PEGylated superparamagnetic iron oxide nanoparticles labeled with $^{68}$Ga as a PET/MRI contrast agent: a biodistribution study

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Abstract The purpose of this study is to evaluate the biodistribution of polyethylene glycol (PEG) coated superparamagnetic iron oxide nanoparticles radiolabeled with $^{68}$Ga in normal mice after intravenous administration of this probe. Three mice were sacrificed at specific time intervals. The biodistribution data revealed high uptake by liver and spleen (60.62 and 12.65 %ID/g at 120 min post injection for liver and spleen, respectively). The clearance of other organs was fast. These results suggest that $^{68}$Ga-PEG-SPIONs has magnificent capabilities for applying in (PET-MRI) as a theranostic agent for detection of liver and spleen malignancies.

Keywords Superparamagnetic iron oxide nanoparticles (SPION) · $^{68}$Ga · Biodistribution · Liver · Spleen · Cancer

Introduction

Over the past decade, many studies have been focused on the development of numerous kinds of nano carriers including quantum dots, liposomes, ultrasound nanobubbles and magnetic nanoparticles which they are able to improve accurate detection of diseases [1, 2]. Among these nanocarriers, superparamagnetic iron oxide nanoparticles (SPIONs) which have a magnetic core and which have been coated with biocompatible coating materials such as dextran and polyethylene glycol (PEG) can be applied as a contrast agent for diagnosis of metastases in the reticuloendothelial system such as liver, spleen, bone marrow [3, 4]. PEG is able to curb the adsorption proteins on their surface. Thus, it can help to prevent from phagocytosis of nanoparticles (NPs) by mononuclear phagocyte system such as macrophage cells [5, 6]. SPIONs are used as a negative contrast agent in magnetic resonance imaging (MRI) and have a lot of medical applications including...
cancer imaging, cell loading/tracking, drug delivery, genes delivery, macrophage imaging and therapy, theranostic applications and observation biological process such as apoptosis [7–10].

68Ga is an appropriate positron emitter isotope which can be used as radiopharmaceuticals such as 94mTc and 18F in positron emission tomography (PET) [11]. 68Ga decays with electron capture (EC = 11 %) and positron emission ($\beta^+ = 89 \%$) and has a short half-life ($T_{1/2} = 67.7$ min) [12]. This radioisotope has useful characteristics such as having high-energy positrons emitted that can produce strong Cerenkov light emission which is able to be applied in luminescence imaging that it can help to optical guidance at surgery. Also, one of the most important advantages of 68Ga is the short half-life which from a dosimetric point of view, avoids long-term exposure to irradiation in patients and the final advantage is related to production this radiopharmaceutical. It can be produced by 68Ge/68Ga generator that is more acceptable in terms of economical and time-saving aspects than the production of it by reactors or cyclotrons [13–16].

67Ga labeled SPIONs radiotracers are used in single photon emission computed tomography (SPECT) imaging and have been assessed in our previous studies [2, 17–19]. In this study, we have designed, done quality control and evaluated the biodistribution of 68Ga-PEGylated SPIONs. The final aim is to confirm that these radiolabeled SPIONs can be used in PET imaging as a radiotracer for identification of malignancies of the reticuloendothelial system (RES).

**Experimental**

**Materials and methods**

68Ge/68Ga generator was eluted with suprapure HCl (0.6 M, 6 mL) in 0.5 mL fractions. The two fractions with the highest 68GaCl3 (700–900 MBq) activity were generally used for labeling purposes. Radio thin layer chromatography (RTLC) was done by Whatman No. 2 paper which was purchased from Whatman (Maidstone, UK) and analysis of RTLC paper was performed by radio detector system (Raytest GmbH; Germany). High-purity germanium (HPGe) detector coupled with a Canberra™ (model GC1020-7500SL) multichannel analyzer and a dose calibrator ISOMED 1010 (Dresden, Germany) were used for counting distributed activity in mice organs. Other chemical materials were purchased from Merck (Darmstadt, Germany). Calculations of counted activity of mice organs were based on the 511 keV peak for 68Ga. The calculated values were expressed as mean ± standard deviation (Mean ± SD) and the data were compared using student t test. United Kingdom Biological Council’s Guidelines on the Use of Living Animals in Scientific Investigations, the second edition was applied for animal studies [20]. SPIONs was obtained from Micromod (Micromod Partikel technologie GmbH) with the lot number of Nanomag®-D-spio, product code: 79-55-201. This product has been surrounded by polyethylene glycol (PEG) and there was NH$_2$ functional group on the surface of it. The mean core diameter is 8.86 ± 1.61 nm and the Fe concentration in NPs are 2.3 mg/mL [3].

**Particles characterizations**

For estimation of the hydrodynamic size of SPIONs sample, we used photon correlation spectroscopy (PCS) of Malvern Zetasizer Nano ZS-90 (Malvern Instruments Ltd., Worcestershire, UK). The size of the NPs was measured before and after radiolabeling.

