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Research Article

Synthesis of ^{99m}Tc-labeled organo-germanium nanoparticles and their *in vivo* study as a spleen imaging agent

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Summary

^{99m}Tc-Labeled organo-germanium nanoparticles ranging in size from 60 to 80 nm were newly developed for a spleen imaging agent. The radiolabeled nanoparticles were prepared with a high labeling efficiency (over 99%) and they also showed an excellent stability at room temperature for 6 h. The biodistribution data of the nanoparticles injected into rats via intravenous routes showed a notably higher accumulation in the spleen when compared to other reticuloendothelial system organs. Gamma image of the rabbits obtained after an intravenous injection of the nanoparticles revealed a localization of the radioactivity mainly in the spleen and the liver. Copyright © 2006 John Wiley & Sons, Ltd.

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Key Words: organo-germanium compound; spleen imaging agent; ^{99m}Tc-complex

Introduction

The spleen is the largest lymphoid organ and located under the left side of the rib cage. As a result of certain diseases (i.e. leukemia, lymphoma, typhoid), the spleen may become very enlarged and it can be ruptured easily by a trauma.[1–3] Isotope spleen scan can be useful in a variety of clinical conditions.[4–7] It is known that ^{99m}Tc, as a radionuclide, has desirable nuclear properties, for example, a short half-life of 6 h and a 140 keV γ-ray emission energy suitable

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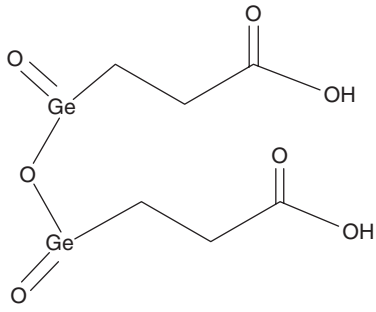

Figure 1. *bis*-Carboxyethylgermanium sesquioxide (Ge-132)

for obtaining gamma images as well as a low price and a general utility, thus it is commonly applied in nuclear medicine as radiopharmaceuticals for a diagnosis and therapy.[8,9] Imaging of the spleen is usually performed with colloids less than 1 μm in size. For example, ^{99m}Tc-sulfur colloid (10–500 nm), ^{99m}Tc-albumin colloid (nanocolloid), and ^{99m}Tc-RBC (red blood cell) have been used to demonstrate the structure of the spleen and any abnormality therein.[10] Bigger particles (e.g. ^{99m}Tc-MAA (macroaggregated albumin), 10–50 μm) formed by an agglomeration accumulate in the lungs, whereas smaller particles (e.g. ^{99m}Tc-ASC (antimony sulfide colloid), 1–13 nm) tend to localize in a bone marrow.[11,12] Thus, the particle size is an important factor to obtain a more specific spleen imaging, since this determines the uptake and retention of the particle in particular organs.

Here we report on an organo-germanium compound, *bis*-carboxyethylgermanium sesquioxide (Ge-132) as a ligand which is labeled with ^{99m}Tc for the development of a new spleen imaging agent. It is well known that Ge-132 (Figure 1) increases the interferon activity and natural killer (NK) cell activity of spleen cells in mice 24 h after an oral administration and it induces a peritoneal macrophage activity.[13,14] Its therapeutic attributes include an immunoenhancement, an oxygen enrichment, free radical scavenging, analgesia and a heavy metal detoxification.[15,16] High germanium concentrations were seen in a spleen after oral administration, and in the spleen and kidney after an i.p. injection.[17,18] We believe that these results can play a positive role in the imaging and treatment of a splenic disease.

Results and discussion

The formation of ^{99m}Tc-Ge-132 nanoparticles and their labeling efficiency and stability study

^{99m}Tc-Ge-132 nanoparticles with a narrow size distribution were formed when Ge-132 and sodium pertechnetate were treated with stannous chloride at pH 4. Direct evidence of the nanoparticle formation was obtained from the TEM

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study which showed spherical nanoparticles with diameters which ranged mostly from 60 to 80 nm (Figure 2). Instant thin-layer chromatography (ITLC) study of the particle also supports the nanoparticle formation. Immobility of the radioactive peak in the MEK and saline mobile phases at the solvent origin of the ITLC, indicated the formation of the particles, otherwise the peak moved to the solvent front in saline. The absence of other peaks other than the peak at the origin in the ITLC denoted that ^{99m}Tc was efficiently labeled with Ge-132 in the form of the nanoparticles without any noticeable generation of soluble ^{99m}Tc-complexes in the reaction medium. To investigate the influences of the pH in the preparation of the nanoparticles, the same reactions were performed in a media with different pHs (Figure 3). The results showed that the labeling yield of the ^{99m}Tc-Ge-132 nanoparticles was over 99% at pH 4 but decreased as the pH increased. These results can be

