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Hayat, SMG, Jaafari, MR, Hatamipour, M, Penson, P and Sahebkar, A (2020) Liposome Circulation Time is Prolonged by CD47 Coating. Protein & Peptide Letters, 27 (1). ISSN 0929-8665

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Liposome circulation time is prolonged by CD47 coating

Seyed Mohammad Gheibi Hayat[1], Mahmoud R. Jaafari[2,3], Mahdi Hatamipour[3], Peter E. Penson[4], Amirhossein Sahebkar[2,5,6*]

[1]*Department of Medical Biotechnology, Faculty Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
[2]*Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran*
[3]*Nanotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran*
[4]*School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, UK*
[5]*Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*
[6]*School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran*

Correspondence:

- Amirhossein Sahebkar, Department of Medical Biotechnology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, P.O. Box: 91779-48564, Iran; Tel: 985118002288; Fax: 985118002287; E-mail: sahebkara@mums.ac.ir; amir_saheb2000@yahoo.com

- Mahmoud Reza Jaafari, Department of Pharmaceutical Nanotechnology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad 91775-1365, Iran.

Running title: CD47-decorated liposomes with prolonged half-life

Source of funding: This project was financially supported by the Mashhad University of Medical Sciences Research Council (Mashhad, Iran). The project was also supported by a grant from cancer research center of cancer institute of Iran (Sohrabi cancer charity, Grant No: 37652-202-01-97) and a Grant No. 961204 of the Biotechnology Development Council of the Islamic Republic of Iran.

Availability of data and material: Data associated with this article are available from the corresponding authors upon a reasonable request.

ORCIDs:

Seyed Mohammad Gheibi Hayat (https://orcid.org/0000-0002-1378-118X)
Mahmoud R. Jaafari (https://orcid.org/0000-0003-3908-6828)
Mahdi Hatamipour (https://orcid.org/0000-0002-0782-1907)
Peter E. Penson (https://orcid.org/0000-0001-6763-1489)
Amirhossein Sahebkar (https://orcid.org/0000-0002-8656-1444)

Abbreviations

4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)

Antibody against PEG (antiPEG)

Area Under the Curve (AUC)

Area Under the Momentum Curve (AUMC)

Artificial Antigen Presenting cells (aAPC)

Clearance (Cl)

Concentration Maximum (CM)

Dimethyl Sulfoxide (DMSO)

Doxorubicin Hydrochloride (Dox)

Food and Drug Administration (FDA)

High Performance Liquid Chromatography (HPLC)

Human Monocyte Cell Line (THP-1)

Hydrogenated Soya Phosphatidylcholine (HSPC)

Layer Chromatography (TLC)

Liposomal Doxorubicin (LD)

Mean Residence Time (MRT)

Methoxypolyethyleneglycol (Mw 2000)-distearylphosphatidylethanolamine (mPEG2000-DSPE)

Mononuclear Phagocytic System (MPS)

Nuclear Magnetic Resonance (NMR)

Pegylated Liposomal Doxorubicin (PLD)

Polyethylene glycol (PEG)

Polyethylene Glycol–polylactic Acid (PEG–PLA)

Red Blood Cells (RBC)

Reticuloendothelial System (RES)

Self Peptide (SP)

Self peptide Liposomal Doxorubicin (SP-LD)

Signal Regulatory Protein Alpha (SIRPα)

Abstract

Introduction:

Bio-degradable nano-particles have many applications as drug delivery vehicles because of their good bio-availability, controlled release, low toxicity and potential for encapsulation. However, the most important obstacle to nanoparticulate drug delivery is elimination by macrophages which reduces the residence time of nanoparticles in the blood. To overcome this problem, the surface of the nanoparticle can be passivated by coating with Polyethylene glycol (PEG). However, the use of PEG has its own disadvantages. CD47 receptor acts as a self marker on the surface of many cells and inhibits phagocytosis. This study used a CD47 mimicry peptide as a substitute for PEG to fabricate "stealth" nanoliposome with reduced macrophage clearance.

Methods:

Doxorubibin was used as a model drug because of its inherent fluorescence. Doxorubicin-containing liposomes were coated with different percentages of CD47 mimicry peptide (0.5% and 1%). PEG-functionalized doxorubicin-containing liposomes, were used as a comparator. The liposomal formulations were intravenously injected into mice. Serum was collected at pre-defined time points and tissue samples were taken at 24 hours. Fluorescence was used to determine the concentration doxorubicin in serum, heart, spleen, kidney, liver and lung tissues.

