A Novel Method to Radiolabel Stealth Liposome through 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-DTPA with ^{99m}Tc and Biological Evaluation

Mirel Cabrera[a], Alejandra Medrano[b], Nicole Lecot[a], Marcelo Fernández[a], Maria Moreno[c], Jose A Chabalgoity[c], Juan Pablo Gambini[d], Omar Alonso[d], Henia Balter[a] and Pablo Cabral[a,*]

[a]*Arera de Radiofarmacia, Centro de Investigaciones Nucleares, Facultad de Ciencias, Universidad de la República. Mataojo 2055, 11400 Montevideo, Uruguay*

[b]*Departamento de Ciencia y Tecnología de Alimentos, Facultad de Química, Universidad de la República. General Flores 2124, 11800, Montevideo, Uruguay*

[c]*Departamento de Desarrollo Biotecnológico, Instituto de Higiene, Facultad de Medicina, Universidad de la República. Av.Alfredo Navarro 3051, 11600, Montevideo, Uruguay*

[d]*Centro de Medicina Nuclear, Hospital de Clínicas "Dr. Manuel Quintela", Facultad de Medicina, Universidad de la República. Av. Italia s/n, 11600, Montevideo, Uruguay*

Abstract: *Purpose*: To study surface technetium labeling of stealth DTPA-Liposome and to evaluate its potential as a molecular imaging tracer for both normal and melanoma-bearing mice.

The radiolabeling yield of liposomes was greater than 90% and showed good chemical and biological stability. Biodistribution studies in normal mice showed blood clearance with hepatic and renal depuration. Melanoma-bearing mice showed a similar pattern of biodistribution with high tumor uptake allowing tumor imaging.

The developed method of surface radiolabeled DTPA-PEG-Liposomes with ^{99m}Tc was effective and stable *in vivo*.

Keywords: Liposome, nanomedicine, diagnostic oncology.

INTRODUCTION

Nanotechnology is a multidisciplinary field, which covers a vast and diverse array of devices: these devices include nanovectors for the targeted delivery of anticancer drugs and imaging contrast agents [1].

Liposomes are hollow microspheres that develop spontaneously when water is added to a dried lipid mixture [2-3]. These nanoparticles have been used as delivery vehicles since the 1960s, and their role as imaging agents has been explored [4-5]. In nuclear medicine, radiolabeled compounds are administered to patients for diagnostic or therapeutic purposes. Diagnostic radiopharmaceuticals are labeled with a radionuclide emitting gamma radiation (photons). Photons with the appropriate energy can penetrate tissues and can be detected outside the body and visualized using a gamma camera [6].

In order to use liposomes as a diagnostic tracer, they should preferably need to be labeled with technetium-99m (^{99m}Tc). This is due to the appropriate physical properties of ^{99m}Tc, such as its short half –life (6h) and gamma photon emission of 140 keV, which render it suitable for effectively imaging patients [7].

Liposomes can be radiolabeled by simply adding the ^{99m} TcO_4^- to the aqueous medium that is used to hydrate the lipids. However, this method has a major disadvantage; it has a low labeling efficiency (generally lower than 10 %) [8]. Therefore, strategies have been developed to radiolabel preformed liposomes with high efficiency. Two different approaches for radiolabeling preformed liposomes can be distinguished: firstly, the radionuclide or a carrier molecule can be transported through the lipid bilayer and trapped in the internal aqueous phase of the liposome [9, 10]. Secondly, liposomes can be labeled by coupling the radiolabel to the lipid bilayer, either directly to the surface or through a chelator [11-13], as the one we performed through Diethylene triaminepentaacetic acid (DTPA).

The term "steric stabilization" has been introduced to describe the phenomenon of polymer mediated protection. Chemical modification of liposome with certain synthetic polymers, such as PEG, is the most frequently used approach to enhance *in vivo* longevity

*Address corresponding to this author at the Arera de Radiofarmacia, Centro de Investigaciones Nucleares, Facultad de Ciencias, Universidad de la República. Mataojo 2055, 11400 Montevideo, Uruguay; Tel: 0059825250800; Fax: 005985250895; E-mail: pcabral@cin.edu.uy

ISSN: 1927-7210 / E-ISSN: 1927-7229/13

of drug carriers. On the biological level, coating liposomes with PEG prevents drug carrier interaction with opsonins and slow down their capture by the mononuclear phagocyte systems (MPS) [14, 15]. During angiogenesis, the vascular system undergoes phenotypical changes. Often, the vasculature is characterized by an increased capillary permeability, a characteristic that has been used for passive targeting of liposomes to diseased sites [16]. Liposomes are thought to accumulate in tumor tissue owing to the leaky vascular barrier around the tumor [17].

Radionuclide imaging after [^{99m}Tc]–DTPA-PEG-Liposome injection would provide a noninvasive method for tumor diagnosis. This paper will focus on the strategy for surface radiolabeling of stealth liposome with the chelator DTPA and its potential use as an *in vivo* non- invasive melanoma scintigraphic diagnostic agent.

