Int. J. Pharm. Investigation, 2023; 13(1):100-105.
www.jpionline.org

Original Article

Site-Specific Delivery of Doxorubicin Using Cell-Penetrating Peptide for Lung Cancer Chemotherapy

Laxmikant Gautam, Suresh P. Vyas*

Drug Delivery Research Laboratory, Department of Pharmaceutical Sciences, Dr. Harisingh Gour Vishwavidyalaya (A Central University), Sagar, Madhya Pradesh, INDIA.

ABSTRACT

Recent studies have focused heavily on tumor-oriented nanocarriers mediated by cell-penetrating peptides (CPPs). However, the loss of CPPs in normal tissues and enzymatic degradation in circulation frequently prevented the use of CPPs *in vivo*. To alleviate these limitations, CPPs needed to be kept immobilized before they arrived at the intended target for receptor-mediated endocytosis (RME). In this study, we developed CAR/DOX-Liposomes with doxorubicin hydrochloride (DOX) entrapped in the hydrophilic core of liposomes using a thin film hydration method, and CAR peptide was subsequently conjugated through SPDP chemistry on the surface of liposomes as targeting moiety to develop and improved targeted cancer chemotherapy. The prepared liposomes were characterized and evaluated for different parameters which were recorded to be vesicle size 275.2±5.65 nm, polydispersity index 0.260±0.85, Zeta-potential -33.90±2.42 mV, and % entrapment efficiency 83.96±2.56 %. In addition, Transmission electron microscopy (TEM), and Atomic Force Microscopy (AFM) studies were conducted to assess morphology and *in vitro* drug release performed. Further, the Cell line study of the CAR/DOX-Liposomes was studied over the lung cancer cell line HOP-62 and the comparison IC_{50} values were determined. The study establishes that CAR/DOX-Liposomes offer specific delivery of DOX to the heparan sulfate receptor(s) exclusively overexpressed on the cancer cells.

Keywords: Lung Cancer, DOX, Heparan sulfate, CAR peptide.

Correspondence

Prof. Suresh P. Vyas

Department of Pharmaceutical Sciences, Dr. Harisingh Gour University Sagar-470003 Madhya Pradesh, INDIA.

Email id: spvyas54@gmail.com

ORCID ID 0000-0001-8388-8321

Received: 03-10-2022;
Revised: 11-10-2022;
Accepted: 28-10-2022.

INTRODUCTION

Cell annexation is an innate biological mechanism that cells use to form and constrain the structure of their extracellular matrix in order to develop, regenerate, and safeguard bodily tissues.[1] Cancer is a disease that is frequently detected in people of all ages. Currently, the major category of cancers is breast cancer, lung cancer, prostate cancer, cervical cancer, colon cancer, skin cancer, oral cancer, etc.[2,3] The treatment for cancer and prognosis thereafter is critically dependent on an early diagnosis. There are several conventional treatment methods available, including radiation, chemotherapy, and surgical intervention. The cancer cell generates its environment for its growth which includes excess cell proliferation, low pH, hypoxia, formation of neo-blood vessels (neoangiogenesis), high temperature, high enzymatic activity, etc. Different nanotechnological drug delivery system(s) including liposomes,[4] vesosomes,[5] nanoparticles,[6] mesospheres,[7] nanosponges,[8] superparamagnetic iron oxide nanoparticles (SPIONs), quantum dots[9], etc., have been explored as the delivery platform for bioactive(s) i.e., doxorubicin, paclitaxel, cisplatin, methotrexate, etc. These drugs could be targeted to the site of action using nanomodules resulting in an improved therapeutic index. DOX is an anthracycline antibiotic used to treat leukemia, lung cancer, breast cancer, and ovarian malignancies. DOX mechanisms as an anticancer agent are as follows: (i) production of free radicals and the resulting impairment of cellular membranes, proteins, and DNA; and (ii) interaction with DNA and disruption of topoisomerase-II-mediated DNA repair. Semiquinone, an unstable metabolite that is produced from DOX in a process that produces reactive oxygen species, is formed when DOX is oxidized. This process causes DNA damage, oxidative stress, and the activation of apoptotic pathways in cells.[10] For a site-specific delivery, the ligand is selected which directly targets the cancer site and inhibits the cancer cell growth, with subsequent programmed cell death. The CAR-peptide[11,12] which is a cell-penetrating peptide was selected for the delivery of DOX-loaded liposomes. CAR peptide is a nonapeptide having a series of 9-amino acid CARSKNKDC[13,14] specifically binds with the heparan sulfate receptor over-expressed on the cancer cells of lung carcinomas and reportedly also inhibits the neoangiogenesis by binding in a site-specific manner.