Results

Tissue biodistribution and serum kinetic studies indicated that compared with PEG, the use of CD47 mimicry peptide increased the circulation time of doxorubicin in the circulation. Moreover, unwanted accumulation of doxorubicin in the reticuloendothelial tissues (liver and spleen), kidney and heart was significantly decreased by the CD47 mimicry peptide.

Conclusion

The use of a CD47 mimicry peptide on the surface of nanoliposomes improved the residence time of liposomal doxorubicin in the circulation. The accumulation of drug in non-target tissues was reduced, thereby potentially reducing toxicity.

Keywords: Liposome; CD47; Kinetic; Half-life; Phagocytosis; Macrophage

1. Introduction

The unique properties of nanoparticles allow them to be used to deliver drugs to a wide range of tissues in the human body. The encapsulation of hydrophobic drugs, nucleic acids and proteins in nano-particles is increasingly employed as a technique to reduce intra-cellular degradation, increase the circulation time and enhance the therapeutic effects of the molecule being delivered. Liposomes, discovered for the first time by Alee Bangham in 1963 [1], are the most studied nanostructures in drug delivery. Liposomes have unique properties such as biocompatibility, biodegradability, delivery capacity for both hydrophilic and hydrophobic cargos and low immunogenicity and toxicity, leading to their widespread use in drug delivery [2].

Rapid uptake of nanoparticles by circulating macrophages is the biggest obstacle to their use in drug delivery. Macrophages are leukocytic cells that can phagocytize or absorb cellular debris, bacteria, and particles by an energy-consuming process of membrane engulfation. Macrophages play an important role in the initial response of the body to the presence of potentially harmful foreign materials [3]. Macrophages substantially affect the tissue distribution of nanoparticles and their residence time in the blood. Moreover, macrophages can induce unwanted inflammatory responses, and interfere with the treatment process. In tissues, particularly in the filtration organs (liver, kidney, spleen, and lung) the mononuclear phagocytic system (MPS) actively clears nanoparticles [4-8]. It has been demonstrated that filtration organs sequester about 95% of systemically delivered nanoparticles [9, 10]. Therefore nanomaterial-based drug delivery is limited by rapid clearance from blood and target delivery sites, and inflammatory responses which are initiated when nanoparticles accumulate in macrophages in the clearance organs [11-16].

The absorption and phagocytic degradation of nano-materials may be reduced by altering the physicochemical properties of the particle surface e.g surface energy, hydrophobicity, hydrophilicity and the presence of steric obstacles such as the grafted polymer brush surface. The hydrophilic molecule, poly(ethylene glycol) (PEG) has been used in a variety of settings to provide a brush-like steric obstacle. This reduces protein absorption and is associated with longer blood circulation times for a number of particles [17]. Doxil®, Pegylated liposomal doxorubicin (PLD), is the first nano-medicine delivery system to have been accepted by FDA, and is based on PEGylated liposomes [18]. NK911 is a DOX-encapsulated micelle from a co-polymer of PEG-DOX-conjugated poly(aspartic acid). Genexol-PM is a paclitaxel-encapsulated PEG-PLA micelle formulation that has received approval for clinical use [19]. Nevertheless, pegylation has some disadvantages and complications. PEG is a large molecule, its existence on the nano-particle (or liposomal) surface can inhibit the interaction of nanoparticles (or liposomes) with cells, and hinder the introduction of liposomes into target cells [20]. Repeated administrations of the PEGylated formulation may speed up their clearance from the blood. It is often argued that PEG is non-antigenic and weakly immunogenic, however the existence of a naturally occurring antibody against PEG (antiPEG) was first demonstrated in 1983 and is relatively common. The antibody has been characterized in animal models. Furthermore, some data have quetioned the usefulness of PEGylation in increasing the circulation time of nanoparticles (or liposomes) in the blood [21-24].

