



Comparison of three remote radiolabelling methods for long-circulating liposomes

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ABSTRACT

Long-circulating liposomes (LCL) are often used as a drug carrier system to improve the therapeutic index of water-soluble drugs. To track these LCL *in vivo*, they can be radiolabelled with ¹¹¹In-oxine. For this labelling method, generally DTPA is encapsulated in the aqueous phase of LCL (DTPA-LCL). Alternatively, LCL can be labelled with ¹¹¹InCl₃ after incorporation of DTPA-conjugated DSPE in the lipid bilayer (DTPA-DSPE LCL). Here, we compared the *in vitro* properties of DTPA-DSPE LCL with those of DTPA LCL and empty LCL. Additionally, we compared the *in vivo* performance of DTPA-DSPE LCL with those of DTPA LCL in mice.

DTPA LCL (88 nm) and empty LCL (84 nm) were labelled with ¹¹¹In-oxine, and DTPA-DSPE LCL (83 nm) were labelled with ¹¹¹InCl₃. Labelling efficiency at increasing specific activity was determined. *In vitro* stability of ¹¹¹In-labelled LCL was determined in human serum at 37 °C. The *in vivo* properties of ¹¹¹In-labelled LCL were determined in mice with a *Staphylococcus aureus* infection in the thigh muscle. Image acquisition, blood sampling and biodistribution studies were performed 1, 4 (blood sampling only), 24, 48 and 72 h p.i. of ¹¹¹In-labelled LCL. DTPA-DSPE LCL could be labelled efficiently at a much higher specific activity compared to DTPA LCL and empty LCL: >90% at 15 GBq/mmol, >90% at 150 MBq/mmol and 60–65% at 150 MBq/mmol, respectively. ¹¹¹In-labelled DTPA-DSPE LCL and DTPA LCL were stable in human serum, regarding label retention, for at least 48 h at 37 °C (>98% retention of the radiolabel). In contrast, only 68% radiolabel was retained in empty LCL after 48 h. *In vivo* targeting of ¹¹¹In-DTPA-DSPE LCL to the abscess was comparable to targeting of ¹¹¹In-DTPA LCL (3.5 ± 0.9%ID/g and 3.4 ± 0.9%ID/g abscess uptake respectively, 48 h p.i.).

In conclusion, labelling of DTPA-DSPE LCL with ¹¹¹InCl₃ represents a robust, easy and fast procedure which is preferred over the more laborious conventional labelling of DTPA-LCL with ¹¹¹In-oxine.

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1. Introduction

Liposomes are small lipid vesicles with an aqueous core, which have been extensively studied since their discovery in the early 60s [1,2]. Coating with polyethylene glycol (PEG) sterically hinders binding of proteins that could act as opsonins, which enables them to escape recognition by macrophages of the reticuloendothelial system, thereby increasing their blood circulation time if given intravenously [3]. These long-circulating liposomes (LCL) have been extensively studied as a drug carrier system to increase the therapeutic index of drugs and reduce their systemic side-effects [4–6]. By virtue of increased

permeability of local vascular endothelium and their small size (80–120 nm in diameter), liposomes preferentially access pathological sites, such as inflamed, infectious or cancerous tissue, a phenomenon referred to as ‘passive targeting’ [7–9]. Retention of liposomes and drug delivery at target sites can also be enhanced by the attachment of antibodies, small molecules or peptides to the surface of the liposomes (‘active targeting’) [10–13]. To evaluate the pharmacokinetics and biodistribution of liposomes and their content *in vivo*, non-invasive imaging methods, such as positron emission tomography (PET) and single photon emission computed tomography (SPECT), are used [7,14]. For this end, liposomes have been radiolabelled with ¹¹¹In, ^{99m}Tc, ⁸⁹Zr, ⁶⁷Ga and ⁶⁴Cu and several remote labelling methods have been described [15–19]. Radioactivity is sequestered in the aqueous core, the lipid bilayer or on the surface of the liposomes. Entrapment of the radionuclides in the aqueous core and insertion in the lipid bilayer may

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require higher temperatures [20,21]. Because this might influence the liposomal properties and because the related labelling procedures are more laborious, surface labelling is the most convenient method to efficiently radiolabel liposomes, although firm attachment of the incorporated radionuclide after surface labelling has not always been determined. Since the half-life of ^{111}In (2.8 days) is sufficient for tracking the radiolabelled formulations during several days and the blood half-life of liposomes is also in the order of days (typically about 48 h), the use of ^{111}In is preferred in our experiments. To allow surface labelling with $^{111}\text{InCl}_3$, DTPA can be derivatized with a phospholipid that can be inserted in the lipid bilayer. Here, we used DTPA conjugated to 1,2-distearoyl-phosphatidylethanolamine (DSPE), which was incorporated in the lipid bilayer. Conventional ^{111}In -labelling of liposomes was achieved by the entrapment of ^{111}In in the aqueous core. In this method the soluble chelating agent diethylenetriamine pentaacetate (DTPA) is encapsulated in the liposomes and allows entrapment of the radionuclide by incubating the liposomes with the lipophilic chelate ^{111}In -oxine, that can pass through the lipid bilayer.

