ScienceDirect

Acta Biomaterialia

Volume 106, 1 April 2020, Pages 301-313

Full length article

Cell-penetrating corosolic acid liposome as a functional carrier for delivering chemotherapeutic drugs

Xuqian Li [a], Andy Samuel Widjaya [a], Jingxuan Liu [a], Xiao Liu [a], Zhiguo Long [b], Yanyan Jiang [a]

Show more

Share Cite

https://doi.org/10.1016/j.actbio.2020.02.013

Get rights and content

Abstract

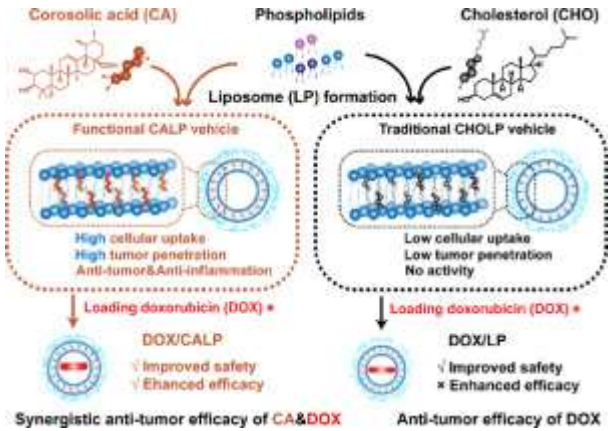
Corosolic acid (CA), a natural pentacyclic triterpenoid, exhibits antitumor and synergistic therapy effect with chemotherapeutic drugs mainly through inhibiting STAT3 activation. In this study, it is found that CA possesses cholesterol-like properties in liposome by regulating membrane phase behavior to form stable cholesterol-free CA liposomes (CALP). Compared with traditional cholesterol liposomes (CHOLP), CALP exhibit stronger membrane fusion and higher cellular uptake, and other functions including inhibition of STAT3 activation and suppression of the recruitment of macrophages to tumor microenvironment. Therefore, CALP is used as a functional carrier, and doxorubicin-loaded CALP (DOX/CALP) based on PEGylated liposomal doxorubicin (DOXIL®) are prepared by replacing its cholesterol with CA. The physicochemical properties and biological activities are compared with those of doxorubicin-loaded cholesterol liposomes (DOX/LP). Both DOX/CALP and DOX/LP possess approximately similar physical properties and exhibit high

stability and low drug leakage as shown by the published data of DOXIL®. Nevertheless, it is noteworthy that DOX/CALP displays higher *in vitro* cellular uptake and tumor spheroid permeation along with stronger cytotoxicity against tumor cells than DOX/LP. Despite DOX/CALP has the same PK parameters, normal tissue biodistribution, and safety profile as DOX/LP, the results of an *in vivo* study in 4T1-bearing mice indicate that the DOX/CALP treatment group exhibit higher tumor accumulation, more significant tumor growth inhibition, and longer life span than the DOX/LP group. Overall, DOX/CALP is a representative example of CA-doped liposomes, suggesting that CALP as a functional drug carrier for solving low efficacy of present liposomal drugs might have promising application potential.

Statement of Significance

An original drug delivery nanocarrier, corosolic acid liposome (CALP), was developed in this study. It was found that CA possesses cholesterol-like function to regulate phospholipid membrane phase behavior. By replacing the cholesterol with CA, the liposomes were converted into high cellular uptake carriers, possessing anti-inflammatory activity and synergism with chemotherapeutic drugs. The variability of CALP formulations enabled to deliver therapeutic agents. The use of CALP to deliver doxorubicin not only significantly enhanced the therapeutic efficacy compared with the classic PEGylated liposomal doxorubicin, but also maintained the improved safety. Because CALP can be obtained by conventional liposome preparation methods, its use as functional drug carriers for solving low efficacy of present liposomal drugs would have promising application potential.