DOI: 10.5530/223097131810

The present study reports on the development of a biodegradable system based on liposomes for efficient chemotherapy of lung cancer tissue. Studies were conducted to characterize the developed formulation(s) for vesicle size, zeta potential, PDI, %EE, and cumulative drug release. Additionally, MTT assay experiments for the cytotoxic capability of the proposed system were conducted using the HOP-62 lung cancer cell lines.

MATERIALS AND METHODS

Lipids i.e. dipalmitoylphosphatidylcholine (DPPC),1,2-Distearoyl-sn-glycero-3-phosphorylethanolamine (DSPE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (ammonium salt) (DSPE-PEG-2000), cholesterol, were purchased from Lipoid GmbH. Frigenstr, Ludwigshafen, Germany. N-succinimidyl 1,3-(2-pyridyl dithiol) propionate (SPDP), purchased from CovaChem, LLC., USA. Doxorubicin hydrochloride (DOX) was received as a gift sample from Neon Laboratories Ltd., Mumbai, India. CAR peptide was purchased from Synpeptide co. Ltd., China. Sephadex G-25, Triton X-100, DMSO, and other chemicals were purchased from Sigma-Aldrich Powai, Mumbai, India. All Analytical-grade chemicals were used as supplied by the manufacturer(s).

Preparation of DOX-loaded CAR-conjugated liposomes

The liposomes were prepared using the thin film hydration method.[15,16] In this process, lipids DPPC, cholesterol, DSPE, and DSPE-PEG-2000 in a ratio of 65:25:5:5 was used for the preparation of liposomes shown in Figure 1. The lipids were dissolved in a solvent mixture of chloroform and methanol (90:10). The solvent was evaporated under a vacuum using a rotatory vacuum evaporator (Rotavap, REMI) until a dry film was formed. Ammonium sulfate (250 mM) was then used to hydrate the dry film.[17] The ammonium sulfate-loaded multilamellar vesicles (MLVs) were thus formed. The prepared MLVs were then transformed into Small Unilamellar Vesicles (SUVs) by using a sonication process. In an ice bath (to avoid overheating), the prepared MLVs were sonicated for 6 min applying a cycle of 20s ON and 20s OFF which resulted in the formation of SUVs. After sonication, for the removal of excess salt, the formulation was dialyzed for 24 hr against a solution of HEPES buffer (145 mM NaCl, 10 mM HEPES, pH 7.4). DOX (10 mg aqueous solution in PBS) was then added, under moderate stirring for 30 min at 55°C. DOX-loaded liposomes were dispersed in SPDP in Dimethyl formamide (DMF) (30 µL) solution and incubated at room temperature for nearly 30 min. Then the excess SPDP was removed by applying ultracentrifugation for 355000 g for 1 hr at 4°C. The sedimented pellet was redispersed in phosphate buffer saline (PBS). To the dispersion of amino-functionalized phospholipids, DOX-loaded liposomes, CAR peptide (2 mg in 200 µL) was added and incubated for 1 hr at room temperature. The excess CAR was separated by centrifugation at 355000 g for 1 hr. The pelleted CAR functionalized loaded with DOX liposomes were redispersed in PBS and stored at 4°C temperature until they were used for characterization.[18,19]

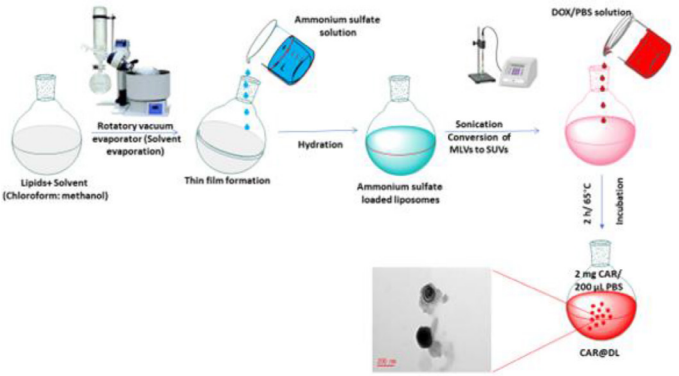

Figure 1: Method of Preparation of DOX-loaded liposomal system

Fourier transform infrared spectroscopy (FTIR)

The developed system was characterized by FTIR spectroscopy using Bruker Tensor-37, FTIR. Prior to IR tests, samples of DOX and CAR/DOX-Liposomes were vacuum-dried for 12 hr. The dried sample was set used for further IR spectral analysis.[20]

Vesicle size, zeta-potential, and PDI

The particle size, polydispersity index (PDI), and zeta potential were determined using Quasi elastic light scattering techniques. CAR/DOX-Liposomes suspension was diluted with 1:100 distilled water and analyzed by photon correlation spectroscopy (PCS) using a NanoPlus Zeta/nanoparticle analyzer (Version 5.01, Particulate Systems, Norcross, GA, UK).