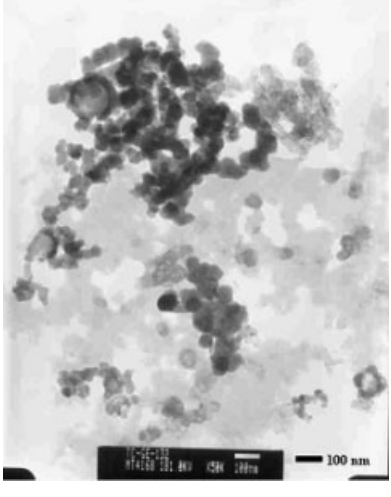

Figure 2. TEM image of the ^{99m}Tc-Ge-132 nanocolloids

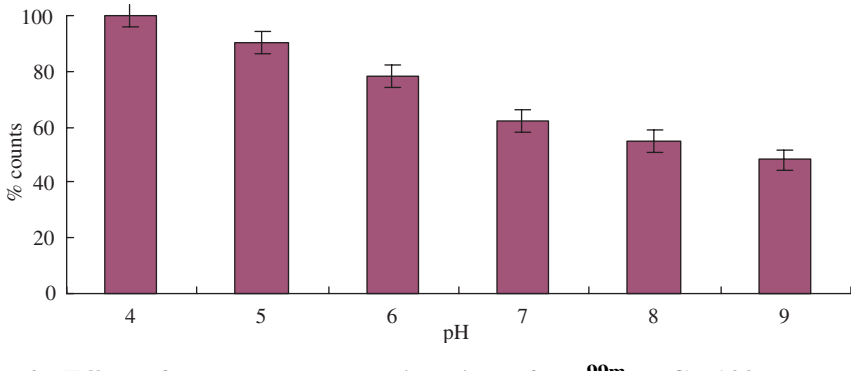

Figure 3. Effect of the pH on the labeling yield of the ^{99m}Tc-Ge-132 nanocolloids

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attributed to the insolubility of the stannous chloride ($SnCl_2 \cdot 2H_2O$) in higher pHs, which leads to a decrease in the labeling efficiency due to its insufficient reduction ability. During the reduction of the [^{99m}Tc]pertechnetate ions by using stannous chloride ($SnCl_2 \cdot 2H_2O$), insoluble SnO_2 colloids are frequently formed as byproducts and these colloids are known to decrease the labeling efficiency of the ^{99m}Tc complexes. However, we presume that the tin colloids may induce and/or facilitate the formation of the ^{99m}Tc-Ge-132 nanoparticles. In order to estimate the stability of the radiolabeled nanoparticles, the ^{99m}Tc-Ge-132 nanoparticles in closed vials were stored at room temperature and the labeling efficiency was determined at 0.5, 1, 2, 3, 4, 5, 6 h, respectively. The results indicated that the radiolabeled nanoparticles are stable up to 6 h without a decrease in the labeling efficiency.

Animal experiments

At 5, 30, and 60 min post-injection the biodistribution data was obtained from the uptake of the particles in the excised organs (blood, kidney, spleen, liver, heart, lung, stomach, and intestine). The biodistribution data showed that these nanoparticles were mainly accumulated in the reticuloendothelial system (RES) organs such as the spleen, liver, and lungs within a half-hour of an injection (Figure 4). Although other nanoparticles such as ^{99m}Tc-sulfur colloid, ^{99m}Tc-albumin colloid and ^{99m}Tc-tin colloid also showed a higher