Graphical abstract



Download : Download high-res image (345KB)

Download : Download full-size image

Introduction

Corosolic acid (CA), a pentacyclic triterpenoid extracted from plant species [1], has been demonstrated as a potent STAT3 inhibitor in many types of tumors such as osteosarcoma [2], glioblastoma [3], and ovarian cancer [4], as well as in stromal cells in tumor environment, including tumor-associated macrophages [3]. CA could also directly induce mitochondria-mediated and caspase-dependent apoptosis in A549 lung adenocarcinoma cells [5]. An animal experiment result indicated that CA impairs tumor development and lung metastasis by inhibiting the immunosuppressive activity of myeloid-derived suppressor cells in murine sarcoma model [2]. More importantly, studies have confirmed that CA displays synergistic antitumor effect with chemotherapeutic drugs such as doxorubicin (DOX), paclitaxel, cisplatin, 5-FU, and the multitarget kinase inhibitor sorafenib [4,6,7]. However, some undesirable properties of CA, such as poor water solubility, low bioavailability, and rapid plasma clearance, limit its *in vivo* application. Furthermore, most results on its antitumor efficacy are from *in vitro* studies. In only two previous *in vivo* studies, the dosage regimens are daily intraperitoneal injection (using dimethyl sulfoxide as solvent) [7] or oral administration at high dosage [2].

Liposomes have favorable properties that enable formulation of highly toxic and/or poorly soluble drugs [8]. Encapsulation of drugs in liposomes could enhance the therapeutic indices and improve the pharmacokinetic characteristics [9]. Cholesterol is often required when preparing liposomes because cholesterol can stabilize the phospholipid membrane by regulating its fluidity [10]. However, cholesterol occupies the limited hydrophobic space in the membrane and thus decrease hydrophobic drug content in bilayer [11], [12], [13].

The same issue arises when we attempt to prepare liposomal CA; it is almost impossible to load CA into liposomes using the formulation of phospholipids and cholesterol (an earlier study in 2019 achieved only ~3.8% mole loading capacity of CA in liposome [14]). Unexpectedly, once cholesterol was removed from the formulation, liposomal CA (CALP) can be easily obtained with favorable stability, and the maximum loading capacity of CA is 38.4% mole ratio in lipid membrane, which is far greater than many hydrophobic components like paclitaxel [12].

Our recent research has demonstrated that co-delivery of multiple therapeutic mechanism agents achieved better antitumor effects [15]. Considering that CA has synergistic antitumor efficacy in combination with chemotherapeutic drugs, we assume that CALP, as the carrier of chemotherapeutic agents, would improve their therapeutic efficacy. In the present study, CA-doped liposomes with different formulations were prepared, and their properties and functions were evaluated. Furthermore, DOX-loaded CALP (DOX/CALP) were designed on the basis of approved DOX liposomes (DOXIL®) by replacing the cholesterol in DOXIL® with corosolic acid, and the DOX/LP

were then prepared as an equivalent of DOXIL®. The physical and chemical properties of DOX/CALP and DOX/LP were compared carefully, and their biological functions and antitumor efficacy were further evaluated both *in vitro* and *in vivo*.

Section snippets

Preparation of liposomes

Cholesterol liposomes (CHOLP) were prepared by thin film hydration methods according to the formulation of DOXIL® [16]. Briefly, hydrogenated soy phosphatidylcholine (HSPC), cholesterol (CHO), and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)−2000] (DSPE-PEG2000) (imported by A.V.T. Pharmaceutical, Shanghai; produced by NFC, Japan) were mixed at molar ratio of 56.3: 38.4: 5.3 and dissolved in ethanol/isopropanol (1/1,v/v), followed by evaporation at 60 °C. The …

Characterization of CALP

Empty CALP and CHOLP were prepared on the basis of formulation of DOXIL®, with a 38.4% molar ratio of CA or CHO. As shown in Fig. 1A and B and Table 1, the Z-average size of CHOLP and CALP were 96 nm and 85 nm, respectively, with a polydispersity index (PDI) less than 0.1, and both liposomes had a negative charge. The morphology of the liposomes observed by TEM showed spheres with uniform size.