In the present study we determined the labelling efficiency, maximal specific activity and the stability of radiolabelling of 3 types of ^{111}In -labelled LCL: (1) DTPA-DSPE LCL surface-labelled with $^{111}\text{InCl}_3$ were compared with those of conventionally labelled liposomes containing (2) DTPA in their aqueous core and (3) empty LCL (without DTPA) labelled with ^{111}In -oxine. Additionally, we compared the *in vivo* performance of ^{111}In -DTPA-DSPE LCL with those of ^{111}In DTPA LCL in female NMRI mice with a focal *Staphylococcus aureus* infection.

2. Material and methods

2.1. Preparation of liposomes

DTPA-DSPE LCL, DTPA LCL and empty LCL (without DTPA) were prepared by injection of an ethanolic lipid solution into an aqueous dispersion medium (water for injection or saline), followed by extrusion, as described previously [22]. Briefly, dipalmitoylphosphatidylcholine (DPPC), 1,2-distearoyl-phosphatidylethanolamine-methylpolyethyleneglycol conjugate-2000 (mPEG2000-DSPE) (both from Lipoid GmbH, Ludwigshafen, Germany) and cholesterol (BUFA, Uitgeest, The Netherlands) (1.85: 0.15: 1 M ratio) were dissolved in ethanol by heating to 70 °C with continuous stirring. For the preparation of DTPA-DSPE LCL also 1.3 mM DTPA-DSPE (Avanti Polar lipids, Alabaster, AL, USA) was dissolved in the ethanolic lipid solution. In the case of DTPA LCL, 6 mM DTPA (Sigma Aldrich, St. Louis, MO) was dissolved in saline (B. Braun, Melsungen, Germany) as an aqueous dispersion medium. After dispersion of the ethanolic lipid solution in the aqueous solution the resulting coarse dispersion was passed through polycarbonate filter membranes with pore sizes of 200, 100 and 50 nm, to adjust the size of the liposomes to 100 nm diameter. Size distribution and polydispersity were determined by dynamic light scattering (triplicate measurements) on a Malvern 4700 system (Malvern Ltd., Malvern, UK). Ethanol was removed by dialysis against saline using Slide-A-Lyzer dialysis cassettes (Thermo Fisher Scientific, Etten-Leur, The Netherlands) with a molecular weight cutoff of 10 kDa and repeated changing of the dialysis medium (phosphate buffered saline (PBS)).

2.2. Radiolabelling procedure

Surface labelling of DTPA-DSPE LCL was performed by incubation of the liposomes (24.7 pmol total lipid–24.7 μmol total lipid) with 3.7 MBq $^{111}\text{InCl}_3$ (Mallinckrodt Pharmaceuticals, 's Hertogenbosch, The Netherlands) in 0.5 M 2-(N-morpholino)ethanesulphonic acid buffer (MES buffer, twice the volume of $^{111}\text{InCl}_3$), pH 5.4, for 30 min at RT. The amount of activity that was added during the labelling procedure ranged from 0.15–15,000 GBq/mmol total lipid. After incubation, 50 mM EDTA was added to a final concentration of 5 mM to chelate unincorporated ^{111}In . Labelling efficiency was determined using instant

thin layer chromatography (ITLC) on ITLC-SG strips (Agilent Technologies, Amstelveen, The Netherlands), using 0.1 M citrate buffer, pH 6.0, as mobile phase and gel filtration on PD-10 Columns (GE Healthcare Life Sciences, Buckinghamshire, United Kingdom).

Labelling of DTPA-DSPE LCL and empty LCL was performed by incubation of the liposomes (247 nmol total lipid–24.7 μmol total lipid and 2.47 μmol total lipid–24.7 μmol total lipid, respectively) with 3.7 MBq ^{111}In -oxine (Mallinckrodt Medical B.V., Petten, The Netherlands) in 0.2 M Tris buffer (50% of the total volume), pH 8.0, for 30 min at RT. So specific activity ranged from 0.15–150 GBq/mmol total lipid. After incubation, 50 mM EDTA was added to a final concentration of 5 mM. The labelling efficiency was determined by gel filtration on a disposable PD-10 column.