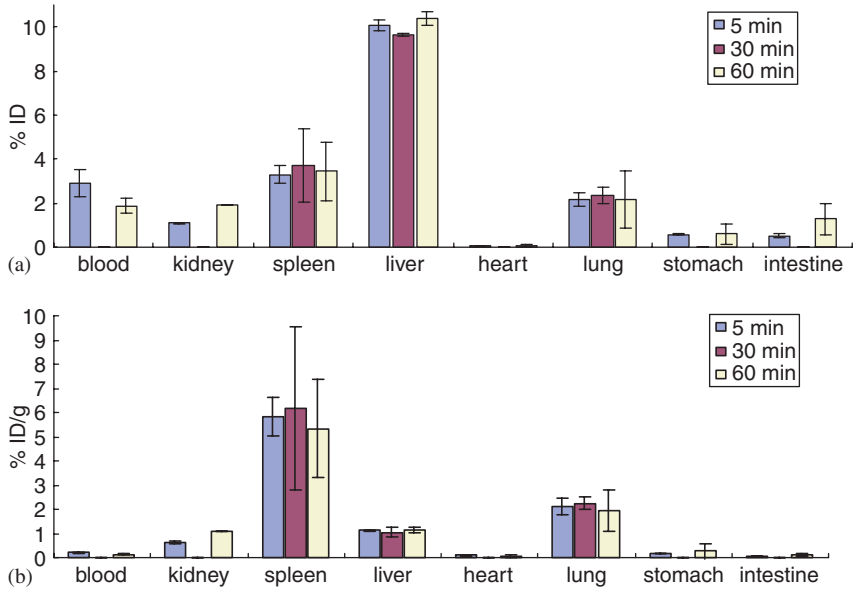

Figure 4. Percent of the injected activity of the ^{99m}Tc-Ge-132 nanoparticles in the organs: (a) expressed as percent injected dose/total tissue; and (b) percent injected dose/g tissue

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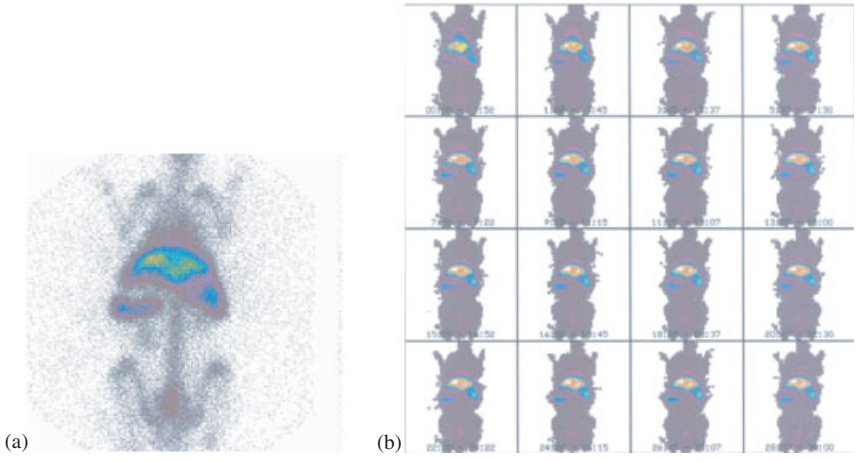

Figure 5. (a) Gamma image at 30 min; and (b) image scans for 30 min of a rabbit after an intravenous administration of the ^{99m}Tc-Ge-132 nanoparticles

accumulation in the RES organs due to their size effects,[19] it is especially notable that a significantly higher spleen uptake among the other RES organs was observed with the ^{99m}Tc-Ge-132 nanoparticles. High accumulation of the radiolabeled nanoparticles in the spleen was further validated by the gamma scintigraphic images of a rabbit as shown in Figure 5. The images, collected with a gamma camera for 30 min after an ear-vein administration of the ^{99m}Tc-Ge-132 nanoparticles, showed that the concentration of the radioactivity was highlighted in the spleen and the liver.

Conclusion

In this paper, we demonstrated that ^{99m}Tc-Ge-132 nanoparticles with a narrow size distribution (60–80 nm) as well as a high stability and labeling efficiency can be prepared by the reaction of *bis*-carboxyethylgermanium sesquioxide (Ge-132) with [^{99m}Tc]pertechnetate and applied as a spleen targeting imaging agent. The biodistribuion data of the nanoparticles showed several more localizations of the radioactivity in the spleen when compared to other RES organs. From the results, the ^{99m}Tc-labeled organo-germanium nanocolloids are considered to have an excellent diagnostic potential for a spleen imaging. Its medical applications are currently under investigation.

Experimental

General

Ge-132 was obtained from Geranti Pharm Ltd. (Seoul, Korea). Unless otherwise noted, all the chemicals were obtained from commercial suppliers and used without any further purification. Sodium pertechnetate ($Na^{99m}TcO_4$)

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was eluted from a $^{99}Mo/^{99m}Tc$ generator (Unitech 500, Samyoung Unitech Co. Ltd., Seoul, Korea) using 0.9% saline and the final radioactivity of the solution was adjusted to 10 mCi.