**Animal models**

Normal mice were purchased from Razi Institute, Karaj, Iran. All mice were sacrificed at specific time intervals after intravenously administration of 68Ga radiolabeled PEGylated USPIOs from their tail veins.

**Radiolabeling of SPIONs with 68Ga**

Polyethylene glycol (PEG) coated SPIONs was purchased from Micromod (Micromod Partikel technologie GmbH). According to the research by Madru et al., SPIONs were labeled with 40–80 MBq of 68GaCl3 [14]. We used 68GaCl3 solution with an activity of 1.15 mCi (42.5 MBq). SPIONs solution includes 200 $\mu$L SPIONs which was dissolved in 800 $\mu$L normal saline, thus, the final volume of our SPIONs solution was 1 mL (1000 $\mu$L).

Radiolabeling procedure was done by the final SPIONs solution and 42.5 MBq of 68Ga-chloride (in 0.6 MHCl and 0.6 M HEPES) were added. The resulting solution stayed at room temperature (RT) for 30 min. After the radiolabeling procedure, product was controlled by RTLC.

**Purification and quality control of 68Ga–SPION**

After the radiolabeling procedure, purification of 68Ga–SPIONs had to be done. For purification, a strong magnetic field was applied, and the magnetic-assorting cell sorting column (MACS® Separation Unit, Miltenyi Biotec Inc, Germany) was attached to this magnetic field and the final solution was run through it [7]. When radiolabeled SPIONs were passing through the LS–MACS column (Miltenyi Biotec) with a high gradient magnet (MACS_ Separation Unit, Miltenyi Biotec Inc, Germany), the labeled NPs were trapped in the column and the rest such as free 68Ga...
radionuclides non-attached to SPIONs and buffer solution were able to pass through the column. After detaching the column from the high gradient magnet, radiolabeled NPs which were trapped in the column had been washed out by normal saline.

**Calculation of labeling efficiency**

In order to evaluate the $^{68}$Ga labeling efficiency (after purification by magnetic assorting cell separation (MACs) column) RTLC was used. This procedure has been done in the presence of saline as the mobile phase. Finally, labeling efficiency was obtained by this equation [21]:

\[
\text{Labeling efficiency} \% = \frac{\text{Total counts} - \text{counts of free}^{68}\text{Ga}}{\text{Total counts}} \times 100 \quad (1)
\]

**Biodistribution of $^{68}$Ga–SPIONs in normal mice**

Biodistribution of $^{68}$Ga-SPIONs was evaluated in normal mice which were purchased from Razi Institute (Karaj, Iran). Little volume (50 $\mu$L) of final $^{68}$Ga-SPIONs which had activity in the range of between 4.4 and 5.2 MBq was injected by intravenously into the tail vein of mice [21]. 5 mice were sacrificed at each specific time intervals (5, 15, 30, 60 and 120 min post injection), and the activity was obtained from organs and expressed as percentage of the injected dose per gram tissue (%ID/g) (Based on the area under the 511 keV peak obtained by an HPGe detector).

**Measurement of activity**

In order to accurate measurement of injected activity, the activity of syringe before and after injection was measured. Measurement of syringe activity and some organ activities such as liver and spleen (for double checking in order to evade the signal saturation of HPGe detector) was done by well-type ionization chamber (model: RAMS-88). All samples were background subtracted. For calculating the activities from the of counts [22, 23]:

\[
A(Bq)_{\text{Tissue}} = \frac{\text{Area}}{t \times \text{Eff} \times \text{Br}} \quad (2)
\]

In this formula, $t$ is the time of counts and \( \text{Eff} \) is the efficiency of the detector for the selected energy and \( \text{Br} \) is the decay yield of selected energy (511 keV) for the \( ^{68}\text{Ga} \) [24]. After calculation of \( ^{68}\text{Ga} \) activity at time $t$, we measured %ID/g ($t$) which is expressed as the percentage of injected activity per gram of tissue (which is equivalent to percentage of injected activity per gram %IA/g) and it could be measured by following formula [25]:

\[
\%ID/g = \frac{A_{\text{Tissue}}}{A_{\text{Total}}} \times 100 \quad (3)
\]

In this equation, $A_{\text{Tissue}}$ is related to the \( ^{68}\text{Ga} \) activity of each sample, $M_{\text{Tissue}}$ is the mass of the sample and $A_{\text{Total}}$ is the total activity of \( ^{68}\text{Ga} \) injected into the mice.

In order to measure the %ID/g for each organ, in each specific time intervals, five mice were dissected and their organs had been extracted from their bodies, then the count of each sample was measured by HPGe to determine the percentage of injected dose per gram. The uncertainty amount of organ’s count was low because each assay collected at least 10,000 counts, for that reason, standard deviation (SD) of results were less than 1 % [26]. In order to the prevention of overestimation and underestimation of in dose calculation, we had tried to have the same geometry and same volume for all samples [26].

**Statistical analysis**

Data have been represented as the mean of three individual observations with a standard error of the mean.