For the *in vivo* imaging and biodistribution experiments both liposomal preparations were labelled with 350 MBq ^{111}In , in order to obtain preparations with a specific activity of 150 MBq/mmol total lipid. The radiochemical purity of both radiolabelled preparations used in this experiment exceeded 95%.

All radiolabelling procedures were performed under metal-free conditions to prevent the interference of contaminating metals with DTPA.

2.3. *In vitro* stability of the radiolabelled liposomes

To examine the *in vitro* stability regarding label retention, radiolabelled liposomal preparations, DTPA-DSPE LCL were labelled with $^{111}\text{InCl}_3$ and DTPA LCL were labelled with ^{111}In -oxine with a specific activity of 150 MBq/mmol total lipid. The radiolabelled preparations were purified by gel filtration chromatography on a PD-10 column. These samples, with a radiochemical purity of 100%, were incubated in human serum (1:1) at 37 °C. At 24 h and 48 h, retention of the radiolabel to the liposomal preparations was determined by gel filtration chromatography on a PD-10 column. In case of ^{111}In -DTPA-DSPE LCL, retention of the radiolabel was also determined using instant thin layer chromatography (ITLC) on ITLC-SG strips, using 0.1 M citrate buffer, pH 6.0, as mobile phase.

2.4. DTPA – challenge assay

DTPA-DSPE LCL, DTPA LCL and empty LCL were radiolabelled with ^{111}In (specific activity of 150 MBq/mmol total lipid), diluted to a volume of 500 μl with saline and subsequently 500 μl DTPA solution, pH 7.4 was added. The final concentration of DTPA in the solutions was 10^{-5} , 10^{-4} , 10^{-3} and 10^{-2} M. Samples were incubated at 37 °C for 24 h and analysed by ITLC (^{111}In -DTPA-DSPE LCL) and by gel filtration chromatography on a PD-10 column (^{111}In -empty LCL and ^{111}In -DTPA LCL).

2.5. Animals

Female NMRI Mice, 4–6 weeks of age were purchased from Harlan (Horst, The Netherlands). Mice were housed in individually-ventilated cages (IVC) under standard laboratory conditions (temperature, 20–24 °C; relative humidity, 50–60%; 12 h light–dark cycle) and food (SNIFF Voer, Soest, The Netherlands) and water were available *ad libitum*. All animals were accustomed to the environment for at least one week before experiments were initiated. All *in vivo* experiments were approved by the institutional animal welfare committee of the Radboud University, Nijmegen, and were conducted in accordance with the principles laid out by the revised Dutch Act on Animal Experimentation (1997).

2.6. Murine thigh muscle infection model

To compare the *in vivo* properties of ^{111}In -DTPA-DSPE LCL with those of ^{111}In -DTPA LCL, both preparations were injected i.v. in mice with an intramuscular *S. aureus* abscess in the thigh muscle [23]. To induce the infection, mice were kept under anaesthesia (1–3% isoflurane in O_2), shaved and injected i.m. with 50 μl of bacterial suspension into the left

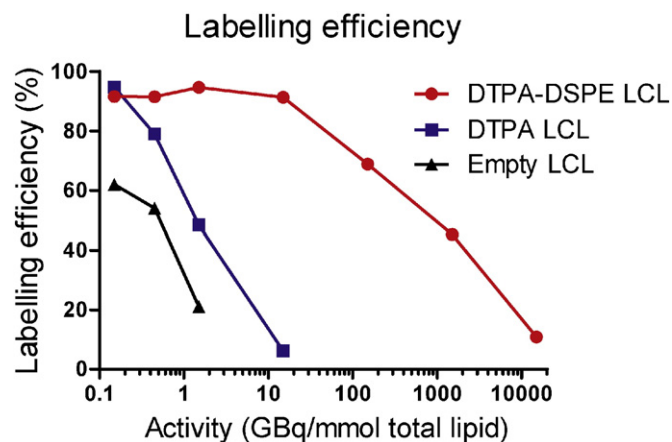


Fig. 1. Labelling efficiency after labelling of DTPA-DSPE LCL (red circles), DTPA-LCL (blue squares) and empty LCL (black triangles) with ^{111}In as a function of added activity (0.15–15,000 GBq/mmol total lipid).

thigh muscle (1.28×10^8 colony-forming units (CFU) per ml, mixed 1:1 with autologous blood).

