IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 11, Issue 2 Ver. III (Mar.- Apr.2016), PP 75-81
www.iosrjournals.org

Effect Of Surface Modification In Conventional Liposome With Lipophilic Agents

Susmita Chandra[1*], Mridula Misra[2],Kakali De[3],Indranil Banerjee[4]
[1,4] *(Cell Biology and Physiology Divn, Indian Institute of Chemical Biology, Kolkata,India)*
[2,3]*(Nuclear Medicine Division, Indian Institute of Chemical Biology, Kolkata, India)*

Abstract: *Conventional liposomes normally shows frequent uptake by reticuloendothelial system(RES). To reduce the RES uptake and to prolong the biological half life researchers aimed to prepare sterically stabilized liposomes containing charged or neutral particles as surface modifier for targeting various organs for therapeutic or diagnostic purposes. Film method followed by ultracentrifugation and sonication was used for the preparation of Ethyl cysteinate dimer(ECD), Phenylalanine(Phe) and Phe-Phe-Cys(ffc) containing lipid vesicles. Phosphatidyl choline(PC) and Phosphatidyl ethanolamine(PE) were used as the phospholipids with Cholesterol(Chol) and Glutathione(GSH), both as positive charge inducer and bilayer condenser. Free and encapsulated radiopharmaceutical content was determined by conventional method and particle size was measured in electron microscopy. Biological studies were performed in rat and imaging studies performed in rabbit.The surface modified radiolabeled liposomes were highly stable and have got high entrapment efficiency. Their in vivo characteristics were found to be significantly different from conventional liposomes and free radiopharmaceuticals or ligands. The higher levels of organ uptake supported the case for lesser RES uptake. Liposomes coated with Phe has the highest uptake in brain. It has got the usefulness as diagnostics as well as a potential Central Nervous System(CNS) targeting agent.*

Key words. *Biodistribution, liposome, Peptide synthesis, stability*

I. Introduction

Liposomes are spherical lipid bilayers that are formed spontaneously when exposed to aqueous media[1,2]. It is possible to label them with various radionuclides in their aqueous interior or their lipid walls. Radiotracers, γ emitters and very short lived Positron Emission Tomography (PET) imaging agents are now a days used to incorporate into carrier systems like liposomes for diagnosis or therapeutic purpose [1,2,3,4]. When incorporated into liposome, these imaging agents may become attached to the surface. Such surface labeled liposomes become structurally different from their normal counterparts resulting in potential change in their *in vivo* behavior [4,2].

In a successful drug-carrier delivery system, the drug should be retained within a stable core being "invisible" to the reticuloendothelial system, and preferably follow a controllable drug release mechanism, resulting in excellent therapeutic efficacy, with few or no adverse effects[6].

The conventional liposomes, which were composed of egg phosphatidylcholine and cholesterol, are easily targeted by plasma opsonins in the blood. Subsequently, reticuloendothelial cells easily phagocytize and remove these liposomes from circulation[7]. Many researchers tried to overcome this limitation by introducing PEG coated sterically stabilized liposome (2^{nd} generation) that can evade phagocytosis[6,8]. As a result these liposomes exhibit extended circulation times with a rate controlled pharmacokinetics by favorably altering the drug absorption, biodistribution, and clearance.

The physicochemical properties of PEGylated liposomes are favorable for developing targeted drug delivery system for therapeutic purposes instead of developing an imaging agent[9]. PEG coating actually hinders binding of the liposome to the targeted drug delivery site[10]. It also results in the entrapped drug being protected from metabolism within the liposome interior and cannot become active or metabolized until released. Due to PEG coating liposomes can face steric hindrances also to reach the transferring receptors during passage through blood brain barrier. Thus it can only follow a passive mechanistic way to cross the blood brain barrier (BBB). The mechanism can be useful when blood brain barrier of tumor affected brain is destroyed but to cross the intact BBB in the early onset of the tumor or any other disease might not be achievable satisfactorily by these PEGylated liposomes[11].

A new approach have been introduced in this study with an objective to modify the surface properties of conventional liposomes, with an aim to achieve a fast efficient uptake of the radiopharmaceuticals through blood brain barrier, efficient attachment and carriage of targeting molecules (receptor binding agents) and also to reach a possible lowest uptake by the reticuloendothelial system. To bring such modifications in the surface properties we selected small peptides acting as receptor analogue, antibody or any other small organic molecule[12,13,14]. In this study our choice is small peptide molecules containing neutral amino acids like

Phenylalanine(Phe), Cysteine(Cys) or only neutral amino acid like Phe, were chosen to be attached on the liposomal surface.