METHODS

Preparation of Liposomes

Liposomes were prepared by the hand shaking method, typically 9 mg of phosphatidylcholine (Avanti Polar Lipids), 4 mg of cholesterol (Sigma Aldrich), 1 mg of 1,2- dimyristoyl-sn-glycero-3-phosphoethanolamine-N-DTPA (Avanti Polar Lipids) and 1 mg of 1,2 dimyristoil-sn-glycero phosphoethanolamine-3-N-metoxi (polyetilenglycol-5000) (Avanti Polar Lipids), were added in chloroform-methanol (2: 1 v/v) medium to a round bottom flask. The lipids were rotaevaporated to dryness in a 60 °C water bath at 120 rpm. The obtained film was hydrated with 5 ml of bidistilled water, at 60 °C per 60 minutes at 120 rpm. Then the multi-lamellar large vesicles (MLV) obtained were extruded through a serie (11, 0.4 µm; 11, 0.1 µm) of polycarbonate filters at 60ºC to form unilamellar liposomes.

Liposome Characterization

Differential Scanning Calorimeter (DSC)

DSC was performed with a Shimadzu DSC 50 device previously calibrated with indium. All determinations were performed at least in duplicate. Hermetically sealed aluminum pans were prepared to contain the freeze-dried liposomes (5 mg). The samples were scanned at 1ºC/ min from 5ºC to 60ºC. As a reference, an empty aluminum pan was used. Enthalpy changes and characteristic transition temperatures were calculated using the Software thermal analysis system TA- 50 WSI.

Droplet Size Distribution

Droplet size distribution was estimated by laser light diffraction and polarized light dispersion using a particle size analyzer Coulter Counter Multisizer (Coulter Electronics Ltd.), the d3,2 and d4,3 indices were determined.

Phospholipids Quantification

Total lipids were calculated by phospholipid quantification of inorganic phosphate determination (Bartlett Test). We performed quantitative determination of inorganic phosphates by a technical development of molybdenum blue color and extent of absorption by a spectrophotometer at 882 nm: the sample was compared with standard solutions of phosphates that receive the same treatment as the liposomal suspension.

Radiolabeling of DTPA-PEG-Liposomes and Labeling Efficiency

Labeling with ^{99m}Tc requires the reduction of pertechnetate; and this was accomplished by the stannous reduction method. In order to label liposomes, 0.1 ml of $SnF_{2\cdot2}H_2O$ was added from 0.69 mg/ml solution to 0.8 ml of DTPA-PEG-liposomes and 222Mbq of $^{99m}TcO_4^-$ and incubated for 20 minutes at room temperature. The labeling yield and radiochemical purity were estimated by ascending instant thin layer chromatography, ITLC (Pall Corporation). The efficiency of labeling was evaluated using different chromatographic systems: a) saline/ITLC-SG, (NaCl, Fluka) b) pyridine: acetic acid: water (3:5:1.5 v/v) /ITLC - SG. The activity of each segment was then expressed as percentage of the total activity on the strip. Also, in order to estimate the labeling efficiency, a size exclusion chromatography (PD-10 column) was performed with saline buffer, NaCl 0.09 %, as eluent, obtaining a 0.5 ml fraction.

Transchelation Studies

Stability and strength of the binding of PEG-DTPA-liposomes were determined by mixing the suspension with an equal volume (0.5 ml) of different solutions of L-Cysteine (SIGMA) (0.1, 10 and 30 mM) in a water bath for 1 and 3 hours at 37°C. The mixture was then spotted on ITLC-SG paper for development in a 0.9 % NaCl. In order to differentiate ^{99m} TcO_4^- of [^{99m}Tc]-Cysteine chromatography was performed in Whatman 1 and methyl ethyl ketone (MEK).

Animal Model

The B16-F1 murine melanoma cell line, obtained from the American Type Culture Collection, was maintained in Dulbecco's modified Eagle's medium (DMEM) (PAA Laboratories GmbH, Pasching, Austria) supplemented with 10% fetal bovine serum (PAA Laboratories GmbH) and 2 mM glutamine (AppliChem GmbH, Darmstadt, Germany). Six-week-old C57BL/6 mice (URBE, Uruguay) were used in all experiments. All the protocols for work conducted with mice were previously submitted to and approved by the National Committee for Animal Experimentation (CHEA, Uruguay). Melanomas were established in mice by subcutaneous injection of 2.5×10^5 B16-F1 melanoma cells in 0.1 ml of phosphate-buffered saline (PBS). After two weeks of inoculation, the tumors were palpable and the mice were ready for biodistribution and scintigraphic studies.

Biodistribution Studies

Biodistribution studies were carried out in normal C57 black mice (n=15) and in C57 black melanoma bearing mice (n=6). The normal mice received a 19-20 MBq dose of the [^{99m}Tc]-DTPA-PEG-liposomes by intravenous (i.v.) injection through the tail vein. The C57 black melanoma bearing mice were administered an i.v. injection through the tail vein of 15-17 MBq dose of [^{99m}Tc]-DTPA-PEG-Liposome.