Therefore, new approaches which do not rely upon PEGylation are required to enhance the bioavailability of drugs carried by liposomes and nanoparticles. CD47 is a protein found on the surface of red blood cells (RBC), which sends a "Don't eat me" message when it is exposed to the SIRPα receptor on the surface of macrophages. CD47 thereby prevents phagocytosis of the RBCs by macrophages. Interestingly, cancer cells transmit the "Don't eat me" signal to the macrophages by expressing the CD47 protein on their surface and

thus defend themselves against the risk of phagocytosis by macrophages [25]. Therefore, it is possible to transmit the "Don't eat me" signal to the macrophages using the CD47 protein on the nanoliposomes surface, and to reduce or prevent the clearance of nanoliposomes by macrophages. Because of the large size of CD47, this study employed a CD47 mimicry protein (Self peptide: SP) instead. Doxorubicin was loaded into nanoliposomes so that it could easily be traced in serum and tissue by virtue of its autofluorescence. This research experimented if it is possible for SP-coated liposomes to reduce phagocytic absorption by macrophages compared with PLD uptake.

2. Materials and methods

2.1 Materials

China Peptides Co. (Shanghai: China) performed the synthesis of SP (cgggCERVIGTGWVRC) which had a molecular weight of 1.69 KD and 95.08% purity. Cholesterol, doxorubicin hydrochloride (Dox) and Dowex® 50WX4-400 were obtained from Sigma–Aldrich (St. Louis: MO). Methoxypolyethyleneglycol (Mw 2000)-distearylphosphatidylethanolamine (mPEG2000-DSPE) and hydrogenated soya phosphatidylcholine (HSPC) were supplied by Lipoid (Ludwigshafen: Germany). Acidified isopropyl alcohol (90% isopropanol/0.075 M HCl) was produced by the addition of 2.5 mL water and 7.5 mL HCl 1 M to 90 mL isopropanol (Merck; Darmstadt: Germany). Doxil® was obtained from Behestan Darou Company (Tehran, Iran).

2.2 Conjugation of the CD47 mimicry peptide to PEG2000-DSPE-Mal

The variables listed below were used to calculate the number of peptide molecules in each liposome. 1. Phospholipid concentrations of Doxil; 13.279 mM, 2. Average diameter of liposomes; 100 nm, 3. Lipid molecules/liposome with the average size of 100 nm; 8×10^4, 4. The number of liposome/ml: 1×10^{14}, 5. Total contents of peptide; 0.4 mM, 6. The number of peptide molecules/ml aliquot of peptide-micelle; 2.4×10^{14}, 7. Number of peptides/liposome (150 & 300 ligand). Thioether linkage between thiol groups of cysteine residue was used to synthesize the peptide conjugation with DSPE-PEG2000-Mal. Mixing of the peptide (dissolved in dimethyl sulfoxide, DMSO) and maleimide-PEG2000-DSPE (dissolved in chloroform) was performed at molar ratios of 1:1. The mixture was exposed to argon gas in order to eliminate chloroform. The volume of the mixture was adjusted to 1 mL by the addition of HEPS buffer pH=7.2 (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid). The ligation reaction was then performed at 37 ˚C for 24 hours.

2.3 Determination of peptide coupling efficiency

The extent of peptide coupling to the surface of the liposome was determined using three methods: Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) and Nuclear Magnetic Resonance (NMR).

2.4 Preparation of liposomes

The extrusion method and thin-film hydration were used to prepare the liposome formulation. Remote loading, using the ammonium sulfate gradient method [26], was employed to encapsulate Dox in the liposomes. The liposome nomenclature and lipid compositions are shown in Table 1.

Briefly, appropriate quantities of lipids in chloroform solution were mixed in round-bottom flasks. Solvent removal was achieved using a vacuum-equipped rotary evaporator and freeze-drying. Pre-warmed

ammonium sulfate (250 mM) was used to hydrate the final thin films to achieve total lipid concentration of 50 mM. Sonication was performed at 60°C for fifteen minutes under an argon atmosphere. The sonicated suspension was then passed across poly-carbonate nano-pore filters with pore sizes of 50, 100, 200, and 400 nm (Avestin: Canada). The external medium was substituted with HEPES buffered sucrose (HBS: 10 mM HEPES; 300 mM sucrose; pH=7.0) by dialysing the cassettes (Pierce: Rockford; IL) with 12–14 kDa molecular weight cut off (MWCO). To achieve optimal temperatures for DOX loading, incubation of aliquots (0.2 ml) of liposomes was initially performed with DOX hydrochloride (1 mg DOX/7 µmol total phospholipid) at 20, 30, 40, 50, 60, and 70°C for 60 minutes, and left to cool to room temperature. Aliquots of 25 µl were transferred to vials with 40 mg of Dowex resin and 1 ml of 10% sucrose (w/v) to remove the un-encapsulated DOX.