We chose Phe because it is known to cross the blood brain barrier with high affinity. In previous studies it was observed that C^{14} labeled phenylalanine (C1 position) has very high uptake in the rat brain in normal condition[15]. A number of receptors are known to be present in the blood brain barrier which has got affinity towards the molecules containing Phenylalanine moiety (e.g. dopamine), like, large neutral amino acid receptors (LNAA)[16].

In our current study we tried to observe the effect of surface modification of liposomes using different lipophilic agents like ECD, Phe, ffc with respect to their organ distribution, in vivo stability, physcochemical properties like size, labeling efficiency and in vitro stability. Since our aim is to pass the surface modified liposomes through intact BBB our study would also emphasize the uptake characteristic of the different liposomes in brain.

II. Material And Methods

All reagents were of either HPLC or analytical grade purchased from Sigma, Merck Germany or SRL, India. ECD kit and $^{99}Mo/^{99m}Tc$ generator was purchased from BRIT Mumbai, India. Nomenclatures L1, L2 L3, and L4 are as follows.

Type of liposomes	Description of composition
L1	^{99m}Tc-Liposome-GSH$^{\#}$
L2	^{99m}Tc-ECD-Liposome-GSH
L3	^{99m}Tc-Phe-Liposome-GSH
L4	^{99m}Tc-ffc-Liposome-GSH*

$^{\#}$Conventional Liposome

*In case of L4 two methods were followed. They are M1 and M2.

M1: ^{99m}Tc-ffc was incorporated into liposome directly during lipid film hydration which is similar to the method used for L1, L2 and L3

M2: Small peptide PhePheCys(ffc) was attached to liposome following Kung-Redeman method and then radiolabeled with ^{99m}Tc during hydration.

Synthesis and analytical studies of tripeptide and radiolabeling

The tripeptide ffc was semi-automatically synthesized on a peptide synthesizer (PS3 Protein Technology Instruments, NULAB, USA) following standard Fmoc amino protection chemistry using trityl resin detailed method was already described in our previous paper[16]. Analytical purity of ffc was determined by ITLC silica gel plates and reverse phase high performance liquid chromatography(RP-HPLC, Waters, USA)[17].

Preparation of Liposome

Liposomes were prepared by film method using the molar ratio 7:2:2 of egg phosphatidyl choline (PC)(Sigma, USA), phosphatidyl ethanol amine (PE) and cholesterol followed by hydration with with 200 mM GSH in Dulbecco's Phosphate buffered saline (PBS; pH 7.4) at room temperature and purified according to size with dialysis membrane of desired porosity. For preparation of Phenylalanine coated Liposomes the lipid film was treated with 1.0mg/ml phenylalanine during hydration with PBS (pH 7.4) and incubated for 30 minutes.

Radiolabeling of ECD, Phe and ffc for preparation of Radiolabeled Liposomes and Analytical studies

^{99m}Tc-ECD was prepared according to the protocol described in the Kit supplied by BRIT [18]. The tripeptide ffc was radiolabeled by standard $SnCl_2$ method described previously[16]. Normal liposome and Phe coated liposome was directly labeled with ^{99m}Tc by tin(Sn) reduction to generate L1 and L3 respectively.

Technetium labeled ECD and ffc were added to liposomes during lipid film hydration to generate L2 and L4-M1(Method for ^{99m}Tc-ffc incorporation into liposome during lipid hydration will be known as M1). In another set ffc was incorporated into liposome by Kung Redeman method (M2) and then radiolabeled using standard Sn reduction process to prepare L4-M2. Briefly in Kung Redeman method sebacic acid (0.08 nmol) and dicyclohexyl carbodiimide (0.042 mmol) were dissolved in methylene chloride and incubated under flush of nitrogen for 48 hr at room temperature, with stirring. The anhydride formed used without further purification and treated with 0.038 mmol of PC and triethyl amine(0.108 mmol) in chloform. The reaction was allowed to proceed for few hours at room temperature and its progress was monitored by TLC (Chlororform : Methanol : Water (65:25:4) (v/v).

To stop the reaction 5 ml of chloroform and 4 ml of 0.02 M phosphate buffer and 0.02m citrate buffer was added and the upper organic phase was collected. The product in the organic phase was dried and used directly for incorporation of the peptide into liposome by activating it with EDCI (1-ethyl-3-(dimethyl

aminopropyl) – carbodiimide, 2.5mg/ml). The reaction was carried out overnight at 4^oC before separating the products from reactants by usual method.The purity of radiopharmaceuticals was verified by TLC and RP-HPLC[19].