Transmission electron microscopy (TEM)

The CAR/DOX-Liposomes were observed under TEM (Tecnai, Japan). A drop of the sample was placed on a copper grid coated with carbon and dried under air to form a uniform film of the sample. Subsequently, the film was negatively stained using 1% (w/v) Phosphotungstic acid (PTA). After the film of the sample was completely dried, it was examined using a transmission electron microscope. The microphotograph was captured at the appropriate magnifications.

Atomic force microscopy (AFM)

Using an NSC 12(c) cantilever (MikroMasch, Silicon Nitride Tip) using Nano DriveTM version 8 software, the CAR/DOX-Liposomes was also seen using AFM in the acoustic AC mode (also referred to as tapping mode). A steel disc with an adhesive coating was given a drop of CAR/DOX-Liposomes. The dried sample was then pushed into a nitrogen atmosphere after allowing the drop to evaporate. The samples were examined using an atomic force microscope before photomicrographs were obtained at the proper magnification.

% Drug entrapment efficiency

The high-speed centrifugation technique was used to determine the drug content of CAR/DOX-Liposomes. CAR/DOX-Liposomes suspensions (1 mL) were centrifuged using a lab centrifuge (Sigma 2-16 PK Labmate (Asia)) for 3 min at 2,000 rpm. The liposomal pellet was lysed using 0.1% Triton X-100 and the liberated drug was analyzed spectrophotometrically using Shimadzu UV mini 1240 at 480 nm.[4] The drug entrapment efficiency was calculated using the formula:

$$\% \text{ drug entrapment efficiency} = \frac{\text{Amount of drug in liposomes}}{\text{Total amount of drug used in the liposomal formulation}} \times 100$$

In-vitro drug release study

In-vitro release study was carried out in phosphate buffer saline (PBS) at pH 7.4. The 10 mg of DOX was dispersed in 1 mL of PBS (pH 7.4) and then sonicated to produce the free DOX nanosuspension. In a dialysis bag, the drug release experiment was conducted (nitrocellulose membrane, Molecular weight cut-off 12,000 Da). Half an hour before the release study, the dialysis bag was activated by treatment with phosphate buffer solution. The nanosuspension of DOX and the suspension of CAR/DOX-Liposomes (SUVs) were placed in the equivalent amount into different two sets of dialysis-based bags. The bags were emersed in PBS (100 mL) stirred and maintained at 37°C for 72 hr. The samples from dissolution media were taken periodically. The volume of the media was maintained by replacing the withdrawn volume with freshly prepared PBS. At 480 nm, the absorbance was measured using a UV-Visible spectrophotometer.

Cytotoxicity analysis

Free DOX and CAR/DOX-Liposomes were all tested for cytotoxicity on HOP-62 cells using the MTT 3-(4,5-dimethylthiazol-2- yl)- 2,5- diphenyltetrazolium assay. HOP-62 cell lines (2×10^5/well) were plated in a 96-well microtiter plate and allowed to adhere for 24 hr. Freshly prepared media was added and the cells were treated with different concentrations (1, 5, 10, 20, 40, 80 µg/ml) of free DOX, and CAR/DOX-Liposomes and the culture medium treated group was considered as control, incubated at 37°C for 24 hr in a CO_2 incubator. After being washed with PBS 7.4, the cells were treated with the MTT reagent (5 mg/mL) and allowed to co-incubate for 4 hr. Subsequently, co-incubation of the formazan crystals formed was dissolved by adding 100 µL of DMSO. The absorbance was then measured using a microplate reader at 570 nm. Cells treated with PBS served as controls. The degree of cytotoxicity was evaluated followed by IC_{50} for 24 hr of treatment, and the data were recorded with a mean standard deviation (±SD).

Statistical Analysis

All data are presented as mean± SD and were analyzed by ANOVA (GraphPad Prism version 8.0, GraphPad Software, San Diego, CA). In statistical analysis, *p*-value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Preparation and characterization of CAR/DOX-Liposomes