Bacterial suspension was prepared by overnight incubation of *S. aureus* (ATCC 25923) in Brain Heart Infusion (BHI) broth (OXOID Deutschland GmbH, Wesel, Germany). The bacteria were centrifuged (10 min, 2000 $\times g$), the pellet was washed and resuspended in 0.9% NaCl solution and diluted to a final concentration of 2×10^9 CFU/ml. Infection dose was prepared by diluting, controlled by optical density, and verified by plating dilutions on agar plate. Colonies were counted after incubation for 24 h at 37 °C. ^{111}In -DTPA-DSPE LCL and ^{111}In -DTPA LCL were injected 24 h after induction of the infection, followed by microSPECT/CT imaging, blood sampling and biodistribution after dissection.

2.7. Micro-SPECT/CT imaging

NMRI mice were injected i.v. with 15 ± 0.6 MBq ^{111}In -DTPA-DSPE LCL (specific activity 150 MBq/mmol total lipid, 100 μmol total lipid/mouse in 200 μl , $n = 20$) or with 17.1 ± 0.3 MBq ^{111}In -DTPA LCL (specific activity of 150 MBq/mmol total lipid, 100 μmol total lipid/mouse in 200 μl , $n = 20$) 24 h after induction of *S. aureus* infection. Groups of 5 mice were euthanized at 1 h, 24 h, 48 h and 72 h p.i. by CO_2/O_2 suffocation. Subsequently, mice were placed prone in the U-SPECT-II/CT scanner (MILabs, Utrecht, The Netherlands) [24]. Mice were scanned for 45 min using a 1.0-mm-diameter cylindrical pinhole mouse ultra-high sensitivity collimator (UHS-M). SPECT scans were followed by CT scans for anatomical reference (spatial resolution of 160 μm , 65 kV, 615 μA). Scans were reconstructed with MILabs reconstruction software, which uses an ordered-subset expectation maximization algorithm, with a voxel size of 0.4 mm, 16 subsets and 1 iteration. Images were processed using Inveon Research Workplace 4.1 Software (Siemens Preclinical Solutions, Knoxville, TN, USA). After image acquisition, mice were dissected to determine the biodistribution and blood clearance of the radiolabel. At 1 h, 24 h, 48 h and 72 h p.i., tissues of interest (blood, heart, lung, abscess, muscle, spleen, pancreas, kidney, liver, stomach and duodenum) were dissected, weighed and radioactivity was measured in a shielded 3"-well-type gamma counter (Perkin-Elmer, Boston, MA, USA). For the determination of the blood clearance profiles, an additional blood sample was taken via a tail cut at 4 h p.i. Injection standards (1%) were counted simultaneously with the tissue samples to correct for physical decay and to allow calculation of percentage of the injected dose per gramme tissue (%ID/g).

2.8. Statistical analysis

Statistical analysis was performed using the non-parametric, Mann–Whitney *U* tests using GraphPad Prism software (version 5.03; GraphPad Software). All mean values are given \pm standard deviations. All tests were two-sided and a *p*-value of 0.05 was considered significant.

3. Results

3.1. Size distribution of DTPA-DSPE LCL, DTPA LCL and empty LCL

For direct comparison of *in vivo* characteristics, the liposomal preparations should have similar diameter and size distribution. As determined by dynamic light scattering, mean particle sizes of DTPA-DSPE LCL (83 ± 2 nm), DTPA LCL (89 ± 1 nm) and empty LCL (85 ± 2 nm) did not differ significantly (Kruskal–Wallis test $p = 0.08$). In addition to the mean particle size, polydispersity index was reported. This is a value between 0 and 1, 0 indicating monodispersity, whereas 1 indicates maximal variation in particle size. All preparations used had a polydispersity index < 0.11 (0.054 ± 0.003 for DTPA-DSPE LCL, 0.054 ± 0.009 for DTPA LCL and 0.109 ± 0.001 for empty LCL).

