were also performed by use of in-house IDL programs.

It should be mentioned that both linear (for perfusion study) and non-linear relationships between the maximum corrected SI and the concentration of the two nanoparticles were considered in this study.

**Results**

The Fe concentrations measured by atomic absorption spectrometer were 1.29 and 1.94 mg/ml for the carboxydextran- and PEG-coated nanoparticles, respectively; which was used for preparation of the different values of concentrations.

Figure 1 demonstrates the coronal image of the phantom containing different concentrations of the nanoparticles.

The mean non-uniformity coil correction factors for the carboxydextran-coated nanoparticles were calculated as 1.05, 1.07, 1.10, 1.03, 1.03, 1.02, 0.99, 1.00, 1.00, 1.06, 0.98, 0.98, 1.00, 0.97, 1.04, 1.03, 1.01, 1.01, 0.99, 1.02, 1.02, 1.01, 0.99, 0.99 and 0.99 for the vials with the concentrations of 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, and 500 µmolFe/L, respectively.

In addition, the value of non-uniformity coil correction factors for the PEG-coated nanoparticles were calculated as 1.08, 1.10, 1.10, 1.03, 1.03, 1.02, 0.99, 0.98, 1.00, 1.05, 0.98, 0.98, 1.00, 0.97, 1.05, 1.03,

![Fig. 1. Coronal image of the phantom. The position of different concentrations (µmol Fe/L) of the nanoparticles inside the vials is seen in this figure.](http://mjiri.iums.ac.ir)
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0.98, 1.01, 0.98, 1.02, 1.02, 1.01, 0.99, 1.02, and 1.02 for the vials with the concentrations of 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, and 500 µmolFe/L, respectively.

Figure 2 shows the mean corrected SI (for non-uniformity of the coil) from the 9 pix-

Fig. 2. Mean corrected SI (for non-uniformity of the coil) versus concentration of the PEG-coated nanoparticles at different TIs. The error bars show the standard deviation of the SI from 9 pixels in each vial.

Fig. 3. Mean corrected SI (for non-uniformity of the coil) versus concentration of the carboxydextran-coated nanoparticles at different TIs. The error bars show the standard deviation of the SI from 9 pixels in each vial.
els in each vial versus the concentration of the PEG-coated nanoparticles at TIs of 300-900 ms. The maximum non-linear corrected SIs of 290.44 ± 6.90 and 335 ± 8.80 (mean ± SD) were obtained at a concentration of 500 µmol Fe/L for low TIs of 300 and 400 ms, respectively. In addition, the maximum non-linear corrected SIs of 277.9 ± 13.3, 404.60 ± 12.60, 442.59 ± 9.80, 435.94 ± 9.40, and 148.93 ± 4.60 appeared at a concentration of 400 µmol Fe/L for TIs of 500, 600, 700, 800, and 900 ms, respectively.

As shown in Fig. 2, T1 and T2-shortening effects are dependent on TI and the concentration of the nanoparticles. These effects were seen for all corrected SI versus concentration curves at different TIs. T1-shortening effect appeared at lower TIs (300 and 400 ms) and a concentration of 500 µmol Fe/L and led to an increase in the SI. Also a T2-shortening effect at high concentrations (more than 400 µmol Fe/L) appeared for TIs of 500-900 ms.

Figure 3 indicates the mean corrected SI (for non-uniformity of the coil) versus the concentration of the carboxydextran-coated nanoparticles at TIs of 300-900 ms. The concentration of 400 µmol Fe/L gave the maximum non-linear corrected SIs of 316.21 ± 10.5 and 355.40 ± 11.7 (mean ± SD) for TIs of 300 and 400 ms, respectively. The maximum non-linear corrected SIs of 402.39 ± 10.60, 425.75 ± 11.5, 437.88 ± 12.06, and 453.95 ± 11.9 appeared at a concentration of 200 µmol Fe/L for TIs of 600, 700, 800, and 900 ms, respectively. In addition, the maximum non-linear corrected SI of 386 ± 15.02 was seen at a concentration of 300 µmol Fe/L for a TI of 500 ms. The figure also shows T1 and T2-shortening effects for all TIs. These effects will be dependent on the TI and the concentration of the nanoparticles. A T1-shortening effect dominated at lower concentrations and led to an increase in the SI. The T1-shortening effect appeared at lower TIs (300 and 400 ms) and concentration of 400 µmol Fe/L. Also a T2-shortening effect