Entrapment efficiency of $^{99m}TcO^{4-}$, ^{99m}Tc-ECD, ^{99m}Tc-Phe and ^{99m}Tc-ffc in liposome by sucrose gradient and chloroform extraction method

Free concentrations of $^{99m}TcO^{4-}$, ^{99m}Tc-ECD, ^{99m}Tc-Phe and ^{99m}Tc-ffc from L1/L2/L3/L4(M1/M2), were determined using a modification of the method described by Arulsudar et al by using sucrose gradient method (swinging bucket type, 24000g Sorvall) and Modified Bligh–Dyer Method. Briefly, the liposomal suspension (L1-L4) were mixed with 15ml (80%w/v) sucrose solution. 12 ml (20%w/v) sucrose solution was gradually layered on it. This uppermost sucrose layer was again covered with buffered saline and centrifuged at 24,000g (swinging bucket type, Sorvall) at 4^0C for 30 minutes. The unentrapped ECD from the lowest sucrose layer was collected and diluted with 0.1N Sodium Hydroxide to measure the absorbance at 210 nm. The liposomes were collected from the interface between saline and 20% sucrose layer.

The entrapped drug present in liposome was also estimated by Modified Bligh–Dyer Method with slight modification. Briefly, a mixture containing 0.5ml of L1/ L2/L3/L4 suspension, 3ml of chloroform, 1.5ml methanol and 1ml saturated sodium chloride solution was centrifuged at 1200 rpm for 10 minutes. The free drug content was estimated from the aqueous layer (diluting with 0.1N sodium hydroxide) by UV visible spectrophotometer at 210 nm. Radioactive count was measured in Gamma ray spectrometer (GRS,ECIL, India) to obtain the percent of entrapped ^{99m}Tc. Entrapment efficiency was calculated from the difference between the initial amount of radiopharmaceutical added to liposome and that present in the unentrapped form and was expressed as the percentage of the total amount of radiopharmaceutical added to liposome[20,21].

Stability of ^{99m}Tc-Liposome-Drug complex.

Stability of ^{99m}Tc-liposome–drug complex was determined up to 24 hrs in vitro at 37^0C using 0.9% sodium chloride and rat serum by ascending thin layer chromatography[22].

Electron microscopy and size determination

To find out the size and ultrastructure of the liposomes and its entrapped composition, Transmission Electron Microscopy (TEM, Technai,USA) of the liposomal suspension via negative staining was performed. The size and lamellarity of the liposome was also determined from this study.

Biodistribution and imaging with Liposome and its derivatives

All the animal experiments were performed following the institutional as well as the international (NIH) guidelines. Rats (50) were maintained in light and dark cycle with proper feeding. Female Sprague–Dawley rats (300$\pm$20g) were anesthetized with Urethane (2 mg/kg Body weight) intramuscularly (IM). Each radiopharmaceuticals (1-2mci, 0.5ml) were injected intravenously (IV) through femoral vein and sacrificed at 2, 5, 15, 30, 60, and 120 min (n=5). Immediately the organs were collected and radioactivity was measured in a double-channel well counter (gamma spectrometer,ECIL,India) correcting the background and physical decay[8,23,24]. Scintigraphic imaging was also performed in rabbit using similar technique[2,25] under gamma camera (GE Hawkeys,USA,Software:Xeleris) at Regional Radiation Monitoring Centre, Kolkata.

Plasma clearance study

Plasma clearance of all raiopharmaceuticals was calculated by open two compartment analysis from multiple samples after a single injection[26]. In brief, anaesthetized rats were cannulated at both the femoral veins to inject radiopharmaceuticals (37 MBq [1 mCi]) through one side and collect the blood (0.2ml) from other side at 2, 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes post injection[25].
(Further detailed methodology are given in Supplementary File).

III. Results

Characterizations of Liposomes

The liposome prepared by film method were single unilamellar vesicles (SUV) having size range 10-200 nm (Fig. Ia-d) measured by TEM. Liposomal formulation of mean particle size obtained with variation of sonication time and speed have been given in the supplementary Table1. Sonication for 30 seconds at 3 Hz produces liposomes of average particle size of 100nm which can be reduced up to 90% with increase in sonication speed (5 Hz) and time up to 5 minutes (Supplementary Table 1). Very high sonication speed (>5 Hz) or too long sonication time may lead to malformation of the liposomal vesicles (Supplementary Fig.1).