3.2. Labelling efficiency

Labelling efficiencies of DTPA-DSPE LCL, DTPA LCL and empty LCL with ^{111}In were determined. Increasing amounts of activity per mmol total lipid were added, ranging from 0.15 GBq/mmol total lipid to 15,000 GBq/mmol total lipid (Fig. 1). Labelled at a specific activity of 0.15 GBq ^{111}In per mmol total lipid, the labelling efficiency for DTPA-DSPE LCL as well as for DTPA LCL was $> 95\%$. The labelling efficiency of DTPA-DSPE LCL was $> 90\%$ up to a specific activity of 15 GBq/mmol total lipid, while the efficiency of the labelling of DTPA LCL markedly dropped when labelled at specific activities higher than 0.15 GBq/mmol total lipid. The labelling efficiency of empty LCL was least efficient (62%) when labelled at a specific activity of 0.15 GBq/mmol total lipid. The labelling efficiency further decreased, when labelled at higher specific activities. The maximum specific activity which can be achieved by surface labelling is more than two orders of magnitude higher than that of LCL with encapsulated DTPA or without DTPA (13.65 GBq/mmol, 0.14 GBq/mmol and 0.09 GBq/mmol total lipid, respectively).

3.3. Radiolabel retention *in vitro*

Since we aim to use these formulations *in vivo*, we assessed their stability toward retention of the radiolabel *in vitro* by incubation of ^{111}In -labelled LCL in 50% human serum (Fig. 2). This analysis showed that

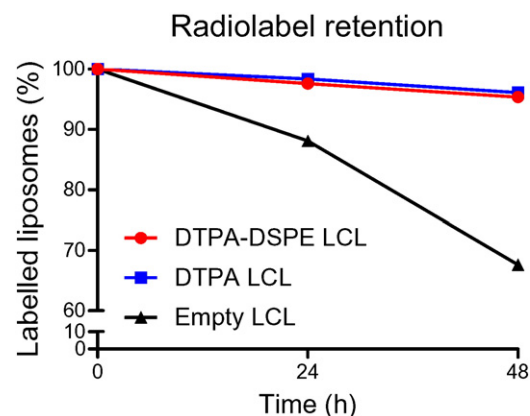


Fig. 2. Stability of DTPA-DSPE LCL (red circles), DTPA-LCL (blue squares) and empty LCL (black triangles) in 50% human serum at 37 °C.

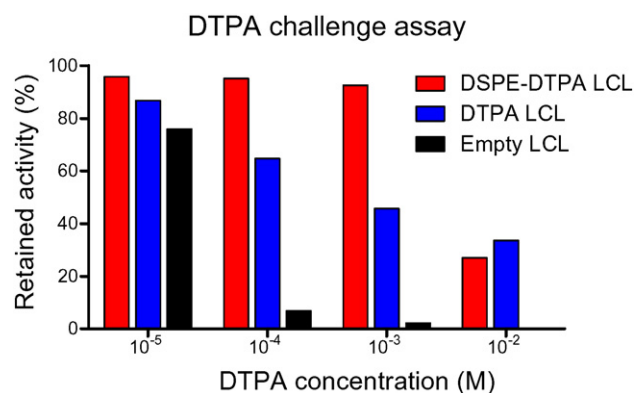


Fig. 3. DTPA challenge assay in which ^{111}In -labelled LCL (DTPA-DSPE LCL = red bars, DTPA-LCL = blue bars and empty LCL = black bars) were incubated in DTPA solutions for 24 h at 37 °C. Activity that retained to the LCL was measured and is shown as a percentage of total activity.

more than 95% of the label remained associated with the liposomes after 48 h of incubation, indicating that both DTPA-DSPE LCL and DTPA LCL were stably labelled with ^{111}In . In case of the empty LCL, 88% of the

label was associated with the liposomes after 24 h and only 68% after 48 h, indicating that the ^{111}In -labelling of this formulation yielded inferior results.

To further characterize the stability of the ^{111}In -labelled liposomal preparations, the radiolabelled formulation was exposed to increasing DTPA concentrations (10^{-5} M to 10^{-2} M). We evaluated the transchelation of the radiometal from labelled LCL to DTPA in the solution after 24 h of exposure at 37 °C (Fig. 3). At the maximum DTPA concentration tested (10^{-2} M), the amount of ^{111}In transferred to DTPA in solution was high for all labelled formulations. The amount of ^{111}In that remained associated with the DTPA-DSPE LCL was high (93%) up to a DTPA concentration of 10^{-3} M. This was significantly higher as compared to ^{111}In associated with DTPA LCL (46%) and empty LCL (2%).