Fig. 4. The maximum non-linear SI versus TI for the PEG- and carboxydextran-coated nanoparticles. The figure shows that the maximum non-linear SI of the carboxydextran-coated particles is slightly higher than the PEG-coated particles at the similar TI. The error bars show the standard deviation of the SI from 9 innermost pixels from center of each vial.
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Fig. 4 indicates the maximum non-linear SI versus TI for the PEG- and carboxydextran-coated nanoparticles. The figure shows that the maximum non-linear SI of the carboxydextran-coated particles was higher than that for the PEG-coated particles at a similar TI.

Figure 5 shows the maximum concentration of contrast agents that gave $R^2 = 0.99$ versus TI for the PEG- and carboxydextran-coated nanoparticles. The maximum linear relationship between concentrations and the corrected SI that gave an $R^2$ equal to 0.99 was 228.18 and 205.65 µmol Fe/L at low TI (300 ms), respectively, for the PEG- and carboxydextran-coated nanoparticles. In addition, these values were reduced to 92.15 and 81.83 µmol Fe/L at the highest TI (900 ms) for the two coated nanoparticles. The figure also shows that an increase in TI led to a decrease in the range of the linearity.

**Discussion**

The strength of the SI will be changed by many factors such as the magnetic field strength, the pulse sequences parameters, imaging protocols, the dose, injection rate, bolus volume of the contrast agent, and the tissue topology (17-25). The relationship between the SI and the concentration of the contrast agent has been defined as Eq. 1 for IR sequences (16).

PEG is a polymeric surfactant which is used as the iron oxide nanoparticles coating material because of its high solubility, stability in aqueous solutions, biocompatibility, and prolonged blood circulation time. PEG can minimize or prevent opsonization of the nanoparticles. Thus, it is an appropriate material for MR angiography and perfusion imaging (26). On the other hand, carboxydextran is a biocompatible organic polymer which decreases the occurrence of allergic reactions and has a long circulation time in blood. The long blood half-life of both coated nanoparticles provides enough time for acquisition of imaging data.

The T1 and T2 shortening effects will ap-
pear on images. The T1-shortening effect is dominant at low concentrations of contrast agent and both T1 and T2 can be affected at high concentrations (see Figs. 2 and 3). Therefore, the relationship between the SI and concentration is linear at low concentrations and non-linear at high concentrations.

Figs. 2 and 3 show the concentration which led to the maximum non-linear SI at different TIs for the two coated nanoparticles. As can be seen from the figures, the T2-shortening effect was started from concentrations of 400 and 500 µmol Fe/L for the carboxydextran- and PEG-coated nanoparticles, respectively, at TI = 300 ms. That effect appeared from concentration of 200 and 400 µmol Fe/L for the carboxydextran- and PEG-coated nanoparticles, respectively, at high TI (600-900 ms). The T1-shortening effect is seen at lower concentrations.

According to Fig. 4, the maximum non-linear SI of the carboxydextran-coated nanoparticles is slightly higher than that of the PEG-coated nanoparticles at similar TIs because of the difference in coating types.

Analysis of the concentration-time curve reveals some information about perfusion and hemodynamic parameters of a tissue. Since MRI cannot measure concentration of contrast agent, if the relationship between SI and concentration of contrast agent to be linear, the concentration will be measured indirectly from SI. Therefore SI-time curve can be used as concentration-time curve (27). This possibility is provided by considering the R² concept, which gives the linear relationship between SI and concentration. Fig. 5 shows that the maximum linearity of the carboxydextran-coated nanoparticles was lower than that of the PEG-coated nanoparticles for similar TIs. Therefore, the PEG-coated nanoparticles are better than the carboxydextran-coated nanoparticles as T1 contrast agents for perfusion measurement because of the high SI. According to the similar core size of the iron oxide nanoparticles in both contrast agents, the difference in linearity is due to differences in the two coating types of the nanoparticles.