Radiolabeling, analysis and entrapment efficiency

^{99m}Tc-ECD and three other radiopharmaceuticals(L1,L2,L3) which were entrapped in liposomes by reverse phase evaporation technique with the mean labeling efficiency of (90-95)%, were extremely stable at pH 7.4. After incubation of all the liposomes with 0.9% saline and serum for 24 hr it was revealed that L1-L3 was highly stable in vitro (Fig. II). A maximum of 8% radioactivity was dissociated after 24hr incubation either in saline or serum. L4-M1 was found to be 10-20% less stable as compared to others (L1,L2,L3) however L4-M2 scored better than L4-M1. Labeling efficiency was at least 5% higher in case of L4-M2 than L4-M1. The TLC results of L1, L2, L3, L4(M1/M2) didn't show any evidence of particle accumulation after 20 minutes of incubation (data not shown). But After 24 hr the particles start to coalesce resulting into slower movement towards the solvent front. Similar characteristics were observed if the sonication speed or time is too high or low. As L4-M2 would be chosen for further biological studies for its superior physicochemical characteristics than L4-M1 and would be denoted by L4. The stability was improved in L4-M2 because of an amide link between the primary amine residue of ethanol amine and carboxyl residue of the peptide. This actually leads to a functional stability and incorporation homogeneity.

Biodistribution studies

Biodistribution of ^{99m}Tc-ECD (data not shown), L1, L2, L3 and L4 were performed to determine the % mean uptake of radioactivity per gram of tissue of normal rats. The percentage injected dose (ID/g) of tissue in various organs at 5 minutes and 2hr for different liposomes were shown in Table I. For most of the liposomal formulations highest uptake in the brain was observed within 5-15 minute PI after which gradually starts falling after 30 minutes. Brain uptake of L2 was reduced at 5minutes post injection (PI) but total retention period was higher as compared to liposome free Tc-99m-ECD. Similarly brain uptake was also decreased in L4 when ^{99m}Tc-ffc was incorporated into liposome. Among L1/L2/L3/L4, L3 showed a good promising uptake and retention in brain. ECD, Phe and ffc containing liposomes were cleared from the circulation and accumulated in the liver, spleen and in the kidneys(Supplementary Table 1-6).

The initial uptake of ^{99m}Tc-ECD, L1, L2, L3 and L4 in kidney, liver and spleen was substantially higher. A little enhancement of liver uptake was observed after 5 minute which decreases after 15 minute. Faster clearance of radioactivity from the blood through kidney was observed for ^{99m}Tc-ECD while L1/L2/L3/L4 showed high accumulation in blood for the first 15 minutes following which rapid clearance was observed. Rapid clearance of L2 and L4 as compared to others from the blood through kidney may have a similar lowering effect of brain uptake (Fig. III).

The calculated $T_{1/2}$ of L1 was 20 minutes, L2 was 17 minutes, L3 was 25 minutes, and L4 was 22 minutes while that of ^{99m}Tc-ECD is 0.8 minutes. Similar results were observed in imaging studies of rabbit.

IV. Discussion

It was reported that the extent of interaction of charged molecules with lipid membranes depends on both electrostatic attraction at the head group level and polar part of the membrane or on the non polar interaction between the lipid bilayer and lipophilic residue of the drug [21,27].

ECD (log P 1.64), Phe and ffc are lipophilic in nature. So there is a high possibility that they can easily cross the blood brain barrier by diffusion mechanism or via LNAA receptor[29]. In our previous study we have observed considerable and good uptake of ^{99m}Tc-ffc into brain which is quite comparable to standard ^{99m}Tc-ECD distribution and uptake[16,29]. That is why we have chosen ECD, Phe and also ffc to be incorporated into liposome to see whether the incorporation leads to any change in the uptake property.

The liposomal formulation was radiolabeled with ^{99m}Tc with an efficiency of more than 97% and high stability till 24 hr. The experimental data revealed that there was hardly any detachment of the radioisotope from the complex within 24hr of preparation. After incubation of more than 24hr the liposomes were found to degrade and release component like cholesterol with 2% to 8% decrease of the radioactivity which were detected by TLC. It signifies not only their high in vitro stability but also its suitability for in vivo use as imaging and therapeutic agent.

^{99m}Tc-ECD and ^{99m}Tc-ffc are characterized by their high brain uptake and fast clearance from blood[16] but incorporation into liposome changes its distribution pattern by upgrading the blood circulation time while decreasing the brain uptake to some extent (Table I).

The brain uptake of the liposomal formulations were mostly found higher at 5-15 minutes time range which remains almost steady for another 30 minutes and decreases slowly thereafter. The brain uptake was highest at 2 minute PI (1.2% ID/g) with the standard ^{99m}Tc-ECD, which decreases sharply after 15 minutes[28]. The brain uptake however decreased when ^{99m}Tc-ECD was incorporated into liposome (L2) (Table I). The uptake was significantly improved in L3, where, Phe was incorporated into liposome.