2.5 Liposome characterisation

A Dynamic Light Scattering (DLS) instrument (Nano-ZS; Malvern, UK) was used to characterize liposomes in terms of the size, zeta-potential, and poly-dispersity index. The phospholipid content was measured using a method based on the Bartlette phosphate assay. Spectrofluorimetry (ex: 470 nm/em: 590 nm, PerkinElmer LS45) was used to measure the concentration of DOX in liposomes by comparison with a calibration curve produced from DOX in acidified isopropanol. This procedure included the addition of 25 µl of the liposomes to vials with 40 mg of 1 ml of 10% sucrose (w/v) and Dowex resin, which was stirred for 1 min. Afterwards, dissolution of 0.1 ml of the supernatant was performed in 2 ml of the acidified isopropanol (90% isopropanol/0.075 M HCL). Then, the DOX fluorescence of the solution was measured. Likewise, a spectrofluorometer (ex: 600 nm/em: 665 nm) was used to measure the Dox content of liposomes in acidified isopropanol. It should be noted that the Dox concentration was determined before and after purification in order to determine the encapsulation efficacy of DOD. The formula below was used to calculate the proportion of Dox encapsulated:

$$\%Dox\ encapsulated = \frac{\text{Dox encapsulation after purification}}{\text{Dox encapsulation before purification}} \times 100$$

2.6 Measurement of cellular uptake

Cellular uptake of all liposomal formulas was examined in the J774.1 cell line. The cells were seeded into 24-well plates at cell densities of 100,000-200,000 cells/well on the 1st day. On the 2nd day, the cell medium was removed. All liposomal formulas were mixed with growth medium which was added to the plates at a normalized DOX concentration of 10 µM (5 µg/mL). The plates were incubated at 37 °C for 24 h. No drug was added to the control group wells. The cell medium was eliminated after 24 h, and ice-cold PBS (pH 7.0) was used to wash the cells 4 times. The cells were then lysed with 1 mL of DMSO. Centrifugation of the supernatants was performed at 140,000 rpm for 10 min, and the supernatants were gathered to obtain the cell lysates. A spectro-fluorometer at λex = 496 nm, λem = 592 nm to was used to measure the DOX fluorescence intensity of the cell lysates in order to calculate the DOX concentration. The mean (± SD) of 3 wells in all treatments for all experiments was used to calculate the intracellular uptake of DOX.

2.7 Bio-distribution

Mice (balbC) with an average weight of 25 to 30 g and about four weeks old were purchased from the Pasteur Institute of Iran (Tehran, Iran) and were kept with ad libitum access to food and water. The Ethics

Committee for Animal Experiments of the Mashhad University of Medical Sciences gave approval for all experimental protocols involving animals .

Mice were categorized into four groups (four mice in each group) for bio-distribution. The injection of all liposomal formulations was performed through the tail vein in a single dose (15 mg/kg doxorubicin equivalent) of either LD, 0.5%SP-LD, 1%SP-LD, or PLD formulations (equivalent volumes of approximately 200 uL of dextrose 5%). Contol mice received 200 uL of dextrose 5%.

Retro-orbital bleeding (~0.5 mL) was used to collect blood samples at three and six hours after dosing. The mice were killed to harvest tissue at 24 hours. Heart puncture was performed in order to collect the blood sample. Sdditionally, spleen, lungs, kidneys, heart, and parts of the liver were dissected, weighed, and placed in 2 mL polypropylene microvials (Biospec; OK) containing 1 mL of the acidified isopropanol and zirconia beads. MiniBeadbeater-1 (Biospec; OK) was then used to homogenize tissue. Blood was left to coagulate at 4˚C and was centrifuged for ten minutes at 14,000 rpm. At the next step, the serum was isolated, and dilution of a sufficient volume was done in 1 mL acidified isopropanol. The homogenized tissue samples and sera were maintained at 4 ˚C overnight to extract the drug. The samples were then centrifuged and the supernatant was assayed to measure concentration of Dox spectro-fluorimetrically (Ex; 470 nm; Em = 590 nm). Serial dilutions of Dox in tissues and sera extracts of the control mice were used to prepare the calibration curve.

2.8 Pharmacokinetics

Centrifugation of the blood samples was performed immediately at 1078 g for ten minutes to obtain plasma. The concentration of Dox was then measured in different groups. The mean residence time (MRT) was calculated by dividing the area under the curve (AUC) by the area under the momentum curve (AUMC). PK solver ADD-IND in Excel Microsoft was used to calculate the pharmacokinetic variables [27].