**Results**

Figure 1 shows size distribution of NPs solution. It shows that the size distribution of prepared superparamagnetic iron oxide NPs is fairly narrow. The hydrodynamic size of radiolabeled NPs was 85 nm.

As it is shown in Fig. 2, the \( R_f \) values of free \( ^{68}\text{Ga} \) and \( ^{68}\text{Ga} \)-SPIONs were 0.9 and 0.0, respectively. The purity of \( ^{68}\text{Ga} \)-SPIONs was about 66.25 % post labeling but after passing through the MACs column, it was increased to 98.96 %. Hence, labeling efficiency of SPIONs with \( ^{68}\text{Ga} \) has improved after passing the solution trough the Macs column.

The accumulation of \( ^{68}\text{Ga} \)-PEG-SPIONs in different organs has been demonstrated in Table 1 and Fig. 3. These results approved that the most radiolabeled NPs had been accumulated in RES.

Static fused images PET/CT for assessment of accumulation of \( ^{68}\text{Ga} \)-radiolabeled SPIONs resulted in high uptake in liver and spleen as shown in Fig. 3.

**Discussion**

Hydrodynamic size of PEGylated SPIONs has an important role in their biodistribution. The range of hydrodynamic size of NPs is between 10 and 100 nm for showing optimum functions [4, 7, 9]. For the ultra-small NPs (size under 10 nm), these NPs are able to pass basal lamina of kidneys,
so, they can be excreted by renal excretion. On the other hand, coated NPs with size above 100 nm will trap in the RES. Therefore by optimizing the design of the NPs with the size of 20–80 nm, we can achieve purposes of prevention of phagocytosis and trap the SPIONs in RES and renal excretion [27–29]. In this study, we have assessed organ biodistribution and compared the results with other related studies. As we mentioned before the hydrodynamic size, coating and charge of the NPs will affect their biodistribution faith [27]. Several studies demonstrated that with decreasing the size from 200 to 70 nm, the uptakes become 27 times higher [30]. Therefore, these radiolabeled PEGylated SPIONs with a hydrodynamic size of 85 nm are not able to pass the renal glomerulus. Also, PEG coating has a susceptibility to restriction on adsorption of plasma proteins. Consequently, the opsonization of this kind of

Fig. 1 a TEM image of SPIONs b diagram of size distribution of Clemex image processing for related micrographs. c The PCS histogram of 68Ga-SPIONs indicating their hydrodynamic magnitudes of about 85 nm

Fig. 2 The results of RTLC analysis of free 68Ga and 68Ga-PEG-SPIONs. a Free 68Ga. b RTLC of 68Ga-PEG-SPIONs before purification. c RTLC of Purified 68Ga-PEG-SPIONs after passing through the MACs column

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NPs by phagocyte system has decreased and their circulation half-life has improved which helps to accumulation in targeted cells and other kinds of hepatic cells except Kupffer cells. Moreover, by increasing circulation half-life, they are accumulated in inflammatory and cancerous tissues by enhanced permeability and retention (EPR) effect [7, 27, 31, 32]. According to Table 1, the clearance of radiolabeled NPs from blood was fast and these NPs gradually accumulated in the target area (liver and spleen). In general, the highest amounts of uptake were related to liver and spleen in comparison with the other organs. This accumulation was based on the hydrodynamic size of radiolabeled NPs and coating of them [32]. Dai et al. showed that PEG-SPIONs with hydrodynamic size around 11.7 nm has appropriate dispersibility in water, colloidal stability, and biocompatibility and the highest amount of accumulations were observed in liver, spleen, and intestine. On the contrary accumulation of other organs such as kidney, heart, and lungs were low [33]. In another study carried out by Madru et al., they demonstrated, PEG-SPIONs with a hydrodynamic diameter of 30 nm were labeled with $^{68}$Ga. They demonstrated that the most accumulation was in sentinel lymph nodes (SLN). Hence, it can be applied as a multimodal imaging probe and assessment of SLN by PET/MRI and in addition, can be used in surgery for optical guidance during resection of SLN. Because of Cherenkov light with high energy from $^{68}$Ga, therefore, detection of SLN can be facilitated. Furthermore, Hoffman et al. in their research demonstrated that more than 50 and 20 % of their PEGylated NPs were accumulated in liver and spleen [7]. Finally, results of this research verified that the accumulation of radiolabeled PEG-SPIONs depends on kind of coating and hydrodynamic size. Although, it has low effect by of the radionuclide types. It has been shown that $^{68}$Ga-PEG-SPIONs can be transferred by the lymphatic system and finally accumulated in liver and spleen [14, 34].

**Conclusion**

We obtained 100 % radiolabeling efficiency in labeling the SPIONs with $^{68}$Ga. On the other hand, the biodistribution data has shown that $^{68}$Ga-radiolabeled SPIONs had a good accumulation in liver and spleen. In conclusion, they have the capability to apply in PET/CT or PET/MRI imaging as a contrast agent for diagnosis of liver and spleen malignancies.

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**Compliance with ethical standards**

**Conflict of Interest** The authors declare that they have no conflict.

**References**

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