3.4. MicroSPECT/CT imaging

To investigate the *in vivo* targeting properties of the liposomal formulations, ^{111}In -labelled liposomes were injected i.v. into NMRI mice with a focal *S. aureus* abscess in the left thigh muscle. ^{111}In -labelled LCL were injected 24 h after induction of the infection and SPECT/CT imaging was performed 1 h, 24 h, 48 h and 72 h after injection. ^{111}In -DTPA-DSPE LCL and ^{111}In -DTPA LCL accumulated in abscess, liver and spleen, but little background activity in other tissues (Fig. 4). Image acquisition

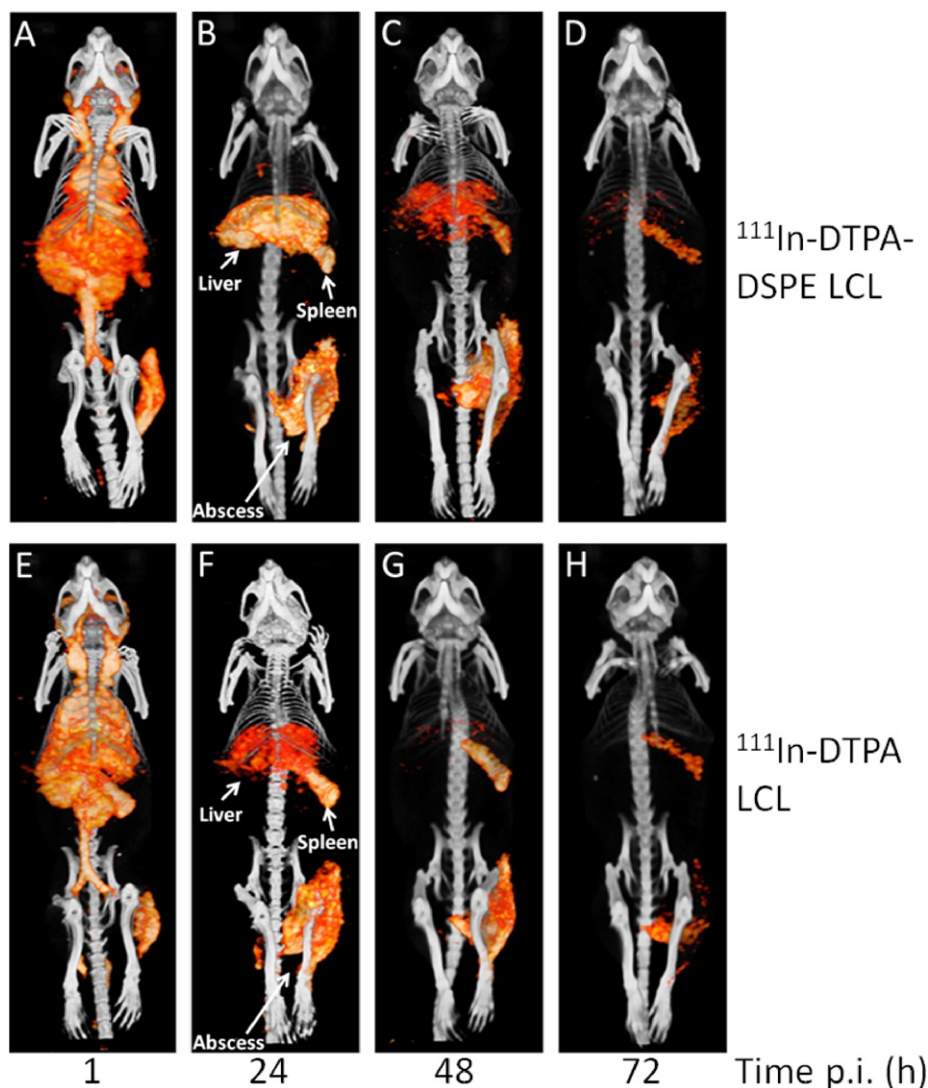


Fig. 4. MicroSPECT/CT imaging with ^{111}In -DTPA-DSPE LCL (A–D) and ^{111}In -DTPA-LCL (E–H) at 1 h, 24 h, 48 h and 72 h p.i. to visualize the targeting properties of the radiolabelled liposomal formulations in mice infected with *S. aureus*. Representative images at all time points are shown.

at 1 h p.i. also visualized the larger vessels and the heart (Fig. 4A and E). Whole body distribution of both preparations was comparable, with exception of the hepatic uptake, which was higher for the ^{111}In -DTPA-DSPE LCL.

3.5. Biodistribution studies

Biodistribution studies of mice that were injected with ^{111}In -DTPA-DSPE LCL and ^{111}In -DTPA-LCL showed that the abscess targeting capabilities were comparable for both formulations at all time points, which is in line with the SPECT/CT imaging results (Fig. 5A). The most striking difference in uptake of the formulations was observed in the liver. Hepatocal accumulation of ^{111}In -DTPA-DSPE LCL was significantly higher at all time points ($p = 0.008$, Fig. 5B), while the uptake in the spleen was comparable (Fig. 5C). Additionally, blood levels, based on quantification of the radiolabel, decreased at later time points, which was reflected by visualization of the large vessels and heart (Fig. 5D).