The liposome in the present study was essentially prepared using selected lipids (DPPC, CH, DSPE) along with DSPE-PEG-2000. The latter was used for the PEGylation of liposomes. The liposomes were initially prepared as MLVs using the conventional lipid film hydration method[15]. The MLVs were subjected to sonication in order to transform into SUVs. The initial hydration is affected by using an ammonium sulfate solution (250 mM). After removal of excess ammonium sulfate by dialysis, ammonium sulfate loaded liposomes suspension was added with DOX solution and incubated at 55°C for 1 hr. The temperature correspondence to the transition temperature (TG) of the lipid mixture used in the preparation of liposomes. At TG and due to concentration gradient DOX diffused into the aqueous core of liposomes and got entrapped as sulfate complex, thus leading to excessively high entrapment efficiency of 83.96±2.56 %. The free DOX was separated from the DOX-loaded liposomes using centrifugation. They were redispersed and activated through SPDP chemistry. This results in the activation of the amino group of DSPE. After removal of excess SPDP by centrifugation, the pelleted amine-activated DOX-loaded SUVs were co-incubated with CAR at 25°C for 1 hr in order to allow CAR conjugation. The CAR/DOX-Liposomes were separated and used for further studies.

FTIR analysis

The FTIR spectrum of the DOX and CAR/DOX-Liposomes samples is shown in Figure 2. The primary distinctive bands of the drug in the liposomes were visible, particularly those that resulted from the following: The carbonyl stretching vibration -C=O at 1734 cm^{-1}, the -CH_2 bending vibration -CH_2 at 1470 cm^{-1}, the symmetric and antisymmetric PO^{2-} stretching vibrations, and the -CH_2 symmetric and antisymmetric stretching vibrations of the acyl chain at 2,850 cm^{-1} and 2920 cm^{-1}, respectively are banding vibrations at 1090 cm^{-1} and 1220 cm^{-1}, respectively. The confirmation of the CAR conjugation with the amino group of DSPE is confirmed by the peak of -NH-CO- at 1685 cm^{-1}.

FTIR analysis of different peaks there appears to be consistent and corresponds to those reported in the literature for SPDP and CAR. However, the disappearance of the peak that corresponds to -COOH of CAR and -NH_2 of DSPE suggests the formation of a covalent link between the -NH_2 group of DSPE and the carboxylic terminal of SPDP that subsequently conjugates with CAR peptide. However, no peak correspondence to the hydrogen

link was recorded. Thus, It may be inferred that there is no link between DOX and phospholipids.

TEM and AFM

Further, studies using the TEM demonstrated that CAR/DOX-Liposomes were spherical. The TEM image of liposomes, however, appeared opaque, spherical, and uniform in size. The opaqueness of the electron beams may be accounted for by the effect of conjugation. The imaging performed using atomic force microscopy also supported the formation of homogeneous liposomes. In the micrographs, there was no evidence of liposome aggregation or fusion, demonstrating that remote loading did not lead to vesicular deformity or morphological distortion. The uniform size, spherical, and well-defined shape of CAR/DOX-Liposomes are evident in three-dimensional images from (3D) AFM.[21] The CAR/DOX-Liposomes were microscopically (TEM and AFM) shown in Figure 2.

Vesicle size, zeta-potential, and PDI

The average vesicle diameter of CAR/DOX-Liposomes was found to be 275.2±5.65 nm. The polydispersity index of the optimized

