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Note

Biodistribution of dual radiolabeled lipidic nanocapsules in the rat using scintigraphy and γ counting

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Abstract

The aim of the present work was to study the biodistribution of a radiolabeled lipidic nanocapsule formulation after intravenous administration in rat by scintigraphy and γ counting. This formulation is expected to be used as anticancer agent delivery devices and as transfection complexes. For this purpose, ^{99m}Tc-oxine was incorporated in the lipidic core, while ^{125}I labeled tensioactive shell of the nanocapsule. First, in vitro stability of radiolabeled nanocapsules was evaluated by dialysis against distilled water and size measurements. Second, the nanocapsule biodistribution was followed after intravenous administration for 3 h by dynamic scintigraphic acquisition and up to 24 h by determining the gamma activity in blood and tissues. Radiolabeling was efficient and stable in vitro. After intravenous injection blood radioactivity decreased with an early half disappearance time of about 45 min for both radioisotopes. Liver and intestine radioactivities raised up to 24 h. The relatively long remanence in blood of the tracers which is probably due to the presence of PEG at the nanocarrier surface seems promising for the use of these solvent free lipidic nanocapsules as carrier of lipophilic drugs.

Keywords: Biodistribution; Lipidic nanocapsules; Radiolabeling; Technetium-99m; Iodine-125; Scintigraphy

A novel formulation of lipidic nanocapsules, without use of organic solvents, was recently developed in our laboratory (Heurtault et al., 2000). These are composed of a liquid lipidic core (medium chain triglycerides) surrounded by a tensioactive shell (2-hydroxy-stearate of polyethylene glycol and lecithin) and dispersed in an aqueous medium. They are prepared by a two-step method using inversion phase temperature properties. In order to adapt the carrier to various therapeutic goals, their size can be controlled (20–100 nm). These particles, prepared with innocuous excipients, present an alternative system to polymeric nanoparticles, emulsions and liposomes, and can be used as intravenously injectable drug carrier. Cellular membranes and biological barrier crossing is expected for application as lipophilic anticancer agent delivery devices or as transfection complexes in gene therapy. The main purpose of

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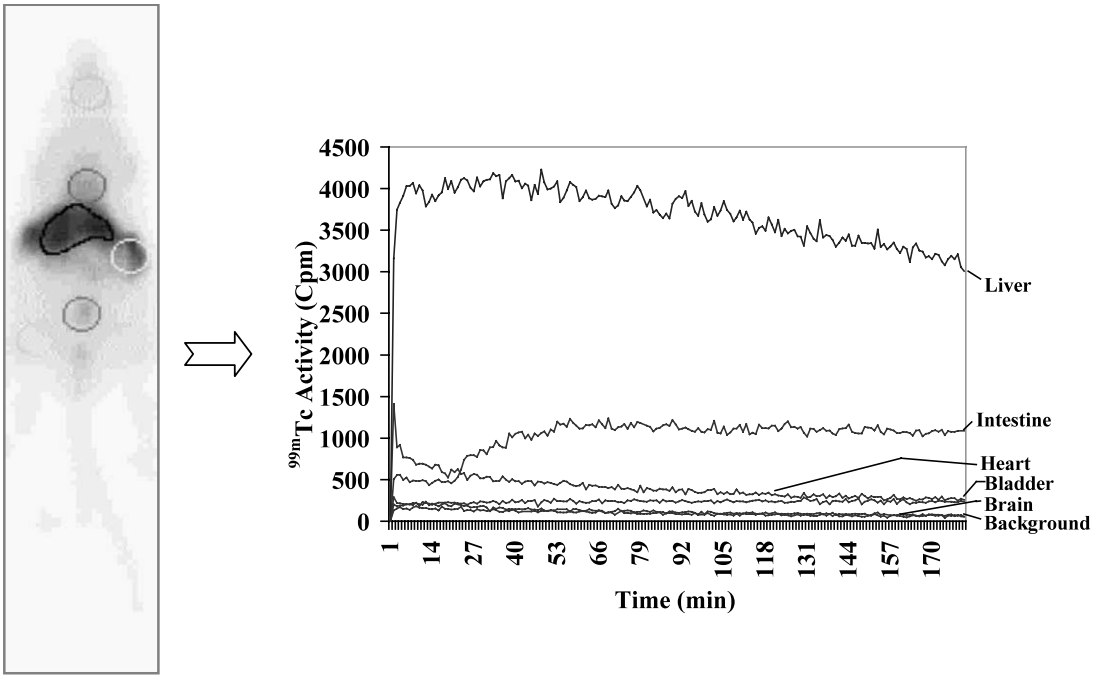

Fig. 1. Scintigraphic image of rat after intravenous injection of dual radiolabeled nanocapsules. Determination of regions of interest for different organs.