2.8 Statistical analyses

GraphPad Prism5 (GraphPad Software; San Diego: CA) was used to perform statistical analyses. The level of doxorubicin concentration in spleen, plasma, lung, heart, liver, and kidney is provided as the concentration value (written as mg of drug or metabolite/ml of plasma or gram of tissue). Each value is written as the mean+SEM. Analysis of variance, Bartlett, and Student–Newman–Keuls tests were used to compare experimental groups.

3. Results

3.1 Peptide coupling efficiency

The results of TLC, HPLC and NMR showed the formation of thioether bond between the cysteine (available at end of the peptide) and maleimide (available in DSPE-PEG (2000) Maleimide) (Data not shown).

3.2 Liposome characterisation

The particle diameter of the liposomes was about 110 nm (Polydispersity index <0.09) in all groups, and SP did not affect the observed liposome size (Table 1).

The efficiency of Dox encapsulation of each preparation was more than 96%. Leakage stability experiments did not indicate a significant difference in the release of Dox from peptide-modified or non-modified liposomes during 48 h incubation at 37˚C in the presence of 30% FCS. Liposomes showed the least Dox leakage, and more than 90% of the encapsulated Dox remained encapsulated.

3.3 Cellular uptake of Dox

Phagocytosis was 18% lower in the 1%SP-LD compared with the PLD group, showing a significant difference between the two groups ($P<0.05$). Furthermore, phagocytosis was significantly different between the 0.5%SP-LD group and the 1%SP-LD group ($P<0.05$). However, there was no significant difference between PLD and 0.5%SP-LD groups (Figure 1).

3.4 Bio-distribution of Dox

Biodistribution of each liposomal formulation was estimated by gauging intrinsic autofluorescence signals of doxorubicin in spleen, liver, kidney, heart, and lung in mice (Figure 2). Doxorubicin accumulation was significantly reduced in most tissues in the 1%SP-LD group.

2.5 Pharmacokinetics

The results obtained from the biodistribution of BalbC mice (Figure 3) show that the content of serum doxorubicin at 0.5 h after injection was significantly different between the SP-LD and PLD groups and LD group. However, there was no significant difference among 0.5%SP-LD, 1%SP-LD, and PLD groups. Although there was no significant difference between 0.5%SP-LD and PLD at 6 h after injection, a significant difference was reported between 1%SP-LD and PLD. In addition, there was a significant difference between 0.5%SP-LD and 1%SP-LD. The difference between the groups was more substantial at 24 h after injection. The concentration of doxorubicin in the serum of the 1%SP-LD group was nearly double that of the PLD group.

Table 2 presents other pharmaco-kinetic parameters such as area under the curve (AUC), concentration maximum (CM), mean residence time (MRT), clearance (Cl), and half-life ,t1/2.

4. Discussion

Much progress has been made in the field of nanotechnology and liposomal drug delivery for the treatment of various diseases [28-31]. Using functional ligands like CD47 coud be an appropriate strutegy to overcome drug delivery limitations with liposomes [32].

The CD47 protein is found on the surface of most cells in the body, and contributes to apoptosis, proliferation, adhesion, homeostasis, and cell migration. One of the most important roles of CD47 protein is to transmit the "Don't eat me" signal through the SIRPα ligand on the macrophage membranes. The present study used the CERVIGTGWVRC peptide sequence as a Self-Peptide (SP) on the surface of liposomes. This process aims to prevent liposomal clearance by macrophages *via* transmitting the "Don't eat me" signal to macrophages. Results obtained from HPLC and NMR demonstrated that the SP is well linked to DSPE-PEG (2000) Maleimide. In vitro results also showed that the use of the CD47 mimicry peptide prevents phagocytosis in macrophages. Moreover, in vivo results demonstrated that if SP-LD is used, this will result in a longer residence time in the blood circulation, as compared to PLD. Furthermore,

SP-LD accumulation was much lower in liver, spleen and lung tissues. This likely reduces the severity of side effects, which occur when nanoparticles containing drugs accumulate in non-target tissues.

Tissue biodistribution results in different tissues revealed that there is a significant difference between SP-LD and PLD in liver, spleen, and lung tissues. Greater accumulation of PLD was observed in these tissues. For example, doxorubicin concentrations in spleen tissue were 40% lower in the 1%SP-LD group compared to the PLD group. The same comparison for the liver tissue indicated that the level of doxorubicin in the 1%SP-LD group declined by 36% compared to the PLD group. This phenomenon occurred because the tissue macrophages in the RES system, result in greater absorption and uptake of the PLD form in these tissues. However, since phagocytosis is inhibited by SP, the SP-LD form undergoes much less phagocytosis and uptake into RES tissues.