The normal mice were sacrificed by cervical dislocation at 2 (n=5), 4 (n=5) and 24 h (n=5) after the injection. Melanoma bearing mice were sacrificed by cervical traction at at 4 (n=3) and 24 h (n=3). Biodistribution of liposomes was determined after weighing and measuring the radioactivity in organs and tissues in a solid scintillation counter NaI (Tl) 3"x3" crystal detector associated with an ORTEC single channel analyzer at fixed time intervals. The percentage mean uptake per tissue was calculated and uptake ratios were generated

Liposome Blockade of the Reticuloendothelial System

In order to saturate reticuloendothelial system (RES) cells, liposomes without radiotracer (called "cold liposome"), were injected 30 min before injection of [^{99m}Tc]-DTPA-PEG–liposomes. Groups of 5 mice per concentration per time point were used in the study. Cold liposomes were administered to C57 black mice through the tail vein at a concentration of 0.75 mg (n=10) and 1.5 mg (n=10). Control group (without blockage) consisted of 10 C57 black mice. Biodistribution measurements were carried out at 1 and 3 h post injection of a 8-10 MBq dose of [^{99m}Tc]-DTPA-PEG-liposome.

Study of Liposomal Metabolization

Four female C57 black mice (weighing 19-22 g) were administered purification fractions of ^{99m}Tc-DTPA-liposmes-PEG (by column PD-10). Each mouse received a dose of 3 MBq. Biodistribuition was carried out after 1 h post-injection: afterwards urine was removed by manual extraction. The urine from 4 mice was collected for analysis by molecular exclusion, in which 400µL of urine were eluted with NaCl 0.9% using a PD-10 system, collecting 0.5 ml fractions. The chromatographic systems used for their analysis were the same as the ones used to evaluate the radiochemiacal purity of ^{99m}Tc-PEG-DTPA-liposomes. These studies were performed using a drop of urine, that was left to dry and followed its development.

Scintigraphy

Scintigraphic imaging was performed in normal C57 black mice, weighing 25-30 g and C57 black melanoma bearing mice, weighing 25-30 g using a gamma camera (Sophy DSX, Sopha Medical, Buc, France) connected in series with a dedicated computer system (Mirage Segami, Columbia, MD). Mice were injected with 18 MBq of [^{99m}Tc]-DTPA-PEG-Liposome through the tail vein and were anesthetized by intramuscular injection of Ketamine- Xylazine at the time of imaging (100-10 mg/ Kg). The mice were imaged after 4 and 24 h of administration of [^{99m}Tc]-DTPA-PEG-Liposome.

RESULTS

Liposome Characterization

The sample of liposomes had a phospholipid concentration of 1.2174 mg/ml and had a nanoscopic size D(4,3) of 0.155 µm. The transition event was found to occur at 37.2 ◦C with an enthalpy of 301 mJ/g, as shown in Figure **1**.

Liposome Radiolabeling

The radiolabeling yield of [^{99m}Tc]-DTPA-PEG-Liposome was superior to 90%.

In saline/ITLC-SG the [^{99m}Tc]-DTPA-PEG-Liposome was retained at the point of application ([^{99m}Tc]-DTPA-PEG-Liposome Rf = 0; $^{99m}TcO_2.H_2O$ Rf = 0; and the Rf of $^{99m}TcO_4^-$ was 0.9-1).

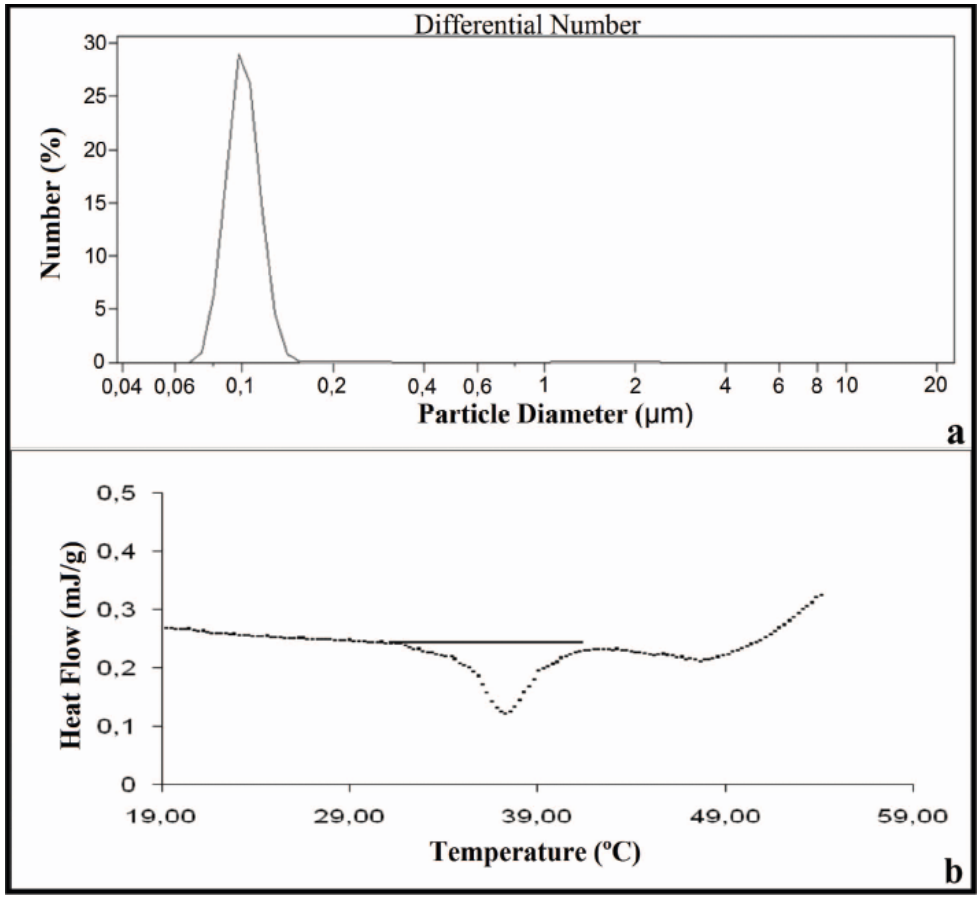

Figure 1: (**a**) Particle number distribution of the liposome using a particle size analyzer Coulter Counter Multisizer. (**b**) Representative DSC of liposome formulation for heating scan. Phase transitions of liposomes: ΔH: 301 mJ/g; Td: 37.5 ºC.