Fig. 2. Organ time activity curves after intravenous injection of dual radiolabeled nanocapsules in rats.

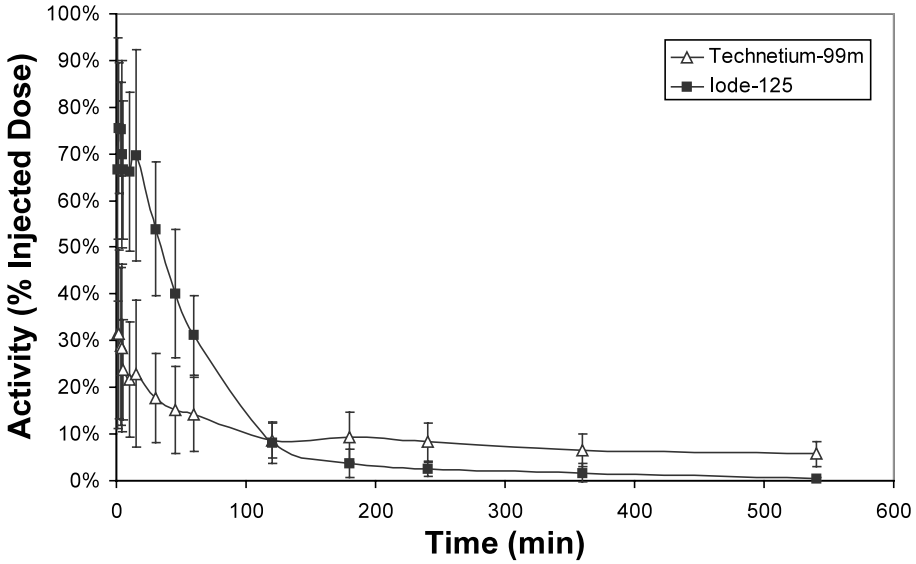

Fig. 3. Evolution of blood activity during 9 h expressed in percentage of injected dose. Each data point is mean ± range for seven rats.

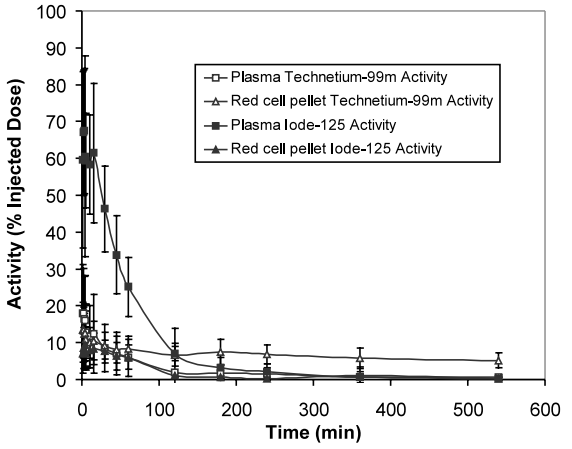

Fig. 4. Evolution of radioactivity blood repartition during 9 h expressed in percentage of injected dose. Each data point is mean ± range for seven rats.

this work was to label the nanocapsules and determine their in vivo fate in rats after intravenous administration, using scintigraphy and γ counting. In order to study their biodistribution, ^{99m}Tc-oxine was incorporated in the lipid core while ^{125}I was used for shell component labeling. After preliminary study about radiolabeling stability, animal investigations were performed by scintigraphy during 3 h, then rats were sacrificed at different times up to 24 h and organs were removed and counted.