These antiphagocytotic effects are consistent with results reported by Tasi et al. who used biotin and streptavidin, and linked CD47 to the polystyrene beads. These CD47-coated beads were then exposed to the human monocyte cell line (THP-1). The researchers observed that the rate of phagocytosis was reduced by 50% compared to the control sample (polystyrene without CD47) [33]. Moreover, Bruns et al. (2015) observed antiphagocytic effects of the CD47 receptor in artificial antigen-presenting cells (aAPC). These authors succeeded in designing a "don't eat me" aAPC using CD47-Ig on a classical aAPC [34].

Increasing the residence time of SP-LD in the circulatoin due to the inhibition of macrophage phagocytosis may allow achieving better therapeutic outcomes. For example, $T_{1/2}$ value for 1%SP-LD($C_{t=24}$=8.86) increased by about 29% in comparison to PLD($C_{t=24}$=6.86) formulation. Moreover, the clearance rate of 1%SP-LD (0.0077) formulation decreased by 25% compared to PLD(0.0102), representing a slower removal of 1%SE-LD formulation of the blood circulation compared to the PLD formulation. Additional pharmacokinetic parameters such as ACU and MRT showed that SE-LD clearly outperforms PLD. The results of this study demonstrate that this SP may defend LD against phagocytic activity of macrophages, and improve in-vivo pharmacokinetics and tumor distribution. This research used 150 and 300 peptides on 0.5%SP-LD and 1%SP-LD formulations, respectively. Comparison between the two groups indicated more acceptable effects with use of 300 peptides compared 150 peptides. The theoretical SP density on liposomes is of a similar order of magnitude (350 molecules/mm^2) to the observed physiologic situations on human red blood cells (250 molecules/mm^2) (20299253).

5. Conclusion

One of the most important problems associated with nanoparticle-based drug delivery is the rapid removal of nanoparticles by macrophages in the circulating blood, and the consequent short residence time of drug-carrying nanoparticles. A deep understanding of the interaction between macrophages and nanoparticles in can be used to improve drug delivery strategies. The interaction between the CD47 receptor and SIRPα (on the macrophage membrane) is very important because it inhibits phagocytosis by transmitting a message to the macrophages. The use of the CD47 receptor mimicry peptides on the nanoparticles' surface can inhibit phagocytosis by macrophages, and increase the residence time of nanoparticles in the circulation. Increasing the residence time of nanoparticles containing drug in the blood circulation may improve the efficacy of treatment. Our results suggest that CD47 coating reduces the accumulation of nanolipsomes in the tissues, which can reduce the risk of off-target toxicty of drugs in non-tumor tissues. Future stuidies are warranted

to confirm the present findings using other drugs and tumor models. Moreover, detailed toxicological studies are encouraged to compare the organ toxicities following administration of PLD and optimized SP-LD liposomes.

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Figure legends

Figure 1. 24-hour intra-cellular Dox uptake in J774.1 cells (n=3 experiments, 3 wells/treatment.
Abbreviations: LD: Liposomal Doxorubicin, PLD: Pegylated Liposomal Doxorubicin, SP-LD: Self Peptide Liposomal Doxorubicin, SP-PLD: Self Peptide Pegylated Liposomal Doxorubicin