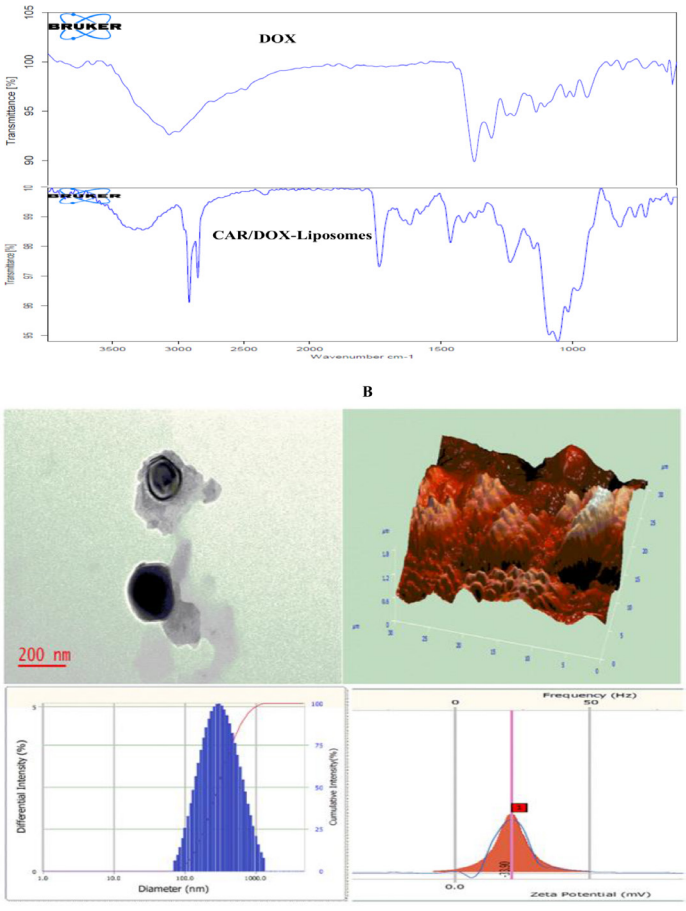

Figure 2: (A) FTIR analysis of DOX and CAR/DOX-Liposomes, (B) Physicochemical characterization of CAR/DOX-Liposomes; Transmission electron micrograph (upper left) appeared opaque particulates image which could be due to the conjugation effect, 3-dimensional atomic force micrograph (upper right), vesicle size (lower left), zeta-potential (lower right).

Table 1: Average vesicle size (nm), Zeta-potential (mV), PDI and % Entrapment efficiency of CAR/DOX-Liposomes (*n*=3, ±SD).

Formulation	Average vesicle size diameter (nm)	Zeta-potential (mV)	PDI	% Entrapment efficiency
CAR/DOX-Liposomes	275.2±5.65	-33.90±2.42	0.260±0.85	83.96±2.56

formulation of CAR/DOX-Liposomes was 0.260±0.85. The low polydispersity index i.e., less than 0.3 as obtained for CAR/DOX-Liposomes indicates narrow size distribution of liposomes. The zeta-potential recorded for the CAR/DOX-Liposomes was -33.90±2.42 mV. The liposomes displayed a negative charge with optimal potential subscribing, and greater stability. The data are reported in Figure 2 and Table 1.

% Entrapment efficiency

The enhanced 83.96±2.56% drug entrapment efficiency was measured. In case of active loading is facilitated due to s gradient-dependent diffusion of ammonium sulfate specifically ammonia gas and likewise influence of DOX within the liposomes core and formation of protonated DOX following interaction with sulfate ions could have offered better retention and maintenance of DOX gradience. The finding is the well agreement with those reported in the literature.[22-24]

% Cumulative drug release

The drug release was conducted on Plain DOX and CAR/DOX-Liposomes using dialysis bags with defined and molecular cutoffs. The study was conducted for 72 hr. It was recorded that almost 100% drug was diffused out of the bags for plain DOX nanosuspension, while in the case of CAR/DOX-Liposomes the release of the drug as DOX was recorded to be constantly slow when in 72 hr 65.32 ± 1.26 % of the drug was diffused out from dialysis media (dissolution media). In the case of liposomes, the release was sustained shown in Figure 3A, which could be beneficial for improved drug pharmacodynamic effect.[25]

Cytotoxicity analysis

In vitro cytotoxicity of DOX and CAR/DOX-Liposomes is determined in HOP-62 (Human Lung Adenocarcinoma) cell lines. Irrespective of the fact whether it is a free drug or CAR/DOX-Liposomes the toxicity was explicitly concentration-dependent. CAR/DOX-Liposomes (~4.46) and DOX (~9.30) both exhibited IC_{50}. However, there was a remarkable difference in cytotoxicity as well as IC_{50} in the case of CAR/DOX-Liposomes recorded. It was significantly higher than plain DOX when tested on an equivalent dose basis ($p<0.05$) recorded in Figures 3B and C. The results as recorded after 24 hr suggest that over a prolonged period of time there has been continuous accumulation as well as the sustained cytotoxic release of drug