^{99m}Tc (pertechnetate form) was obtained as a sodium solution by from ^{99}Mo generator (Cis Bio International). The labeling requires the reduction of pertechnetate by the stannous method in presence of a lipidic chelator, oxine, soluble in nanocapsule lipidic phase (mainly tricaprylin).

^{125}I labeling was performed by the Bolton Hunter method (Nen Life Science, Dupont) to the phosphatidylethanolamine (9% of the crude lecithin) through acylation reaction on free amine group. Efficiency was determined by gel filtration on LH20-100-Sephadex® (Sigma Chemical Company).

The procedure for the preparation of nanocapsules was concordant to the French patent no. 0002688 (Heurtault et al., 2000) one. All the components were mixed under magnetic stirring (Lipoid® S75-3, Solutol® HS 15, Labrafac®WL 1349, NaCl and water), with a rise from room temperature to 85 °C and a progressive cooling from 85 to 60 °C at a rate of 4 °C/min. Three temperature cycles (85–65–85–65–85–70 °C) were applied to obtain the inversion process.

All the compounds required for the radiolabeling (oxine, ^{99m}Tc, SnF_2, ^{125}I radiolabeled phosphatidylethanolamine) were mixed to the raw materials before starting the magnetic stirring.

Stability assays were performed by dialysis against distilled water and size measurements by photon correlation spectroscopy (PCS) using a Malvern Autosizer® 4700 (Malvern Instrument S.A.) fitted with a 488 nm laser beam at fixed angle (90°) and at 25 °C.

Biodistribution was evaluated following 1 ml intravenous injection of labeled nanocapsules (3.7 MBq ^{99m}Tc and 0.085 MBq ^{125}I, 4-h dialysis) to healthy Wistar rats (250–300 g). Images were obtained with a γ camera DSXI (S.M.V.I) by dynamic acquisition in the ^{99m}Tc window (140 Kev ± 20%) during 3 h after injection. They were analysed by selecting regions of interest over different organs. The activity of each organ was normalised according to the total body activity. Time activity curves were generated.

The blood content was estimated as a function of time on seven rats injected by 3.7 MBq ^{99m}Tc and 0.085 MBq ^{125}I and preleaved intravenously up to 24 h. Plasma was separated from cells by centrifugation at $3000 \times g$ for 6 min in gel filled Microtainer® microtubes. All samples were counted for activity.

After sacrifice (5, 15, 30, 90 min, 3 and 24 h post-injection) by exsanguination, organs were removed, washed with saline, weighted and gamma counted (Auto-Gamma 5000 series, Packard). Samples of bone marrow were obtained from the intact femur. Results were expressed as percentage of injected dose per gram and percentage of injected dose per organ.

At 4, 12, 24 and 48 h faeces and urines from rats placed in metabolic cages were collected and counted.

The mean ^{125}I labeling efficiency of phosphatidylethanolamine after passage through the LH20 column was 40 ± 5%. Radioactivity losses from the nanocapsule suspension determined after a 4-h dialysis against distilled water were around 27 ± 19% for ^{99m}Tc and 20 ± 9% for ^{125}I. Hydrodynamic diameter of the initial suspension was

65 ± 20 nm and no change was observed after dialysis.

Dynamic acquisition imaging showed, in the earlier times, hepatic retention after vascular phase followed by digestive elimination (small bowel activity beginning at 30 min). No significant activity was detected in the brain (Figs. 1 and 2).

Investigations on whole blood samples revealed an early half disappearance time of about 47 ± 6 min for ^{125}I and about 41 ± 11 min for ^{99m}Tc (Fig. 3). This relatively long residence time is probably due to the presence of PEG at the nanocapsule surface. ^{125}I was mainly located in plasma while the main part of ^{99m}Tc activity was found in red cell pellet (Fig. 4). This discrepancy between Iode-