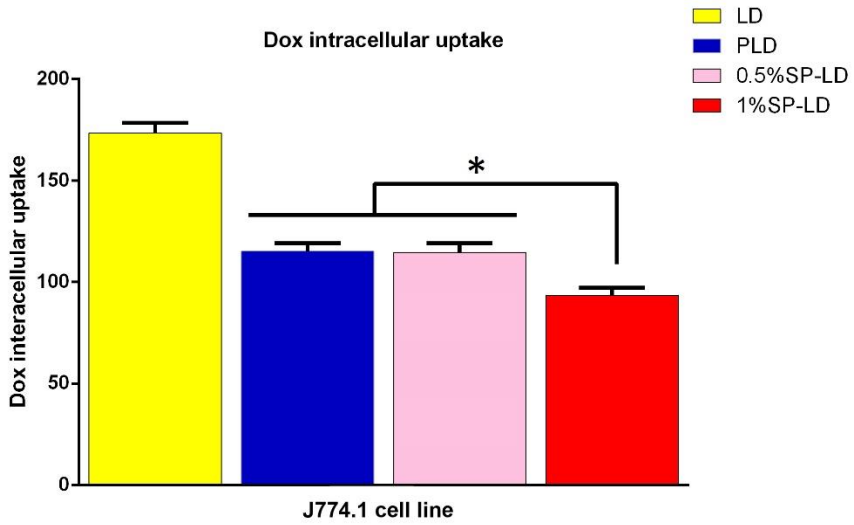


Figure 2. Concentrations of DOX in tissues at 24 h after injection of LD, PLD, 0.5%SP-LD, and 1%SP-LD at the equivalent DOX concentration of 15 mg/kg. Data are shown as mean ± standard deviation (n = 4). *indicates signifcant diferences ($p < 0.01$)

Abbreviations: LD= Liposomal doxorubicin. PLD= Pegylated liposomal doxorubicin. SP-LD= Self peptide liposomal doxorubicin.

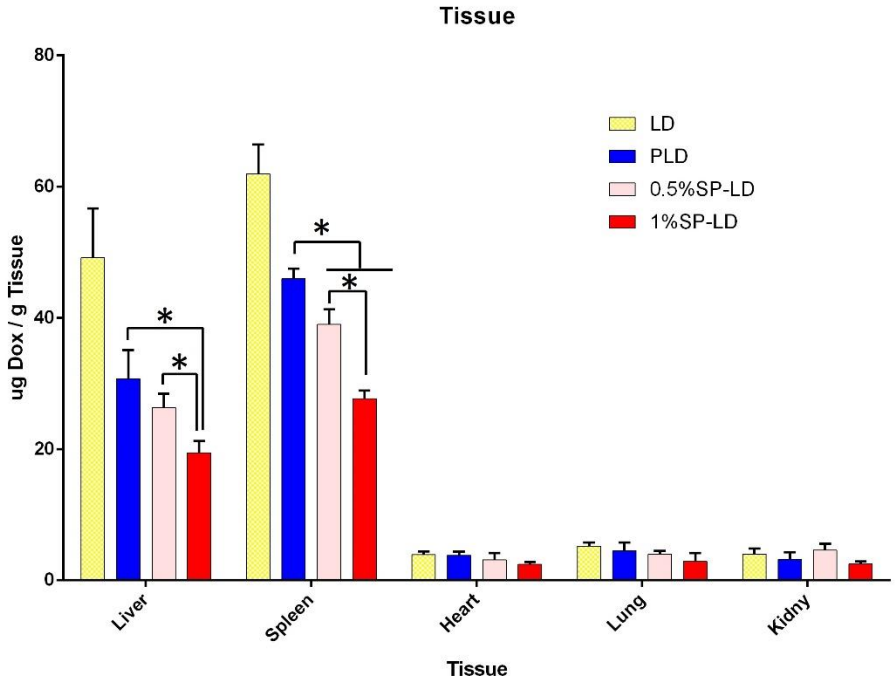
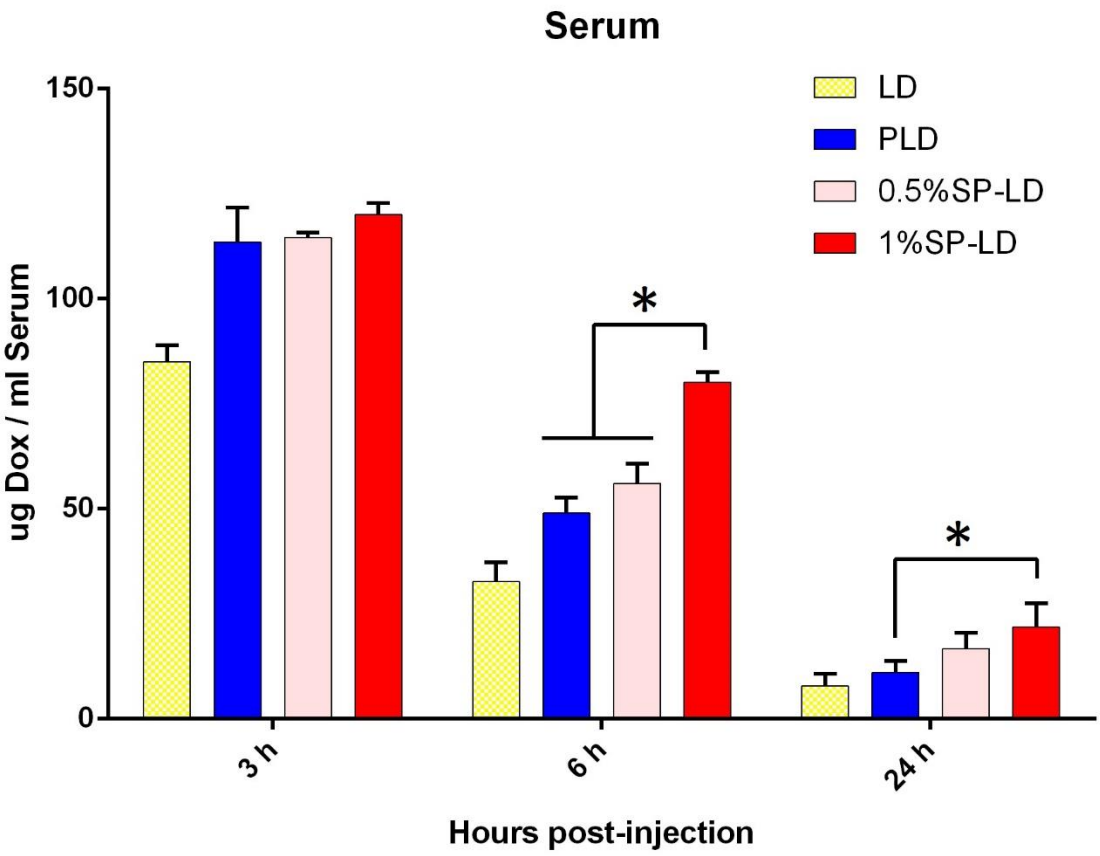


