99mTc-Human Serum Albumin-Nanoparticle for Sentinel Lymph Node Identification

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Submitted 4 March 2019; Revised 27 March 2018; Accepted 29 March 2019; Published 13 May 2019
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Abstract
The sentinel lymph node is the first lymph node in a lymph node basin to receive lymphatic drainage from a primary tumor. An accurate identification and characterization of sentinel lymph node is very important as it helps to decide the extension of surgery, the tumor staging, and establishment of the most adequate therapy. Technetium-99m labeled Human Serum Albumin nanoparticle (99mTc-HSA nanoparticle) was prepare as a radiopharmaceutical with particle size between 100 - 200 nm that is used in lymphoscintigraphy technique for tracing lymphatic system and identifies the sentinel lymph node. Biodistribution study of 99mTc-HSA nanoparticle was conducted in mice to identify the accumulation of this agent in sentinel node and the other tissue. 99mTc-HSA nanoparticle showed good accumulation in sentinel node about 1.29 ± 0.90 %ID with value of popliteal extraction 89.55 ± 8.52 at one-hour post injection. This result was indicated that 99mTc-HSA nanoparticle is very promising compound to be further explored as sentinel lymph node imaging agent.

Keywords: Lymphoscintigraphy, 99mTc-HSA-nanoparticle, sentinel lymph node

1. Introduction
The lymphatic system produces lymphocytes and monocytes in the lymph glands which plays a role in the body’s defense against infections. Lymphosintigraphy is a method of tracing lymphatic flow and lymph nodes after injection of a radiopharmaceutical that is absorbed by the lymphatics which provides the results of scintigram imaging from the lymphatic system. This technique will identify blockage points in the lymphatic system and evaluate lymphedema; a condition where the blockage in the lymphatic system causes accumulation of lymph fluid in the soft tissue so that causes enlargement in the arm or leg of the patients. In the case of breast cancer, lymphscintigraphy is very useful to identify the tendency of breast cancer potentially metastasize with present or absence of radiopharmaceutical accumulation in sentinel node (SLN); the first lymph node to receive lymphatic drainage from a tumor site. This technique is very helpful for medical practitioners to determine cancer stadium and decide cancer treatment plan for biopsy or therapy. A lymphosintigraphy technique depends on particle size and surface characteristics of the injected radiopharmaceutical particles which influence the rate of colloid drainage from the injection site to the dermal lymphatic capillaries and phagocytosis by lymph node macrophages. Some radiopharmaceuticals containing particles have been used for lymphoscintigraphy such as 99mTc-sulfur colloid (10-1000 nm), filtered 99mTc-sulfur colloid (10-50 nm), 99mTc-antinomy colloid (5-20 nm) and 99mTc-human serum albumin (2-3 nm). However, all of these radiopharmaceuticals have a lack as an ideal radiopharmaceutical...
for lymphosintigraphy which inquiring requirement that easy to migrate from injection site and have good retention within sentinel node. Radiopharmaceutical with a small particle size will easily transported through the lymph vessel but has a short retention time, while larger particle will retained in the injecting area and making discomfort to the patient because extension of the duration time for lymphosintigraphy. To solve this problem, our laboratory developed Human Serum Albumin (HSA) radiopharmaceutical in the form of nanometer spherical particles which has ideal particle size (100-200 nm) made from the albumin protein and labeled by technetium-99mm radioisotope.

In previous studies, 99mTc-HSA-nanoparticles have successfully labeled with radiochemical yield more than 90%. Furthermore, 99mTc-HSA nanoparticles showed good accumulation in mesenteric node in inflammation mice on biodistribution study. 99mTc-HSA nanoparticles also gave sufficient results as radiopharmaceutical for bone marrow imaging.

The aim of this study was to determine biodistribution of 99mTc-HSA nanoparticles and its accumulation in the popliteal and lumbar lymph nodes as a SLN model in mice. The results of this study are expected to present biological data that is very useful for 99mTc-HSA nanoparticles as radiopharmaceuticals for to be used to identify SLN.

2. Materials and methods

The main material used in this study is the HSA nanoparticles, and [99mTc] Pertechnetate (Ansto), Whatman 3 MM paper, methanol, HCl (E. Merck), and saline. Mice (Mus musculus) with a uniform body weight (±30 g) used as animal model. Ketamine HCl (ketalar/Pfizer) and xylazine (seton 2%/Calier) are used for anesthesia procedures. Radioactivity was measured using dose calibrator (Victoreen), TLC-scanner (AR-2000, BIOSCAN) and a Single Channel Analyzer with NaI(Tl) detector (Ortec, Model 4890).

2.1. Preparation of 99mTc-HSA nanoparticles

99mTc-HSA nanoparticles was prepared according to the procedure described by Oekar et al. At first 50 μL HSA-nanopartikel was added 200 μL Sn-pyrophosphate solution (containing SnCl₂ 37.5 mg as reductor and 5 mg Na-pirofosfat as co – ligand which diluted in 5 mL of water). pH of the mixture was neutralized by addition of HCl 0.1 N then incubated for 15 minutes. Furthermore, 0.3 – 0.5 mL (1 mCi) of freshly eluted pertechnetate solution (99mTcO₄⁻) was added into the vial, then mixed and incubated for other 15 minutes in room temperature.

Radiochemical yield of 99mTc-HSA nanoparticles and other metabolites of 99mTc were determined by thin layer paper chromatography method. Chromatography systems are developed in Whatman 3 MM/NaCl 0.9%, Whatman 3 MM/Metanol 95% and Whatman 3 MM/HCl. The radiochemical yield was calculated as the ratio of the radioactivity of the labeled product to the total radioactivity.