In pyridine: Acetic acid: water /ITLC-SG the [^{99m}Tc]-DTPA-PEG-Liposome migrates with the solvent front ([^{99m}Tc]-DTPA-PEG-Liposome Rf 0.9-1; $^{99m}TcO_4^-$ Rf 0.9-1; and the Rf of $^{99m}TcO_2.H_2O$ was 0).

In size exclusion chromatography (PD-10 column), the [^{99m}Tc]-DTPA-PEG-Liposome was obtained at 2.5-3 ml of elution and $^{99m}TcO_4^-$ at 7 ml of elution.

In Vitro Stability

The stability of the [^{99m}Tc]-DTPA-PEG-Liposome complex in the presence of different concentrations of cysteine showed that, at 0.1 mM cysteine, 90% of ^{99m}Tc was bound to DTPA-PEG-Liposome 3 h later. With 10 and 30 mM cysteine, 87% and 78% of ^{99m}Tc was bound to DTPA-PEG-Liposome respectively 3 h later (Figure **2**).

Biodistribution Studies

Biodistribution of [^{99m}Tc]-DTPA-PEG-Liposomes in normal C57 black mice are shown in Tables **1-2** after 2, 4 and 24 hour post-injection.

The results showed hepatic uptake (27.1% ± 6.9, 23.4% ± 2.9, 24.0%± 5.5 at 2, 4 and 24h respectively) and urinary excretion (10.0%± 1.3, 4.9% ± 0.6, 6.4% ± 0.5 at 2, 4 and 24h respectively) in normal C57 black mice (Table **1**)

Melanoma-bearing mice showed a similar biodistribution pattern (Table **2**). Significant tumor uptake was observed 4 h after injection (1.579 ± 1.112) with a tumor: muscle relation of 4.

Liposome Blockade of the Reticuloendothelial System

A decrease in hepatic uptake at 1 hour post-injection of 70% was observed in the mice that had cold liposomes in relation to the control group. Also, activity in blood diminished by 33% and 59% for groups with 0.75 and 1.5 mg of cold liposomes blockage respectively. We also observed an increased urinary excretion of 35% and 157% in respect to the control group with 0.75 and 1.5 mg of cold liposomes blockage respectively.

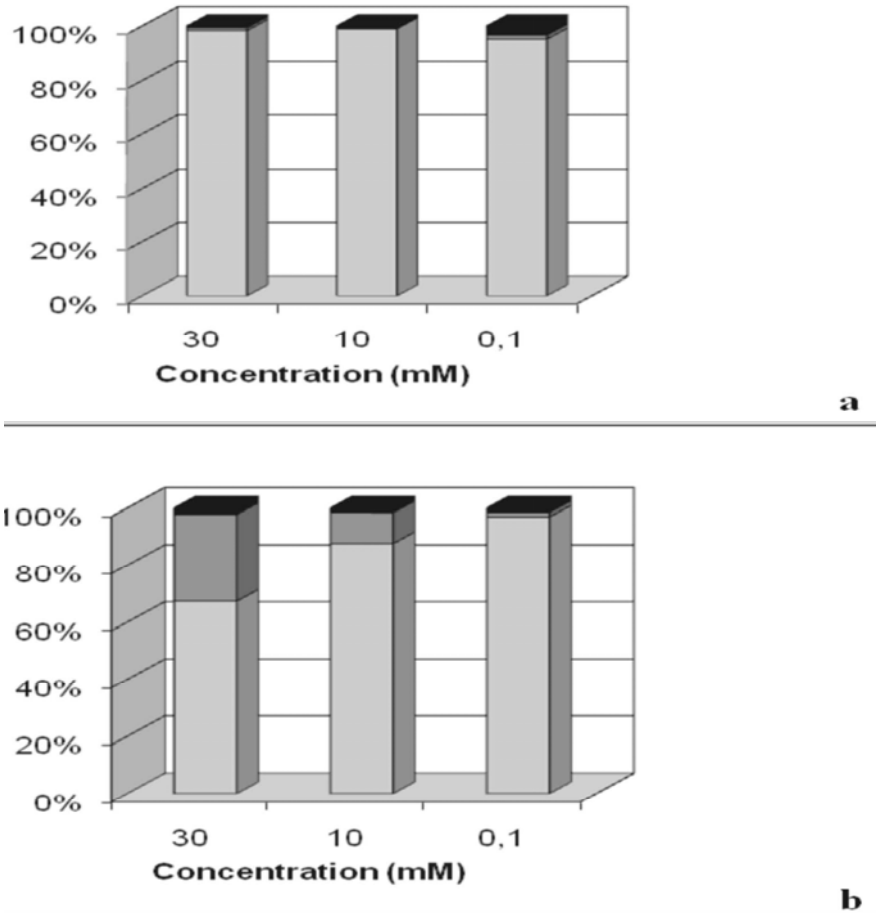

Figure 2: *In vitro* stability study of [^{99m}Tc]-DTPA-PEG-Liposome. L-Cysteine challenge with different concentrations performed at 1 (**a**) and 3 h (**b**) at 37°C in a water bath.
■ $^{99m}TcO_4^-$, ▒ [^{99m}Tc]-Cysteine, □ [^{99m}Tc]-DTPA-PEG-Liposome.