Figure 3. Plasma concentration of DOX (at 3 time points (3,6, & 24 h) after administration of LD, PLD, 0.5%SP-LD, and 1%SP-LD at the equivalent DOX concentration of 15 mg/kg.. Data are shown as mean ± standard deviation (n = 4). *indicates signifcant diferences ($p < 0.01$).

Abbreviations: LD= Liposomal doxorubicin. PLD= Pegylated liposomal doxorubicin. SP-LD= Self peptide liposomal doxorubicin.



Tables

Table 1. *Physical features of various formulations of liposome. Each value represents means ± SD (n=3).*

Liposome	Liposome composition (molar ratio)	Z-average size (nm)	Pdi[a]
LP	HSPC, Chol (62: 38)	107 ± 0.9	0.01 ± 0.01
PLD	HSPC, Chol, mPEG$_{2000}$-DSPE (61.5: 38: 0.5)	110 ± 1.1	0.12 ± 0.02
0.5%SP-LD	HSPC, Chol, pept-mPEG$_{2000}$-DSPE (61.5: 38: 0.5)	113 ± 1.2	0.05 ± 0.03
1%SP-LD	HSPC, Chol, pept-mPEG$_{2000}$-DSPE (61: 38: 1)	112 ± 1.5	0.09 ± 0.04

[a] Values are written as the mean±SD of 3 independent liposome samples (n=3).
[b] Poly-dispersity index (PDI)
Abbreviations: LD: Liposomal Doxorubicin, PLD: Pegylated Liposomal Doxorubicin, SP-LD: Self Peptide Liposomal Doxorubicin, SP-PLD: Self Peptide Pegylated Liposomal Doxorubicin

Table 2. *Plasma pharmacokinetic variables after the* intravenous *injection of 15 mg/kg of* LD, PLD, 0.5%SP-LD, and 1%SP-LD *to BalbC mice (n=3).*

Formulations	C_{max}[a] (ug/ml)	AUC[b] (μg/ml*h)	Cl[c] (mg)/(μg/ml)/h	MRT[d] (h)	$T_{1/2}$[e] (h)
LD	85.01	1000	0.0139	6.60	6.82
PLD	113.52	1349	0.0102	7.00	6.86
0.5%SP-LD	114.58	1434	0.0091	9.43	8.34
1%SP-LD	120.1	1668	0.0077	10.83	8.86

[a] Concentration Maximum, [b] Area Under Curve, [c] Clearance, [d] Mean Residence Time, [e] Half life
Abbreviations: LD: Liposomal Doxorubicin, PLD: Pegylated Liposomal Doxorubicin, SP-LD: Self Peptide Liposomal Doxorubicin, SP-PLD: Self Peptide Pegylated Liposomal Doxorubicin