L1 and L4 showed a clear difference with L2 and L3 in the uptake of rat brain at different time points (Table I). L1 is supposed to interact more with RES resulting into lesser organ availability and in case of L4 the

responsible factor may be its molecular size and in vitro stability which play some important role to subdue its uptake efficiency into the brain.

Only a negligible amount of labeled complex was found to be present in the stomach which shows no evidence of pertechnetate leakage from the complex in the system. An inverse relationship between liposome clearance by RES and prolonged circulation time of liposomes was reported previously [8]. In turn, there appears to be a direct correlation between prolonged circulation time of different liposomes and their localization in brain. The blood concentrations of all liposomal formulations were significantly high till 2hrs. The increase in circulation time and increased organ availability of the liposomes may be due to the addition of glutathione as well as their smaller particle size (d<200 nm) or due to surface modification made by ECD, Phe or ffc, which results into reduction in recognition by RES.

The variation in particle size is very important for establishing its in vivo distribution pattern. For very small particles(20-50nm) the brain as well as kidney uptake was high and for medium size particles (50-80nm) the liver and kidney uptake was significant. Particle more than 80nm has significant uptake in liver and spleen. Bigger ones take longer time for clearance from blood and shows very low uptake in the brain(0.05%). Reports suggest that the high level of liposomes in the blood was only observed for small liposomes ($d \leq 200$ nm); larger liposomes ($d > 200$ nm) showed high level of accumulation in spleen and low concentration in blood. Thus, the effect of PC/PE-liposome(GSH) to prolong its circulation time is limited to smaller size. The small liposomes ($d < 100$ nm) might be expected to penetrate through the fenestrations and gain access to hepatocytes because the endothelial lining of the liver sinusoids includes fenestrae with an average diameter of 100 nm[19]. The radioactivity recorded in the highly perfused organs such as liver and spleen could be accounted for the combined activity of the circulating blood passing through the organs as well as particle uptake by cells of RES [7].

The in vitro plasma release studies performed by incubating the liposomes with 80% plasma at 37°C showed that more than 60% of the drug is retained in the liposomes after 24 hr. This finding indicates that sufficient drug will be present in the liposomes for releasing the drug to the target organs as an imaging as well as therapeutic agent. Urine samples from L1-L4 injected rats showed only 8-10% breakdown product i.e. free pertechnetate indicating sufficient in vivo stability.

The conventional liposomes bearing a different surface charge has long been considered as a factor contributing to decreased or increased liposome circulation times and enhanced uptake by RES in vivo because of the direct interaction between the negatively charged group on the liposome surface with cell surface proteins [11,30]. From the experiments performed although it is quite difficult to find out the exact cause of changed uptake in brain when ^{99m}Tc/^{99m}Tc-ECD/^{99m}Tc-Phe/^{99m}Tc-ffc was entrapped in the liposome but it may be attributed to the improvements in lipid composition and size by surface remodeling.

For incorporation of radioisotopes or tagged molecules into liposomes we need a favourable interaction between the nonpolar head and the lipophilic part of the molecule or between the polar endings. In our experiment most of the liposome incorporated complexes are lipophilic in nature and they get embedded in the nonpolar layers. The neutral lipophilic amino acid has got affinity towards the LNAA receptors. In many organs like brain, liver LNAA receptors are responsible for taking up small neutral molecules, neutral amino acids, small peptides containing neutral amino acids like Phe, cysteine etc[16].

Most of the brain imaging agent when used in brain tumor detection shows high uptake at a very late stage when the blood brain barrier is almost damaged. LNAA receptors are found to be up regulated during onset of tumors in the brain. So by designing LNAA receptor based molecules one can aim at developing diagnostic agent which can give an indication of early onset of brain tumors [20, 23].

V. Figures And Tables

Table I: Percentage (%) uptake per gram of organ of rat for L1, L2, L3 and L4 with Particle size 30-50nm

Organs	% uptake per gram of organ, mean (+ SD) (n=5)							
	5 min				2 hr			
	L1	L2	L3	L4	L1	L2	L3	L4
Brain	0.57 (0.06)	0.90 (0.24)	0.98 (0.06)	0.85 (0.03)	0.45 (0.02)	0.80 (0.67)	0.65 (0.02)	0.34 (0.04)
Blood	5.43 (0.94)	5.09 (0.99)	3.99 (0.28)	2.64 (0.28)	0.80 (0.67)	3.44 (0.32)	1.18 (0.75)	1.09 (0.45)
Liver	7.44 (0.67)	6.85 (0.28)	7.45 (0.83)	4.29 (0.36)	4.43 (0.36)	2.66 (0.51)	3.86 (0.18)	2.09 (0.47)
Kidney (Total)	9.33 (0.73)	8.98 (0.97)	10.10 (0.28)	9.97 (0.37)	6.45 (0.64)	3.54 (0.38)	3.44 (0.33)	4.03 (0.44)