Table 1: Biodistribution of C57BL/6 Mice Weight 24.6 ± 3.1g. Receiving 19-20 MBq of [^{99m}Tc]-DTPA-PEG- Liposome

Biodistribution	Normal mice		
	2 h (n = 5)	4 h (n = 5)	24 (n = 5)
Blood	2.65±0.18	2.98±0.00	1.71±0.02
Liver	26.99±6.94	23.36±2.86	24.04±5.50
Heart	0.42±0.23	0.37±0.04	0.46±0.24
Lungs	4.34±0.80	2.40±0.15	1.90±0.90
Spleen	6.89±0.09	3.52±1.02	13.30±2.77
Kydneys	10.04±1.33	4.85±0.60	6.37±0.48
Thyroid	0.53±0.22	0.47±0.09	0.80±0.16
Muscle	0.19±0.08	0.17±0.03	0.14±0.03
Bone	0.76±0.30	0.37±0.10	0.03±0.57
Stomach	0.48±0.31	1.25±0.30	0.39±0.26
Guts	5.57±1.95	3.17±0.09	8.36±5.93

Data presented as % ID/g ± SD.

Table 2: Biodistribution of B16-F1 Melanoma-Bearing C57BL/6 Mice Weight 25.6 ± 5.6 g. Receiving 15-17 MBq of [^{99m}Tc]-DTPA-PEG- Liposome

Biodistribution	4 h (n = 3)	24 h (n = 3)
	^{99m}Tc-DTPA-PEG-Liposome	^{99m}Tc-DTPA-PEG-Liposome
Blood	6.91±2.31	5.31±2.43
Liver	58.21±7.25	53.49±4.83
Heart	2.17±0.31	2.21±0.61
Lungs	5.97±0.99	5.36±0.79
Spleen	10.26±5.13	3.76±1.27
Kydneys	8.75±1.65	4.55±1.88
Thyroid	1.07±0.26	1.69±0.69
Muscle	0.40±0.09	0.89±0.43
Bone	0.76±0.37	1.79±0.04
Stomach	0.42±0.17	2.25±1.27
Guts	1.23±0.79	4.93±1.47
Tumor	1.58±1.11	1.29±0.09

Data presented as % ID/g ± SD.

At three hours liposomes had a decreased hepatic uptake, being more pronounced (50%) in the group with 1.5 mg of blocking. We observed a slower blood clearance in the group with 0.75 mg of blockage than in the other two groups. The urinary excretion instead presents comparable levels of activity in the control group and in the group with 0.75 mg of blockage, while the group with 1.5 mg of blockage presents an increase in urinary excretion over 100%.

Liposome Metabolization

The PD-10 profile does not match the profile of labeled liposomes, being compatible to fragments of

Table 3: Biodistribution of C57BL/6 Mice Receiving Tail Vain Injection of 8-10 MBq [^{99m}Tc]-DTPA-PEG- Liposome 30 min After Injection of Differents Amounts of Unlabeled Liposomes, 0.75 mg(n=10) Weight 29.6 ± 5,4g; 1.5 mg (n=10) Weight 21.7 ± 3,0g; Control Group (n=10) Weight 23.4 ± 3.0g

Biodistribution	1 h (n = 5)			3 h (n = 5)		
	Without blockage	0.75 mg blockage	1.5 mg blockage	Without blockage	0.75 mg blockage	1.5 mg blockage
Blood	40.45±8.41	27.01±3.13	16.47±1.79	8.02±0.42	10.39±1.50	5.08±0.40
Liver	53.64±0.03	14.30±0.86	16.04±5.02	22.64±3.03	19.39±3.60	11.83±1.63
Heart	0.53±0.05	0.43±0.10	0.46±0.13	0.32±0.01	0.28±0.06	0.09±0.01
Lungs	0.88±0.01	1.07±0.04	1.29±0.19	0.61±0.11	0.40±0.14	0.27±0.04
Spleen	2.58±1.01	1.61±0.81	6.32±0.41	3.31±0.42	1.21±0.29	1.37±0.13
Kydneys	12.30±0.01	4.05±1.21	4.48±0.50	3.45±0.28	3.08±0.30	1.45±0.20
Thyroid	0.20±0.04	0.42±0.16	0.40±0.14	0.25±0.03	0.13±0.01	0.08±0.00
Muscle	3.20±2.21	6.13±0.69	8.07±3.59	6.00±0.92	4.77±0.55	1.33±0.29
Bone	0.14±0.08	0.25±0.06	0.29±0.07	0.19±0.04	0.28±0.05	0.12±0.03
Stomach	1.19±0.13	0.37±0.08	0.56±0.21	0.50±0.03	0.50±0.12	0.19±0.00
Guts	5.39±0.40	4.93±0.13	5.78±1.81	7.94±1.15	8.11±0.56	4.05±0.17
Urine + Bladder	13.99±5.05	19.82±7.19	36.29±16.50	34.25±2.57	37.30±8.75	72.81±1.39

Data presented as % ID ± SD.