4. Discussion

In the present study we demonstrated that both DTPA-DSPE LCL and DTPA LCL could be efficiently labelled with ^{111}In , though the maximal specific activity of DTPA LCL might be a limited factor. Empty LCL were labelled less efficiently. We also showed that ^{111}In -labelled DTPA-DSPE LCL and DTPA LCL, retained the label quantitatively in human serum for at least 48 h at 37 °C. Additionally, we demonstrated that both ^{111}In -DTPA-DSPE LCL and the conventionally labelled ^{111}In -DTPA LCL showed efficient targeting to the *S. aureus* infection *in vivo*.

We showed that the surface labelling procedure of DSPE-DTPA LCL with $^{111}\text{InCl}_3$ allows for efficient labelling at high specific activities. The maximum specific activity that can be achieved by surface labelling is two orders of magnitude higher than that DTPA-LCL, 13.7 GBq/mmol

total lipid and 0.14 GBq/mmol total lipid respectively. This might be explained by the higher concentration of activity in $^{111}\text{InCl}_3$ during the labelling procedure (370 MBq/ml) compared to the activity in ^{111}In -oxine (37 MBq/ml). The smaller reaction volume results in a faster reaction, and might influence the labelling efficiency. Additionally, instability of ^{111}In -oxine before it enters the liposomes might also play a role [25,26]. ^{111}In may dissociate from the oxine and will be chelated by EDTA in solution. This is also the case for empty LCL, for which the labelling efficiency and maximum specific activity that could be achieved was even lower (0.09 GBq/mmol total lipid). Due to the absence of a chelating agent in the empty liposomes, ^{111}In -oxine which has crossed the lipid bilayer, is not efficiently trapped in the aqueous core, which results in a less efficient ^{111}In -labelling.

While both the surface labelling and the conventional labelling procedure resulted in a stable preparation in human serum, labelling of empty LCL was not. Since there is no DTPA present in the core of the empty liposomes, the ^{111}In -oxine and the ^{111}In that dissociates from the oxine may leak out of the liposomes and transchelate to serum proteins.

Additionally, the excellent stability of the ^{111}In -DTPA-DSPE LCL was confirmed in a DTPA challenge assay. Most of the incorporated ^{111}In was still associated with the ^{111}In -DTPA-DSPE LCL at DTPA concentrations up to 10^{-3} M. Interestingly, we found that, despite the presence of DTPA, the ^{111}In -labelled DTPA LCL were also less stable than the ^{111}In -DTPA-DSPE LCL. We hypothesise that part of the ^{111}In does not trans-chelate to DTPA in the hydrophilic core, but is non-specifically bound to the lipid bilayer. This part of the ^{111}In -oxine may leak out of the liposomes and transchelate to DTPA in the solution. Again we found that ^{111}In -labelled empty liposomes were not stable. These results imply that *in vitro* ^{111}In -DTPA LCL are less stable than ^{111}In -DTPA-DSPE LCL regarding ^{111}In label retention. This might also be the case *in vivo*.