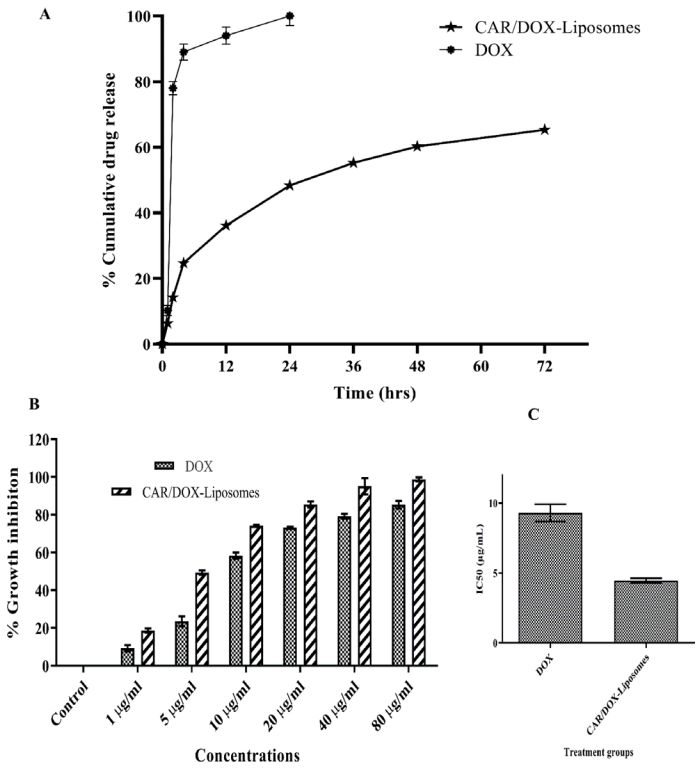

Figure 3: (A) Cumulative % drug release from CAR/DOX-Liposomes as compared to plain DOX through the diffusion-controlled mechanism, the data are represented as mean ±standard deviation in triplicates. (*n*=3); (B) Cytotoxicity assessment using HOP-62 lung cancer cell line for 24 hr (DOX and CAR/DOX-Liposomes) and (C) IC_{50} evaluation. (n=3), (p<0.05).

from accumulated carrier drug composites, which could be accounted for significantly higher toxicity recorded in the case of CAR/DOX-Liposomes.[26] The involvement of receptor-mediated endocytosis may evaluate no specific activity of CAR peptide transmitting it to be site-specific activity could be beneficial in target-oriented chemotherapy using CAR peptide as a targeting ligand.[27] Furthermore, the accumulation of CAR within the cell may interfere with organelles activities ultimately facilitating programmed cell death. Thus, the activity of the drug delivered using CAR could get synthesized as refraction in many folds. Higher toxicity was recorded in the form of IC_{50} value.[28]

CONCLUSION

The present study suggests that the use of DSPE-PEG-2000 and DPPC, DSPE as lipids could help in the engineering of a long circulatory liposomal version for surface functionalization in order to make it site-specific. The active loading procedure could effectively be used for loading water-soluble ionizable drug molecules which on protonation get trapped in the hydrophilic core in incorporated or compromised in diffusion across the hydrophilic domain of the lipid bilayer. Thus, may be used with ensured drug payload. This also transformed the liposomes avail to mitigate drug diffusion. Resulting, in sustained and prolonged drug release. The CAR peptide which by perse biological activity is a non-specific cell-penetrating peptide which could not be used safely in its free form. However, when it is immobilized on the surface of liposomes as a targeted moiety it terms to be site-specific, particularly those which overexpress CD44 receptors for heparan sulfate. The developed system exhibited very strong cytotoxicity which could be escribed to the receptor-mediated endocytosis-based drug carrier composite intracellular accumulation. Subsequently, slow carrier/ carrier moiety released in the cytosol for bispecific anti-cancer activity is accounted for drug while other to the CAR peptide. It may safely be assumed that CAR could have destabilized the endosome before fusion with the lysosome to reach intact with its liposomal entry into the cytosol. The developed formulation was recorded to be physically stable as zeta-potential recorded -33.90±2.42 mV which was optimal for the physical stability of the suspension. In addition, the conjugation of CAR, peptide offered an additional barrier to drug diffusion which could be one of the factors that may be responsible for slowing the drug diffusion of liposomes.[29,30] In inference, the study concludes that the developed system holds very high potential and possibilities to be used in targeted cancer chemotherapy. However, an elaborated system that has been conducted on cells has been seminal in the assessment of its clinical values.

ACKNOWLEDGEMENT

The author Laxmikant Gautam acknowledges ICMR for Senior Research Fellowship (SRF) (45/16/2018-Nan/BMS dated: May 11, 2018).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AFM: Atomic force microscopy; **CPP:** cell-penetrating peptides; **CAR**- CARSKNKDC peptide **DMSO:** Dimethyl sulfoxide; **DOX:** Doxorubicin hydrochloride; **DMF:**Dimethyl formamide; **MLVs:** Multilamellar vesicles; **MTT 3-**(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium assay; **PBS:** Phosphate buffer saline; **PTA:** Phosphotungstic acid; **PDI:** Polydispersity Index; **RME:** receptor-mediated endocytosis; **SPDP:** Succinimidyl 3-(2-pyridyldithio) propionate; **SUVs:** Small Unilamellar vesicles; **SPIONs:** Superparamagnetic iron oxide nanoparticles; **TG:** Transition temperature; **TEM:** Transmission electron microscopy.