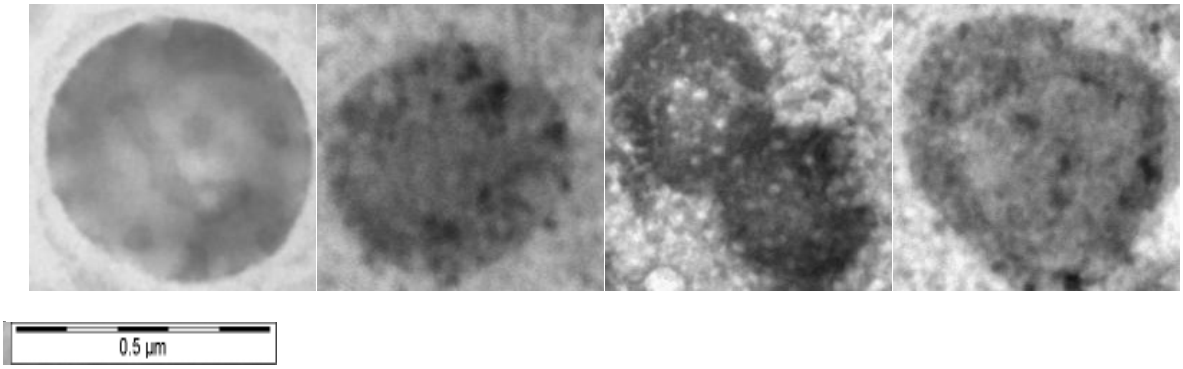


Figure I. Electron Microscopy with negative staining and a) Single Unilamellar Vesicle (SUV) of liposomes (L1) b) vesicles of ^{99m}Tc-ECD-Liposomes.(L2) c) Vesicles of ^{99m}Tc-Phe-Liposomes(L3) d) ^{99m}Tc-ffc-Liposomes (L4)

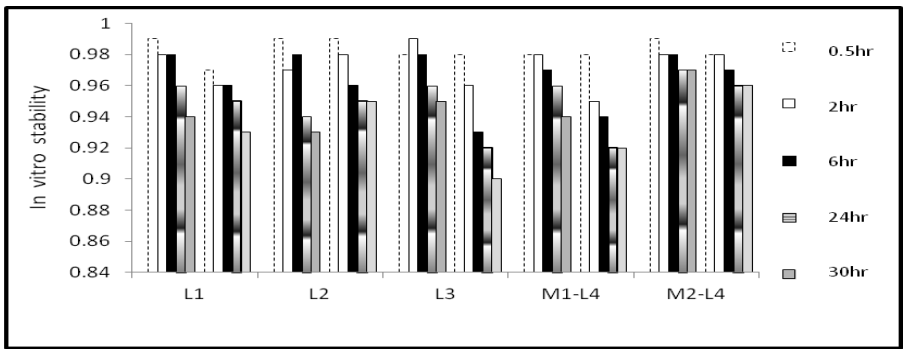
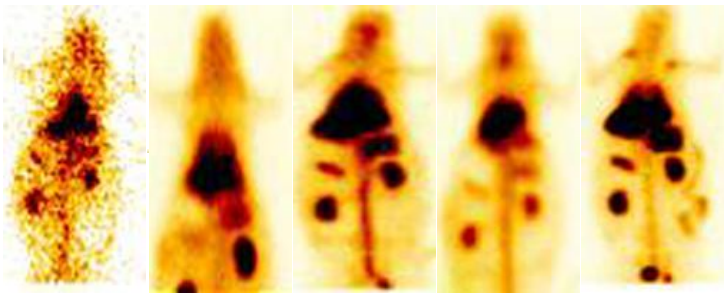


Figure II: Entrapment efficiency of different liposomal formulations expressed as % mean labeling efficiency±SEM at different time points.



Ll L2 L3 M1-L4 M2-L4

ure III: Imaging studies under gamma camera with rabbit(Average body wt 2.5kg) at 5 minute PI. Animals were injected with liposomal particles of size range 30-50nm, with an intravenous injection dosage of 2mg /ml containing radioactivity 3-4 mCi accordingly: a) Conventional Single Unilamellar liposomes (L1) b) ^{99m}Tc-ECD-Liposomes.(L2) c) ^{99m}Tc-Phe-Liposomes(L3) d) ^{99m}Tc-ffc-Liposomes (L4). (Colour scale bar: Higher Colour intensity at the indicates high radioactivity.)