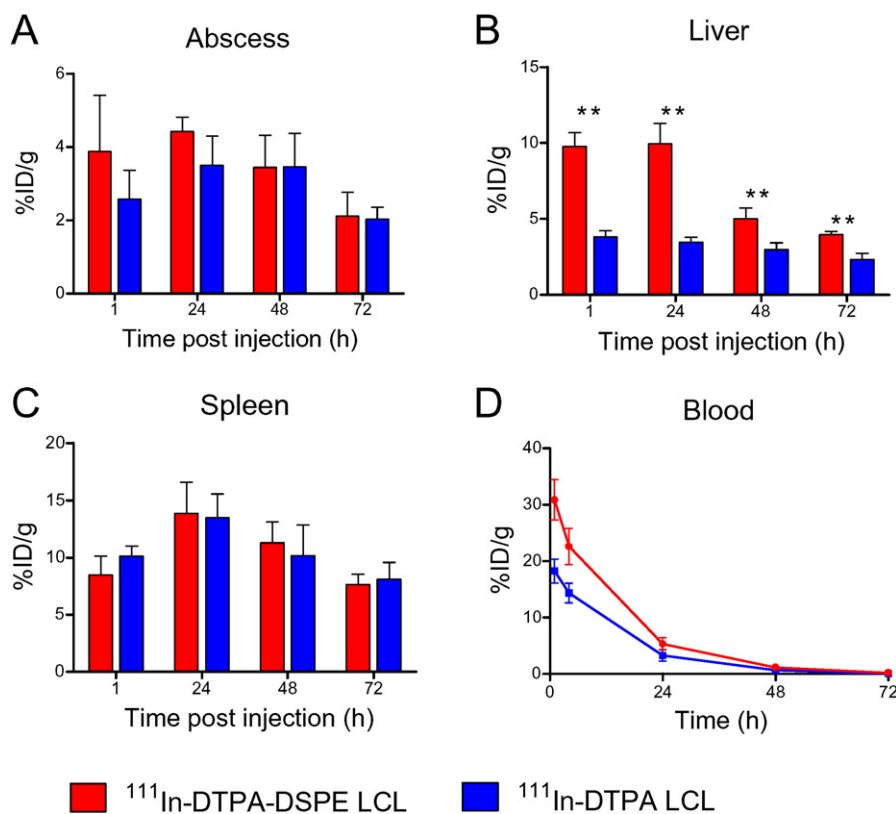


Fig. 5. Tissue uptake in abscess (A), liver (B), spleen (C) and blood (D) after injection of ^{111}In -DTPA-DSPE LCL (red) and ^{111}In -DTPA LCL (blue) expressed in percentage injected dose per gramme tissue. Due to differences in tissue uptake per organ, the y-axes vary in range. Biodistribution studies were determined at 1 h, 24 h, 48 h and 72 h p.i. Blood samples were taken 1 h, 4 h, 24 h, 48 h and 72 h p.i.

To further characterize the radiolabelled liposomal preparations, *in vivo* experiments were performed. Based on the results of the *in vitro* stability assays with empty LCL, we decided not to use this formulation in the *in vivo* experiments.

DTPA-DSPE LCL and DTPA LCL were similarly PEGylated, both were cleared mainly via the hepatosplenic mononuclear phagocyte system (MPS) and they were comparable in size. Based on these characteristics, a comparable biodistribution of intact ^{111}In -labelled formulations is expected. However, we slightly altered the outer layer of the liposomes by addition of DTPA-DSPE. This might change the interaction with serum proteins and increase the propensity of DTPA-DSPE LCL for MPS macrophages, as has also been observed after injection of actively targeted LCL (*i.e.*, LCL with surface-attached targeting ligands) [27].

Additionally, the difference we found in *in vitro* stability of ^{111}In -labelled formulations might also affect the biodistribution of ^{111}In due to label release in the circulation. We found that the tissue distribution of both liposomal formulations was comparable except for hepatic uptake. Accumulation in the liver was significantly higher for ^{111}In -DTPA-DSPE LCL. Part of this uptake might be blood level-driven, since the blood levels of ^{111}In -DTPA-DSPE LCL were higher and the liver is highly vascularised. However, also a higher degree of label release in case of DTPA LCL may have contributed.

When ^{111}In or ^{111}In -oxine is released from the liposomes, transchelation to serum proteins (*i.e.* transferring) can take place. Both ^{111}In chelated to serum proteins (transferrin, albumin) as ^{111}In -DTPA dissociated from the liposomes, will accumulate in excreting organs, leading to rapid clearance of the radionuclide [28]. In contrast, ^{111}In -DTPA-DSPE is released from the ^{111}In -DTPA-DSPE LCL during degradation of the liposomes which have ended up in the MPS cells, like Kupffer cells [29]. Our data show that ^{111}In -labelled DTPA LCL are less stable *in vivo* and as a result, the injected ^{111}In label is cleared faster from the body, leading to lower hepatic uptake at all time points. Splenic uptake was not affected since this uptake is size related and the formulations did not significantly differ in size. Targeting properties of both formulations to the *S. aureus* abscess were comparable.

5. Conclusions

Labelling of DTPA-DSPE LCL with $^{111}\text{InCl}_3$ is a robust, easy and fast procedure which is preferred over the more laborious conventional labelling of DTPA-LCL with ^{111}In -oxine. In addition, the DTPA-DSPE LCL could be labelled more efficiently and at a higher specific activity. The *in vivo* targeting properties to the *S. aureus* infection were comparable for both formulations. Regarding the detachment of ^{111}In label from circulating liposomes, ^{111}In -DTPA-DSPE LCL were extremely stable *in vitro* and may be also more stable *in vivo* compared to the ^{111}In -DTPA LCL. Therefore, ^{111}In -DTPA-DSPE LCL are to be preferred in future imaging studies of the biodistribution and targeting capability of LCL.

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