Preparation and TEM study of ^{99m}Tc-Ge-132 nanoparticles

2 mg (4.4 μmol) of Ge-132 was dispersed in 0.9 ml of a 0.9% NaCl solution (the pH of the solution was pre-adjusted to 4 by addition of 0.1 N HCl). To this solution, 0.1 ml of a $Na^{99m}TcO_4$ solution (10 mCi) and 0.1 mg (0.44 μmol) of a stannous chloride solution in 0.01 N HCl (10 μl) were added and the resulting solution was stirred for 30 min at room temperature to give a ^{99m}Tc-Ge-132 nanoparticle solution. All the solutions were purged with nitrogen gas prior to a mixing to remove the oxygen gas which affects the labeling efficiency. The colloidal solution was sterilized by a membrane filtration (0.22 μm) and kept in sterile reaction vials for a storage in a refrigerator. The particle size of the nanoparticles was measured by transmission electron microscopy (TEM) photographs recorded with a JEOL JEM 1200-EX electron microscope operated at 80 kV. Samples for the TEM were prepared by placing a drop of the colloidal solution on a Formvar/carbon-coated copper grid and allowing the solvent to evaporate.

Labeling efficiency and stability of ^{99m}Tc-Ge-132 nanocolloids

The labeling efficiency and stability of the ^{99m}Tc-Ge-132 nanoparticle were measured with an ITLC system which consisted of an ITLC scanner (EC & G Berthold Linear Analyzer, Germany) and a one-dimensional analysis of the Berthold chroma program. ^{99m}Tc-Ge-132 colloidal nanoparticle solution was spotted onto silica gel coated fiber sheets (Gelman Sciences Inc., Ann Arbor, MI, USA) and the plate was eluted with methyl ethyl ketone (MEK) or physiological saline. The labeling efficiency of ^{99m}Tc-Ge-132 nanoparticle was calculated by comparing the radioactivity of the nanoparticle peak (at the origin in MEK and saline) and the free pertechnetate peaks (at the solvent front in MEK and saline). The labeling stability of the ^{99m}Tc-Ge-132 nanoparticle at room temperature was measured with ITLC for up to 6 h in the same manner (Table 1).

Table 1. ITLC analysis of the ^{99m}Tc-Ge-132 nanocolloids

Chromatographic system		^{99m}Tc species at	
Support	Solvent	Origin	Solvent front
ITLC-SG	MEK	100% of ^{99m}Tc-Ge-132	0% of $^{99m}TcO_4^-$
ITLC-SG	Saline	100% of ^{99m}Tc-Ge-132	0% of $^{99m}TcO_4^-$

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Biodistribution of ^{99m}Tc-Ge-132 nanocolloids in rats

The biodistribution study of the ^{99m}Tc labeled Ge-132 colloidal nanoparticle was performed by using normal, female Sprague-Dawley rats up to 60 min after an i.p. injection of the 0.1 ml/10 μCi ^{99m}Tc labeled Ge-132 colloidal solution. Three rats were sacrificed at each time point (5, 30, and 60 min) and 0.1 g-samples from the blood, kidney, spleen, liver, heart, lung, stomach, and intestine were subjected to a radioactivity measurement which was conducted by a Beckman γ-counter.

Dynamic acquisition and analysis

Six-week old New Zealand white male rabbits (2887.6 ± 101.5 g, $n = 3$) which were anesthetized with ketamine and xylazine, were injected with ^{99m}Tc-Ge-132 nanoparticle solution (3 mCi/0.5 ml) via the left ear vein. All the rabbits were placed in a posterior position. To confirm the dynamic kinetics of the ^{99m}Tc-Ge-132 nanoparticles, whole body dynamic images for 30 min and 16 static images at predetermined time intervals were obtained by using a gamma camera fitted with a low energy all-purpose collimator. A 20% window was centered around 140 keV. Image data was analyzed under a dynamic procedure of the Microdelta system (Simens, USA). The static images were taken at 1.52, 3.45, 5.37, 7.30, 9.22, 11.15, 13.07, 15.00, 16.52, 18.45, 20.37, 22.30, 24.22, 26.15, 28.07, and 30 min post-administration with a Microdot imager (Simens, USA).

Acknowledgements

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