VI. Conclusion

In this research, we finally reached a position to state that the structural and functional difference achieved by the surface modification of liposomes with neutral peptides or amino acids have pronounced effect on their in vivo characteristics.This can change the physiological behavior of both the liposome and the surface ligand. If the binding is strong between the liposome and the interactive agent, their in vivo stability increases. As a result their kinetic control in circulation plays an important role in moving the drug in different organs with various specificity. From the above discussion we find that Liposome surface modified with Phe (L3) is most optimum due to its favorable size, in vitro, in vivo stability, brain uptake efficiency, brain retention pattern and gradual release from brain. This generates the idea that L3 might have a potential use in brain imaging or may also be used as a potential CNS drug delivery agent even during very early onset of tumors when the BBB is expected to remain almost intact like normal condition. So this study might provide an insight into further analysis and effectiveness of the liposome with different surface modifications to use for imaging and therapeutic purposes.

Acknowledgement

We thank the Indian Council of Medical Research (ICMR) for providing financial support to this work. There is no conflict of interest for this work. We are thankful to Peptide chemistry lab of IICB for providing us the facility to peptide synthasis. We are also thankful to Dr. Santanu Ganguly and Bharat Sarkar of for his continuous assistance and support to use Imaging facility at Regional Radiation Medicine Centre, Thakurpukur Cancer Research Centre, Kolkata.

References

[1]. S. Itakura, S. Hama, T. Ohgita and K. Kogure, Development of Nanoparticles Incorporating a Novel Liposomal Membrane Destabilization Peptide for Efficient Release of Cargos into Cancer Cells. PLOS One, 9(10), 2014,1-11

[2]. Z. Vanić, S. Barnert, R. Süss, R. Schubert . Fusogenic activity of PEGylated pH-sensitive liposomes. J Liposome Res 22(2), 2012,148–157.

[3]. J. Banerjee, A.J. Hanson, B Gadam, A.I. Elegbede, S. Tobwala et al. Release of liposomal contents by cell-secreted matrix metalloproteinase-9. Bioconjug Chem 20(7), 2009,1332–1339.

[4]. M.L. Matteucci and D.E. Thrall, The role of liposomes in drug delivery and diagnostic imaging: a review. Veterinary Radiology and Ultrasound, 41(2), 2000,100-107.

[5]. A.L. Petersen, T. Binderup, R.I. Jølck, P. Rasmussen, J.R. Henriksen, A.K. Pfeifer et al. Positron emission tomography evaluation of somatostatin receptor targeted 64Cu-TATE-liposomes in a human neuroendocrine carcinoma mouse model. Journal of Controlled Release. 95(2), 2012,297-308.

[6]. A. Gabizon, D.C. Price, J. Huberty, R.S. Bresalier and D Papahadjopoulos. Effect of liposomes composition and other factors on the targeting of liposomes to experimental tumors: biodistribution and imaging studies. Can Res. 50(19),1990, 6371–6378.

[7]. G.M. Jensen and T.H.Bunch. Conventional liposome performance and evaluation: lessons from the development of vescan. J Lipo Res 17(3-4),2007, 121–137.

[8]. C.M. Lee, Y. Choi, E.J. Huh, K.Y. Lee, H.C. Song, M.J. Sun et al. Polyethylene glycol (PEG) modified ^{99m}Tc-HMPAO-liposome for improving blood circulation and biodistribution: the effect of the extent of PEGylation. Cancer Biotherapy Radiopharmaceutical 20(6),2005, 620-628.

[9]. S. Erdogan, A. Roby and V.P. Torchilin. Enhanced tumor visualization by gammascintigraphy with 111In-labeled polychelating-polymer-containing immunoliposomes. Mol Pharmaceutics70(2),2006, 525–530.

[10]. M.O. Oyewumi, R.A. Yokel, M. Jay, T. Coakley and R.J. Mumper. Comparison of cell uptake, biodistribution and tumor retention of folate-coated and PEG-coated gadolinium nanoparticles in tumor-bearing mice. Journal of Controlled Release 95(3) , 2004, 613–626.

[11]. G. N. Serrano, G. G. Zhanel and F. Schweizer, Antibacterial Activity of Ultrashort Cationic Lipo-β-Peptides. Antimicrobial Agents and Chemotherapy (AAC) 53(5), 2009, 2215-2217.

[12]. X. Liang, G. Mao and K.Y. Ng. Mechanical properties and stability measurement of cholesterol-containing liposome on mica by atomic force microscopy. J Coll and Int Sci 278(1),2004, 53-62.