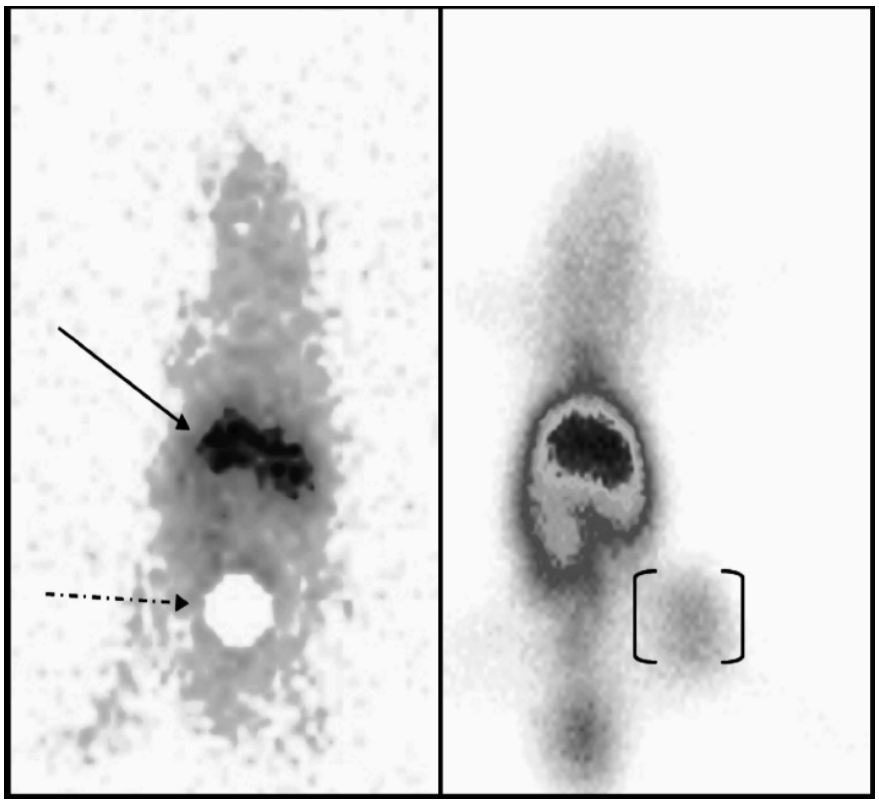

Figure 3: Scintigraphy image of normal and melanoma-bearing mice injected with [^{99m}Tc]-DTPA-PEG-liposome at 4 h post injection.
Solid and dashed arrows point liver and blad (where a mask was placed) respectively. Bracket shows the region where the tumor was located.

liposomes. There was no evidence of $^{99m}TcO_4^-$ as demostrated by chromatographic systems, being ^{99m}Tc attached to lipids derivatized with DTPA.

Radionuclide Imaging

Scintigraphic images at 4 and 24 hours post injection of [^{99m}Tc]-DTPA-PEG-Liposome in C57 black mice showed hepatic uptake and bladder activity due to renal depuration. In melanoma bearing mice, there was also tumor uptake that was evident at 4hs and persisted in the 24 h views.

DISCUSSION

Liposomes have been used as delivery vehicles since 1970's and have also been evaluated as an imaging agent [18-20], being currently used in clinical practice [21].

Initial attempts to target liposomes to tumors proved to be disappointing because the majority of the particles rapidly accumulated in the liver and spleen after intravenous (i.v.) injection. Liposomes are removed from the circulation by phagocytic cells of the RES.

Although partly successful, reduction of the size, inclusion of cholesterol and minimization of charge have shown to be valuable tools to reduce the recognition of the liposomes by the MPS [17].

Early liposome imaging studies employed a direct method of liposome labeling using stannous chloride as a reducing agent to associate reduced ^{99m}Tc with the liposomal surface. This labeling method was subsequently shown to be unstable *in vivo* and led to the development of more effective liposome labeling methods [22].

In the present study, liposomes were designed to allow their conjugation to radionuclides (^{99m}Tc) to be used as *in vivo* non invasive melanoma scintigraphic diagnostic agent. For this purpose a lipid derivatized with DTPA (1,2- dimyristoyl-sn-glycero-3-phosphoethanolamine-N-DTPA) was used in its composition due to its stability and the fact that it does not require the use of a co-ligand. The proposed labeling strategy showed an appreciable blood clearance of labeled liposomes, in accordance with prior reports [23-25], with hepatic and renal elimination. Renal elimination was higher in normal mice (Table **1**)

than in tumor bearing mice (Table **2**). This fact may be due to the retentition of the liposomes by the tumor. The observed renal elimination may be explained by the fact that liposomes are metabolized in the body and the radionuclide remains bound to small fragments of lipid that may be filtered at the glomerulus. We explored this hypothesis, and our results did not find labeled liposomes in urine being in accordance with previous reports [26].