REFERENCES

1. Gautam L, Jain A, Vishwakarma N, Sharma R, Mody N, Dubey S, *et al*. Nanomedicine for the diagnosis and treatment of cancer. Nanomedicine for the Treatment of Disease. Apple Academic Press. 2019. p. 395-422. doi: 10.1201/9780429425714-14.
2. Islami F, Guerra CE, Minihan A, Yabroff KR, Fedewa SA, Sloan K, *et al*. American Cancer Society's report on the status of cancer disparities in the United States, ACS journals. CA Cancer J Clin. 2022;72(2):112-43. doi: 10.3322/caac.21703, PMID 34878180.
3. Shrivastava P, Sharma R, Gautam L, Vyas S, Vyas SP. Nanoparticles and colon cancer. Nano Drug Delivery Strategies for the Treatment of Cancers. Elsevier. 2021. p. 191-223. doi: 10.1016/B978-0-12-819793-6.00009-6.
4. Shrivastava P, Gautam L, Sharma R, Dube D, Vyas S, Vyas SP. Dual antitubercular

drug loaded liposomes for macrophage targeting: development, characterisation, *ex vivo* and *in vivo* assessment. J Microencapsul. 2021;38(2):108-23. doi: 10.1080/02652048.2020.1857861, PMID 33267623.