2.2. In vivo studies

Biodistribution study of 99mTc-HSA nanoparticles was carried out on 10 mice which were anesthetized with a mixture of ketamine HCl (dose 0.16 mL / 200 g BB) and xylazine 2% (dose 0.06 mL / 200 g BB) before the test. A total of 20 μL of 99mTc-HSA nanoparticles with radioactivity of ±30 μCi was injected intradermal into left footpad of the mice. After injection, the footpad was massaged for 30 second. After 60 min injection, a 30 µL solution of the blue dye (patent blue V, SIGMA) in water was administered to the footpad. Ten minutes after the second injection, each animal was sacrificed by decapitation. The popliteal and lumbar lymph nodes, injection site (left paw) and tissues of interest were removed, weighed, and the radioactivity counts were determined with Single Channel Analyzer.

The popliteal extraction (PE) was estimated as follows (9):

\[ \text{PE} = (\% \text{ID popliteal} - \% \text{ID lumbar}) - 100\% (\text{ID popliteal}) \]

3. Results and discussion

99mTc-HSA nanoparticles are
radiopharmaceuticals made from human serum albumin in the form of nanometer (100-200 nm) which are dispersed in water for injection. This radiopharmaceutical has an ideal particle size for lymphosintigraphy techniques.

Quality control of the $^{99m}$Tc-HSA nanoparticles was assessed by thin layer paper chromatography to distinguish and quantify the amounts of radioactive contaminants. $^{99m}$TcO$_2$ was remaining at the point of spotting in Whatman 3 MM/HCl 1 N as the solvent, free $^{99m}$Tc moved with the solvent front in Whatman 3 MM using methanol 95% as the solvent and $^{99m}$Tc-pyrophosphate was found in Rf 0.5-0.6 in Whatman 3 MM/saline (Fig. 1.a, 1.b and 1.c). Therefore, by calculating the free $^{99m}$TcO$_4^-$, $^{99m}$TcO$_2$ and $^{99m}$Tc-pyrophosphate found that $^{99m}$Tc-HSA nanoparticles provide high radiochemical yield > 90% and can be used to carry out in vivo test.

Figure 1.a. Chromatogram profile of $^{99m}$Tc-HSA nanoparticles and TcO$_2$ with Whatman 3 MM/HCl 1 N

Figure 1.b. Chromatogram profile of $^{99m}$Tc-HSA nanoparticles and TcO$_4^-$ with Whatman 3 MM/Methanol 95%

Animal studies were done in animal models as molecular imaging tools to investigate lymph node metastases$^{12,13}$. Animal studies were conducted in accordance with ethical approval of Ethics Committee for Care and Use of Experimental Animal - National Nuclear Energy Agency No:004/KEPHP/BATAN/VIII/2016. Biodistribution of the radioactivity of $^{99m}$Tc-HSA nanoparticles in mice at 1 h post-intradermal injection was shown on Table 1.
Figure 1.c. Chromatogram profile of $^{99m}$Tc-HSA nanoparticles and $^{99m}$Tc-pyrophosphate with Whatman 3 MM/Saline

Table 1. Biodistribution of $^{99m}$Tc-HSA nanoparticles in mice at 1 h post-intradermal injection

| Organ                  | Radioactivity accumulation |
|------------------------|-----------------------------|
| Popliteal lymph node   | 1.29 ± 0.90*                |
| Lumbar lymph node      | 0.11 ± 0.08*                |
| Inguinal               | 0.21±0.17*                  |
| injection site         | 88.37 ±5.07*                |
| Popliteal extraction   | 89.55 ±8.52*                |
| Blood                  | 9.32 ±1.14*                 |
| Liver                  | 6.81 ±1.70*                 |
| Spleen                 | 4.96 ±1.75*                 |
| Stomach                | 3.23 ±0.91*                 |

* %ID; ^%ID/g; * (%ID popliteal-%ID lumbar ) x 100 / (%ID popliteal)

The radiopharmaceuticals for SLN detection should have rapid migration from injection sites to the SLN and prolonged retention of the radioactivity in the SLN$^{5,14}$. In this study, a murine model was used to understand the biodistribution of the radioactivity after intradermal injection of $^{99m}$Tc-HSA nanoparticle. $^{99m}$Tc-HSA nanoparticle showed good accumulation in popliteal lymph node as sentinel node in this experimental model about 1.29 ± 0.90 %ID and accumulation in lumbar lymph nodes, the secondary lymph node in this model was about 0.11 ± 0.08 %ID at one hour post injection. $^{99m}$Tc-HSA nanoparticle also showed radioactivity level in blood after intradermal injection. Since $^{99m}$Tc-HSA nanoparticle was a colloidal particle, it also showed radioactivity level in liver, spleen and stomach.

Popliteal extraction (PE) is a parameter to estimate the selectivity of the radiopharmaceuticals to the sentinel lymph node. The high PE value means that the radiopharmaceutical is specific to the sentinel lymph node. The popliteal extraction (PE) was estimated according to the equation found with value 89.55 ± 8.52 at 1 hour post injection, this value indicated that $^{99m}$Tc-HSA nanoparticle is very promising compound to be further explored as sentinel node imaging agent$^{11}$.

4. Conclusion

In this study, $^{99m}$Tc-HSA nanoparticles could be used as a radiopharmaceutical for mapping lymph nodes and clearly identified the lymph nodes in a short time. $^{99m}$Tc-HSA nanoparticles was also effective for sentinel lymph node identification.
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