To study how biodistribution of liposomes varies with the injected concentration, studies were performed using various amounts of cold liposomes prior to the injection of labeled liposomes. We observed an increase in the blood clearance and a decrease in liver uptake as the amount of the cold liposomes increased. We also observed an increase in renal elimination. Cold liposomes may alter radiolabeled liposome distribution on a concentration basis. These facts may be used as a strategy to produce a liver blockage prior to radiolabeled liposomes administration in order to optimize scintigraphic imaging by decreasing hepatobiliary excretion or also to enhance drug delivery.

Radiolabeled liposomes were evaluated as a non invasive *in vivo* scintigraphic imaging agent in melanoma bearing mice. This tumor is an appropriate model to test radiolabeled liposomes because it presents a well developed vasculature that can allow liposome extravasation through leaky blood vessels [27].

The development of liposomes with long circulating residence time would increase the possibility that they can stay in contact with the tumors and be trapped by them. It has been previously reported that PEG coated liposomes have a longer half life during blood circulation [28]. However, whereas a prolonged circulation half life of liposomes is needed to enhance tumor uptake, a slower blood clearance would hamper tumor visualization at early time points, producing tumor images of a lower quality as the blood background might remain high [29]. On the contrary, our imaging results with a dose of 0.4 mg of radiolabeled liposomes showed tumor uptake even at 4 h post injection. We also observed a fast blood clearance of the radiolabeled liposomes. Even with low doses, PEG liposomes were rapidly cleared from the circulation and were taken up by the RES [30]. This fact allows an increase in tumor visualization even at early time points due to a decrease in the background activity.

These results had a similar pattern of distribution in normal and melanoma-bearing mice.

CONCLUSION

The developed method to surface radiolabel stealth DTPA-Liposome with ^{99m}Tc was effective and showed to be stable *in vitro* and *in vivo*. This labeling method may also be extended to other radionuclides, such as 177Lu to combine diagnostic and radionuclide therapy for cancer treatment. [^{99m}Tc]-DTPA-PEG-Liposome could be used as an *in vivo* non invasive melanoma diagnostic agent, as well as for *in vivo* evaluation of different pharmaceutical formulations that use liposomes as drug carriers

ACKNOWLEDGEMENTS

The authors would like to thank, PEDECIBA (Uruguay), ANII (Uruguay), PDT (Uruguay)

ABBREVIATIONS

PEG = Polyethylene glycol

MLV = Multilamellar large vesicle

REFERENCES

[1] Ferrari M. Review. Nat Rev Cancer 2005; 5(3): 161-71. http://dx.doi.org/10.1038/nrc1566

[2] Torchilin V. Targeted Pharmaceutical Nanocarriers for Cancer Therapy and Imaging. AAPS J 2007; 9: 128-47. http://dx.doi.org/10.1208/aapsj0902015

[3] Maldelmont CG, Lesieur S, Ollivon M. Characterization of loaded liposome by size exclusion chromatography. J Biochem Biophys Methods 2003; 56: 189-17. http://dx.doi.org/10.1016/S0165-022X(03)00059-9

[4] Jain R, Dandekar P, Patravale V. Diagnostic nanocarriers for sentinel lymphnode imaging. J Control Release 2009; 138: 90-102. http://dx.doi.org/10.1016/j.jconrel.2009.05.010

[5] Taki J, Sumiya H, Asada N, Ueda Y, Tsuchiya H, Tonami N. Assessment of p-glycoprotein in patients with malignant bone and solft-tissue using technetium-99m-MIBI scintigraphy. J Nucl Med 1998; 39: 1179-84.

[6] Boerman OC, Laverman P, Oyen WJG, Corstens FHM, Storm G. Radiolabeled Liposomes scintigraphic Imaging. Progress Lipid Research 2000; 39: 461-75. http://dx.doi.org/10.1016/S0163-7827(00)00013-8

[7] Cornelis Sikkink JJM, Reijnen M, Leverman P, Oyen JG, Van Goor H. Tc-99m-PEG –Liposome by Hyaluronate in Rats With Fecal Peritonitis. J Surg Res 2008; 1-6.

[8] Fenske DB, Maurer N, Cullis PR. Encapsulation of weakly-basic drugs, antisene oligonucleotides and plasmid DNA within large unilamellar vesicles for drug delivery applications. In: Torchilin V, Weissig W Liposomes,Oxford University press/Oxford New York 2003; pp. 167-188.

[9] Koukouarakis MI, Koukouarki S, Fezoulidis I, Kelekis N, Kyrias G, Archimandritis S, *et al.* High intratumoral acumulation of stealth doxorubicin (Caelyx®) in

glyoblastomas and in metastatic brain tumors. Br J Cancer 2000; 83: 1281-88.
http://dx.doi.org/10.1054/bjoc.2000.1459

[10] Harrington K, Mohammadtaghi S, Uster P, Vile R, Stewart S. Effective Targeting of Solid Tumors in Patients with Loclly advanced Cancers by Radiolabeled Pegylated Liposome. J Clin Canc Res 2001; l7: 243-54.

[11] Laverman P, Dams E, Oyen W, Storm G, Koenders E, Prevost R, *et al*. A Novel Method to Label Liposomes with ^{99m}Tc by the Hydrazino Nicotinyl Derivative. J Nucl Med 1999; 40: 192-97.

[12] Dams E, Oyen W, Boerman O, Storm G, Laverman P, Kok P, *et al*. ^{99m}Tc-PEG Liposomes for the Scintigraphic Detection of Infection and Inflammation: Clinical Evaluation. J Nucl Med 2000; 41: 622-30.