5. Gautam L, Shrivastava P, Yadav B, Jain A, Sharma R, Vyas S, *et al*. Multicompartment systems: A putative carrier for combined drug delivery and targeting. Drug Discov Today. 2022;27(4):1184-95. doi: 10.1016/j.drudis.2021.12.007. PMID 34906689.
6. Sharma R, Agrawal U, Mody N, Dubey S, Vyas SP. Engineered nanoparticles as a precise delivery system in cancer therapeutics. Engineering of Nanobiomaterials. Elsevier. 2016. p. 397-427. doi: 10.1016/B978-0-323-41532-3.00013-0.
7. Gautam L, Sharma R, Shrivastava P, Vyas S, Vyas SP. Development and characterization of biocompatible mannose functionalized mesospheres: An effective chemotherapeutic approach for lung cancer targeting. AAPS PharmSciTech. 2020;21(5):190. doi: 10.1208/s12249-020-01742-9, PMID 32661573.
8. Banjare N, Gautam L, Behera C, Gupta PN, Vyas S, Vyas SP. Cyclodextrin nanosponges based site-retentive controlled release system for treatment of rheumatic arthritis. J Drug Deliv Sci Technol. 2020;60:101973. doi: 10.1016/j.jddst.2020.101973.
9. Gautam L, Jain A, Shrivastava P, Vyas S, Vyas SP, *et al*. Emergence of novel targeting systems and conventional therapies for effective cancer treatment. Nano Drug Delivery Strategies for the Treatment of Cancers. Elsevier. 2021. p. 1-35. doi: 10.1016/B978-0-12-819793-6.00002-3.
10. Thorn CF, Oshiro C, Marsh S, Hernandez-Boussard T, McLeod H, Klein TE, *et al*. Doxorubicin pathways: Pharmacodynamics and adverse effects. Pharmacogenet Genomics. 2011;21(7):440-6. doi: 10.1097/FPC.0b013e32833ffb56, PMID 21048526.
11. Lin W, Xie X, Deng J, Liu H, Chen Y, Fu X, *et al*. Cell-penetrating peptide-doxorubicin conjugate loaded NGR-modified nanobubbles for ultrasound triggered drug delivery. J Drug Target. 2016;24(2):134-46. doi: 10.3109/1061186X.2015.1058802, PMID 26176270.
12. Gautam L, Shrivastava P, Vyas S. Cell-penetrating peptides (CPPs) and peptide-drug conjugate systems (PDCs): Site-specific delivery of bioactive (s) for effective breast cancer therapy. Targeted Nanomedicine for Breast Cancer Therapy. Elsevier. 2022. p. 509-32. doi: 10.1016/B978-0-12-824476-0.00008-5.
13. Urakami T, Järvinen TA, Toba M, Sawada J, Ambalavanan N, Mann D, *et al*. Peptide-directed highly selective targeting of pulmonary arterial hypertension. Am J Pathol. 2011;178(6):2489-95. doi: 10.1016/j.ajpath.2011.02.032, PMID 21549345.
14. Järvinen TA. Design of target-seeking antifibrotic compounds. Methods Enzymol. 2012; 509:243-61. doi: 10.1016/B978-0-12-391858-1.00013-7, PMID 22568909.
15. Bangham AD, Standish MM, Watkins JCJ. Diffusion of univalent ions across the lamellae of swollen phospholipids. J Mol Biol. 1965;13(1):238-52. doi: 10.1016/s0022-2836(65)80093-6, PMID 5859039.
16. Bangham AD, Horne RW. Negative staining of phospholipids and their structural modification by surface-active agents as observed in the electron microscope. J Mol Biol. 1964;8(5):660-8. doi: 10.1016/S0022-2836(64)80115-7, PMID 14187392.
17. Luo D, Carter KA, Razi A, Geng J, Shao S, Giraldo D, *et al*. Doxorubicin encapsulated in stealth liposomes conferred with light-triggered drug release. Biomaterials. 2016; 75:193-202. doi: 10.1016/j.biomaterials.2015.10.027, PMID 26513413.
18. Santra S, Yang H, Dutta D, Stanley JT, Holloway PH, Tan W, *et al*. TAT conjugated, FITC doped silica nanoparticles for bioimaging applications. Chem Commun (Camb). 2004;24(24):2810-1. doi: 10.1039/B411916A, PMID 15599418.
19. Nahar K, Absar S, Gupta N, Kotamraju VR, McMurtry IF, Oka M, *et al*. Peptide-coated liposomal fasudil enhances site specific vasodilation in pulmonary arterial hypertension. Mol Pharm. 2014;11(12):4374-84. doi: 10.1021/mp500456k, PMID 25333706.
20. Yang Y, Yan Z, Wei D, Zhong J, Liu L, Zhang L, *et al*. Tumor-penetrating peptide functionalization enhances the anti-glioblastoma effect of doxorubicin liposomes. Nanotechnology. 2013;24(40):405101. doi: 10.1088/0957-4484/24/40/405101, PMID 24029287.
21. He Y, Geng Y, Yan Y, Luo XJ. Fabrication of nanoscale pits with high throughput on polymer thin film using afm tip-based dynamic plowing lithography. Nanoscale Res Lett. 2017;12(1):544. doi: 10.1186/s11671-017-2319-y, PMID 28940164.
22. Alyane M, Barratt G, Lahouel MJ. Remote loading of doxorubicin into liposomes by transmembrane pH gradient to reduce toxicity toward H9c2 cells. Saudi Pharm J. 2016;24(2):165-75. doi: 10.1016/j.jsps.2015.02.014, PMID 27013909.
23. Daeihamed M, Haeri A, Ostad SN, Akhlaghi MF, Dadashzadeh SJN. Doxorubicin-loaded liposomes: enhancing the oral bioavailability by modulation of physicochemical characteristics. Nanomedicine (Lond). 2017;12(10):1187-202. doi: 10.2217/nnm-2017-0007, PMID 28447868.
24. Chountoulesi M, Naziris N, Pippa N, Demetzos CJ. The significance of drug-to-lipid ratio to the development of optimized liposomal formulation. J Liposome Res. 2018;28(3):249-58. doi: 10.1080/08982104.2017.1343836, PMID 28627268.
25. Jiang T, Zhang Z, Zhang Y, Lv H, Zhou J, Li C, *et al*. Dual-functional liposomes based on pH-responsive cell-penetrating peptide and hyaluronic acid for tumor-targeted anticancer drug delivery. Biomaterials. 2012;33(36):9246-58. doi: 10.1016/j.biomaterials.2012.09.027, PMID 23031530.
26. Varghese SE, Fariya MK, Rajawat GS, Steiniger F, Fahr A, Nagarsenker MSJDd. Lecithin and PLGA-based self-assembled nanocomposite, Lecithmer: Preparation, characterization, and pharmacokinetic/pharmacodynamic evaluation. Drug Deliv Transl Res. 2016;6(4):342-53. doi: 10.1007/s13346-016-0314-y, PMID 27371394.
27. Järvinen TA, Ruoslahti EJ. Molecular changes in the vasculature of injured tissues. Am J Pathol. 2007;171(2):702-11. doi: 10.2353/ajpath.2007.061251, PMID 17600129.
28. Fu S, Zhao Y, Sun J, Yang T, Zhi D, Zhang E, *et al*. Integrin $\alpha v\beta 3$-targeted liposomal drug delivery system for enhanced lung cancer therapy. Colloids Surf B Biointerfaces. 2021; 201:111623. doi: 10.1016/j.colsurfb.2021.111623, PMID 33636597.
29. Knelson EH, Nee JC, Blobe GCJ. Heparan sulfate signaling in cancer. Trends Biochem Sci. 2014;39(6):277-88. doi: 10.1016/j.tibs.2014.03.001, PMID 24755488.
30. Toba M, Alzoubi A, O'Neill K, Abe K, Urakami T, Komatsu M, *et al*. A novel vascular homing peptide strategy to selectively enhance pulmonary drug efficacy in pulmonary arterial hypertension. Am J Pathol. 2014;184(2):369-75. doi: 10.1016/j.ajpath.2013.10.008, PMID 24401613.

Cite this article: Gautam L, Vyas SP. Site-Specific Delivery of Doxorubicin Using Cell-Penetrating Peptide for Lung Cancer Chemotherapy. Int. J. Pharm. Investigation. 2023;13(1):100-5.