[13]. D.W. Löwik, J.G. Linhardt, P.J. Adams and JCV Hest. Non-covalent stabilization of a beta-hairpin peptide into liposomes. Org and Biomol Chem 1(11),2003, 1827-1829.

[14]. J.W. Park, K Hong, D.B. Kirpotin, G. Colbern and R. Shalaby, J Baselga et al. Anti-HER2 immunoliposomes: enhanced efficacy attributable to targeted delivery. Clin Can Res 8(5),2002, 1172-1181.

[15]. W. M. Pardridge The Blood-Brain Barrier: Bottleneck in Brain Drug Development NeuroRx. 2(1),2005, 3–14.

[16]. S. Chandra, K .De, S. Ganguly, B. Sarkar and M. Misra. Synthesis, Radiolabeling and Biological Evaluation of A Neutral Tripeptide and its Derivatives for Potential Nuclear Medicine Applications. Peptides 30(12), 2009, 2399–2408.

[17]. X. Xu, A. Costa and D.J. Burgess, Pharm Protein encapsulation in unilamellar liposomes: high encapsulation efficiency and a novel technique to assess lipid-protein interaction. Res. 29(7),2012 ;1919-1931.

[18]. R. Dringen, J.M. Gutterer and J. Hirrlinger. Glutathione metabolism in brain metabolic interaction between astrocytes and neurons in the defense against reactive oxygen species. EurJ of Biochem 267(16),2000, 4912-4916.

[19]. R. Hrynyk, J.M. Metselaar, G. Storm and M. Langner. Pharmacokinetic modeling of the in vivo fate of long-circulating liposomes in rats. CMBL 7(2),2002, 285.

[20]. M.M. Kleiter, D. Yu, L.A. Mohammadian, N Niehaus, I Spasojevic, L Sanders et al. A tracer dose of technetium-99m-labeled liposomes can estimate the effect of hyperthermia on intratumoral doxil extravasation. Clin Can Res 12,2006, 6800-6807.

[21]. M. Kinuta, H.Yamada, T. Abe, M Watanabe, S.A. Li, A. Kamitani et al. Phosphatidylinositol 4,5-bisphosphate stimulates vesicle formation from liposomes by brain cytosol. Proceedings of National Academy of Sciences, USA 99(5), 2002, 2842-2847.

[22]. L. You, R. Ferdani and G.W. Gokel. Chloride ion efflux from liposomes is controlled by side chains in a channel-forming heptapeptide. Chem Comm (Cambridge, England) 48(6),2006, 603-605.

[23]. M Korkmaz and AY Ozer. DTPA Liposomes in Diagnostic Imaging. FABAD J Pharmaceut Sci 31, 2006, 210-219.

[24]. K. Vorauer-Uhl, A. Wagner, N. Borth and H. Katinger. Determination of liposome size distribution by flow cytometry. Cytometry 39(2),2000, 166-171.

[25]. R. Saito, J.R. Bringas, T.R. McKnight, M.F. Wendland, C. Mamot, D.C. Drummond et al. Distribution of liposomes into brain and rat brain tumor models by convection-enhanced delivery monitored with magnetic resonance imaging. Can Res 64(7), 2004, 2572-2579.

[26]. N. Arulsudar, N. Subramanian, P. Mishra, K. Chuttani, R.K. Sharma and R.S.R Murthy. Preparation, characterization, and biodistribution study of technetium-99m-labeled leuprolide acetate-loaded liposomes in ehrlich ascites tumor-bearing mice. AAPS Pharm Sci(An Official Journal of American Association of Pharmaceutical Scientists) 6(1), 2004, 45-56.

[27]. E.G. Bligh and W.J. Dyer. A rapid method of total lipid extraction and purification. Canadian J of Biochem and Physiol 37(8), 1959, 911-917.

[28]. Y. Inoue, O. Abe, T. Kawakami, T. Ozaki, M. Inoue and I.Yokoama. Metabolism of ^{99m}Tc-Ethyl cysteinate Dimer in infarcted brain tissue of rat. J Nucl Med 42(5) , 2001, 802-807.

[29]. P. Laverman, O.C. Boerman and G. Storm. Radiolabeling of Liposomes for Scintigraphic Imaging. Methods in Enzymology, 373, 2003, 234-248.

[30]. A. Bao, B. Goins, R. Klipper, G. Negrete and W.T. Phillips. 186Re-liposome labeling using 186Re-SNS/S complexes: in vitro stability, imaging and biodistribution in rats. J Nucl Med 44(12),2003, 1992-1999.