[13] Laverman P, Zalipsky S, Oyen W, Dams E, Storm G, Mullah N, *et al*. Improved Imaging of Infections by Avidin-Induced Clearance of 99mTc-Biotin-PEG Liposomes. J Nucl Med 2000; 41: 912-18.

[14] Papahadjopoulos D, Allen TM, Gabizon A, Mayhew E, Huang SK, Lee KD, *et al*. Sterically stabilized liposomes: improvements in pharmacokinetics and antitumor therapeutic efficacy. Proc Natl Acad Sci USA 1991; 88: 11460-4.
http://dx.doi.org/10.1073/pnas.88.24.11460

[15] Irma AJM, Bakker-Woudenberg. Long-circulating sterically stabilized liposomes as carriers of agents for treatment of infection or for imaging infectious foci. Intern J Antimicrob Agent 2002; 19: 299-11.
http://dx.doi.org/10.1016/S0924-8579(02)00021-3

[16] Raymond M, Schiffelers R, Gjini E, Fens M, Storm G. Targeting Tumor Angiogenesis Using Liposomes. In: Gregory Gregoriadis. Liposome Technology Third Edition Volume III, United Kingdom 2007; pp. 113-122.

[17] Belhaj-Tayeb H, Briane D, Vergote J, Cao A, Moretti J. *In vitro* and *in vivo* study of ^{99m}Tc-MIBI enacpsulated in PEG-liposome: a promising radiotracer for tumor imaging. Eur J Nucl Molec Imaging 2003; 30: 502-509.
http://dx.doi.org/10.1007/s00259-002-1038-4

[18] Tiwari SB, Mansoor A. A Review of Nanocarrier-Based CNS Delivery Systems. Curr Drug Deliv 2006; 3: 219-32.
http://dx.doi.org/10.2174/156720106776359230

[19] Ganta S, Devalapally H, Shahiwala A, Amiji M. A review of stimuli-responsive nanocarriers for drug and gene delivery. J Cont Release 2008; 126(3): 187-204.
http://dx.doi.org/10.1016/j.jconrel.2007.12.017

[20] Richardson VJ, Jeyasingh K, Jewkes RF, Ryman BE. Properties of [^{99m}Tc] technetium labelled liposomes in normal and tumor bearing rats. Biochem Soc Trans 1977; 5: 290-91.

[21] Goyal P, Goyal K, Kumar SG, Singh A, Katare OMP, Mishra DN. Liposomal drug delivery systems. Clinic Appl Acta Pharm 2005; 55: 1-25.

[22] Phillips W. Delivery of gamma-imaging agents by liposomes. Adv Drug Deliv Rev 1999; 37: 13-32.
http://dx.doi.org/10.1016/S0169-409X(98)00108-2

[23] Laverman P, Carstens M, Boerman O, Dams E, Oyen W, Rooijen N, *et al*. Factors Affecting the Accelerated Blood Clearance of Polyethylene Glycol-Liposomes upon Repeated Injection. JPET 2001; 298: 607-12.

[24] Storm G, Belliot SO, Deamen T, Lasic DD. Surface modification of nanoparticules to oppose up take by the mononuclear phagocyte system. Adv Drug Del Rev 1995; 17: 31-48.
http://dx.doi.org/10.1016/0169-409X(95)00039-A

[25] Hwang KJ, Luk K, Beaumier P. Hepatic uptake and degradation of unilamellar sphingomyelin/cholesterol liposomes: A kinetic study. Proc Nati Acad Sci USA 1980; 77: 4030-34.
http://dx.doi.org/10.1073/pnas.77.7.4030

[26] Harashima H, Kume Y, Yamane C, Kiwada H. Kinetic modeling of liposome degradation in blood circulation. Bioph Drug Dispo 1993; 14: 265-70.
http://dx.doi.org/10.1002/bdd.2510140309

[27] Ishida O, Maruyama K, Sasaki K, Iwatsuru M. Size-dependent extravasation and interstitial localization of polyethyleneglycol liposomes in solid tumor-bearing mice. Int J Pharma 1999; 190: 49-56.
http://dx.doi.org/10.1016/S0378-5173(99)00256-2

[28] Leunig M, Huang SK, Berk DA, Papahadjopoulos D. Mirovascular Permeability and Interstitial Penetration of Sterically Stabilized (Stealth) Liposomes in a Human Tumor Xenograft. Cancer Res 1994; 54: 3352.

[29] Hamoudeha M, Kamleh M, Diab R, Fessi H. Radionuclides delivery systems for nuclear imaging and radiotherapy of cáncer. Adv Drug Deliv Rev 2008; 60: 1329-46.
http://dx.doi.org/10.1016/j.addr.2008.04.013

[30] Laverman P, Brouwers A, Dams E, Oyen W, Storm G, Rooijen NV, *et al*. Preclinical and Clinical Evidence for Disappearance of Long-Circulating Characteristics of Polyethylene Glycol Liposomes at Low Lipid Dose. J Pharma Exp Ther 2000; 293: 996-1001.

Received on 05-10-2012 Accepted on 21-11-2012 Published on 31-01-2013

http://dx.doi.org/10.6000/1927-7229.2013.02.01.1