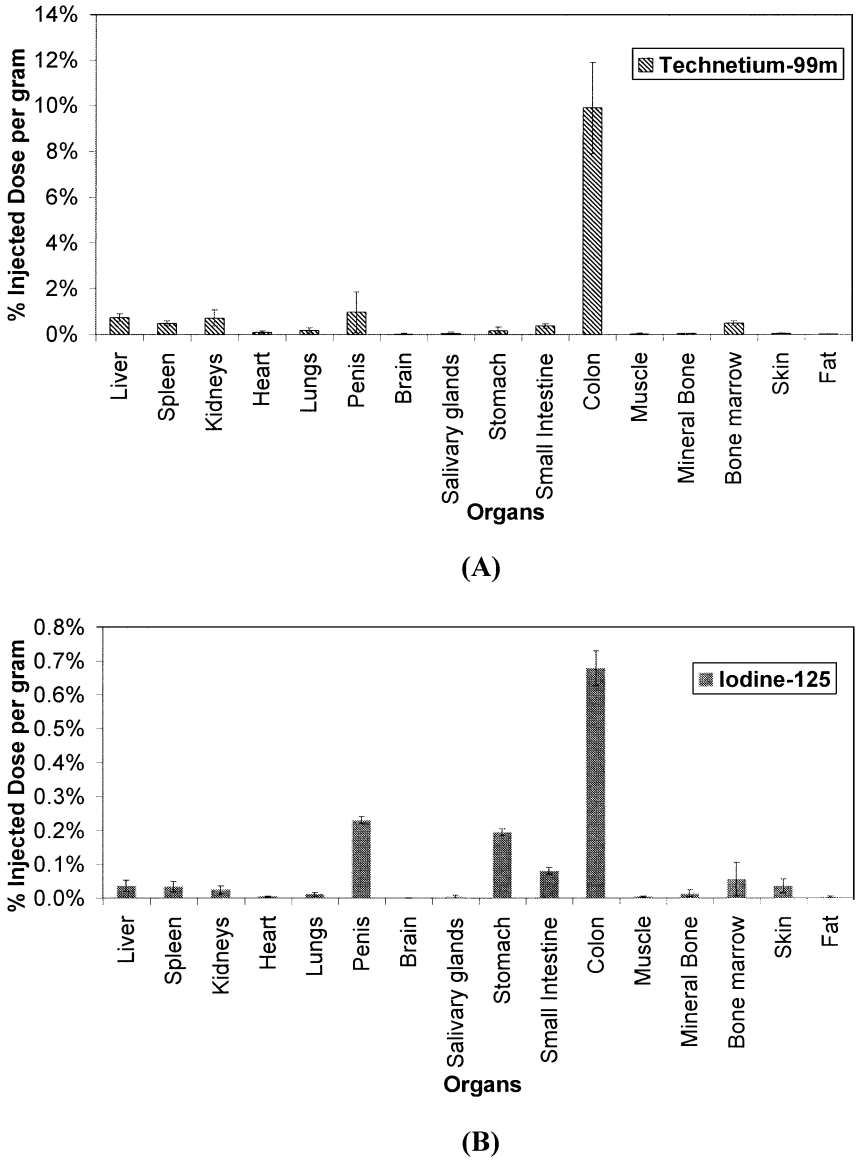

Fig. 5. Tissue distribution of ^{99m}Tc (A) and ^{125}I (B) labeled nanocapsules at 24 h post injection ($n = 7$).

125 and Technetium-99m distribution may be attributed either to exchange of the ^{99m}Tc-oxine complex with blood cell membranes or to adsorption of radiolabeled nanocapsules on blood cells with ^{125}I leakage in plasma.

After sacrifice at 3 h, ^{99m}Tc and ^{125}I activities were mainly found in liver and digestive tract (stomach and small bowel). At 24 h, the colon content was consequent but ^{125}I in stomach was also important (Fig. 5).

At early times, no significant activities were found in urines and salivary glands, in favour of bound forms of both ^{125}I and ^{99m}Tc atoms. Contrarily to ^{99m}Tc, iodine was excreted in urines after some hours up to 24 h.

The different pathways of ^{99m}Tc and ^{125}I activities suggest exchange phenomena of both tracers that needs to be understood more precisely through complementary studies.

Original lipidic nanoparticulate system radiolabeled with Iode-125 and Technetium-99m allows biodistribution studies by scintigraphy and γ counting. Radiolabeling was carried out with a good yield and a satisfactory in vitro stability. In vivo, the relatively long remanence in blood and the digestive excretion of the tracers seem promising for the use of these solvent free lipidic nanocapsules as carrier of lipophilic drugs.

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References

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