Although the R² of the PEG-coated nanoparticles is higher than that of the carboxydextran-coated nanoparticles, the maximum non-linear SI of the carboxydextran-coated nanoparticles was slightly higher than for the PEG-coated nanoparticles at similar TIs.

This study indicates that the TI and the coating type of the nanoparticles are important parameters for measuring SI. They have an effect on the value of both the linear and the non-linear relationship between SI and the concentration of contrast agent (see Figs. 2-5). An increase in TI leads to a decrease in the range of the linearity.

The linear relationship between the concentration of the 20 nm carboxydextran-coated nanoparticles and SI using TIs of 100-400 ms was investigated by Saharkhiz et al. (13). They found the maximum linearity at the concentrations of 139.98 and 76.83 µmol Fe/L for TIs of 300 and 400 ms, respectively; while the concentration values for the maximum linearity in our study with similar TIs were 205.65 and 172.70 µmol Fe/L for the carboxydextran-coated nanoparticles, respectively (Fig. 5). This finding shows that even with similar coating and TIs, the resulted linearity is different due to using different imaging parameters (TR, TE, and matrix size) in the two study. Our previous studies show that the imaging parameters (such as TR and TE) can have an effect on the strength of SI and the relationship between SI and contrast agent concentration (28, 29). Therefore, the results of the two studies are completely different.

Canet et al. reported a linear relationship between the concentrations of AMI-25 (dextran-coated) nanoparticles and SI when they used only a TI of 300 ms (11). They showed a linear relationship between the nanoparticles’ concentration and SI for concentrations up to 200 µmol Fe/L, while it was 228.18 and 205.65 µmol Fe/L for the PEG- and carboxydextran-coated nanoparticles, respectively, at a TI similar to that in our study (see Fig. 5). In spite of the dif-
ference in nanoparticles size and imaging parameters in the two studies, the linearity difference obtained with the dextran-coated nanoparticles in the study of Canet et al. and the carboxydextran-coated particles in the current study was very small. The reason is that carboxydextran is one of the dextran derivatives. In another study, the maximum enhancement of the dextran-coated USPIO nanoparticles was found at 200 μmol Fe/L when a spin-echo pulse sequence with a TR=500 ms and TE=22 ms was applied (12). This finding is in agreement with our results obtained with TIs of 600-900 ms for the carboxydextran-coated nanoparticles, while there was no TI providing similar results with the PEG-coated particles (see Figs. 2 and 3). Chambon et al. (12) also reported maximum enhancement of the nanoparticles at a concentration of 400 μmol Fe/L at a TR=160 ms and TE=20 ms. This is in agreement with our findings for TIs of 300 and 400 ms for the carboxydextran-coated nanoparticles and TIs of 500-900 ms for the PEG-coated particles (see Figs. 2 and 3). The findings indicate the important role of the pulse sequences and imaging parameters in maximum enhancement. On the other hand, regarding the similar core size of both coated nanoparticles in the present study, an influence of the coating material on the maximum SI was seen.

Conclusion
The relationships between the SI and the concentrations of the two coated types (carboxydextran and PEG) of iron oxide nanoparticles were evaluated on T1-weighted MR images.

This study shows that the different coating types of the nanoparticles and different TIs have an effect on both the linear and the non-linear correlation between the SI and the nanoparticles concentration.

The maximum non-linear SI of the carboxydextran-coated nanoparticles is slightly higher than that of the PEG-coated particles. However, the PEG-coated nanoparticles are better than the carboxydextran-coated nanoparticles as a T1 contrast agent for perfusion measurement because they produce a higher maximum linear relationship between the SI and the concentration of the contrast agent at similar TIs (R² = 0.99). Our study shows that an increasing in TI produces a decrease in the range of the linearity.

Conflict of interest
The authors declare that there is no conflict of interest.

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