To determine whether CA has cholesterol-like function that regulates lipid bilayer fluidity, DSC…

Discussion

For the first time, this study finds that CA combined with phospholipids can form stable liposomes through replacing the position of cholesterol in lipid bilayer. The cholesterol-like function of CA in lipid bilayer was first investigated. As shown in Fig. S10, both CA and cholesterol molecule have a hydrophobic rigid body; thus, CA would have a tendency to interact with the palisade structure of the membrane, thus restricting the flexing motion of neighboring fatty acyl chain region, which is…

Conclusion

An original drug delivery nanocarrier, CA liposome, was developed in this study. Our findings are as follows: (1) CA possesses the cholesterol-like function and can form stable liposomes with different phospholipids by replacing cholesterol in liposome formulations; (2) CALP can be easily prepared by conventional liposome preparation methods and achieve a relatively high CA loading; (3) CALP has some unique biological functions, including anti-inflammatory effect, suppression of macrophages,…

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.…

Acknowledgements

The authors gratefully acknowledge the National Natural Science Foundation of China (81371673) and PDH-SPFDU Joint Research Fund(RHJJ2018-05). This study is also supported by The Open Project Program of Key Lab of Smart Drug Delivery (Fudan University), Ministry of Education, China. We thank staff members from National Center for Protein Science Shanghai (NCPSS) for their technical support on TEM and cryo-EM.…

Recommended articles

References (48)

K.J. Nho *et al.*
Corosolic acid induces apoptotic cell death in human lung adenocarcinoma A549 cells *in vitro*
Food & Chem. Toxicolo. Int. J. Publ. Br. Indus. Biol. Res. Assoc. (2013)

S. Hong *et al.*
Effects of triglycerides on the hydrophobic drug loading capacity of saturated phosphatidylcholine-based liposomes
Int. J. Pharmaceut. (2015)

R. van der Meel *et al.*

Extracellular vesicles as drug delivery systems: lessons from the liposome field

J. Contr. Release (2014)

Y.C. Barenholz

Doxil® — the first FDA-approved nano-drug: lessons learned

J. Control. Release (2012)

K. Un *et al.*

Intracellular trafficking mechanism, from intracellular uptake to extracellular efflux, for phospholipid/cholesterol liposomes

Biomaterials (2012)

G. Koopman *et al.*

Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis

Blood (1994)

L. Zhang *et al.*

RGD-modified PEG–PAMAM–DOX conjugates: *in vitro* and *in vivo* studies for glioma

Eur. J. Pharm. Biopharm. (2011)

T.J. Pucadyil *et al.*

Cholesterol: a potential therapeutic target in Leishmania infection?

Trends Parasitol. (2007)

X. Wei *et al.*

Insights into composition/structure/function relationships of Doxil®; gained from "high-sensitivity" differential scanning calorimetry

Eur. J. Pharma. Biopharm. (2016)

L. Silverman *et al.*

In vitro experiments showing enhanced release of doxorubicin from doxil(r) in the presence of ammonia may explain drug release at tumor site

Nanomedicine-UK (2015)

View more references

Cited by (22)

A folic acid-decorated nanoparticles loaded JQ1 for oral squamous cell carcinoma therapy

2024, Chinese Chemical Letters

Show abstract

Replacing cholesterol with asiatic acid to prolong circulation and enhance anti-metastatic effects of non-PEGylated liposomes

2024, Journal of Controlled Release

Show abstract

Quantitative transcriptomic and proteomic analysis reveals corosolic acid inhibiting bladder cancer via suppressing cell cycle and inducing mitophagy in vitro and in vivo

2023, Toxicology and Applied Pharmacology

Show abstract

A multi-bioresponsive self-assembled nano drug delivery system based on hyaluronic acid and geraniol against liver cancer

2023, Carbohydrate Polymers

Show abstract

Corosolic acid-modified lipid nanoparticles as delivery carriers for DNA vaccines against avian influenza

2023, International Journal of Pharmaceutics

Show abstract

Enzyme/GSH/pH-responsive hyaluronic acid grafted porous silica nanocarriers bearing Ag<inf>2</inf>S QDs for fluorescence imaging and combined therapy

2023, Carbohydrate Polymers

Show abstract

View all citing articles on Scopus

View full text

© 2020 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

ELSEVIER

All content on this site: Copyright © 2024 Elsevier B.V., its licensors, and contributors. All rights are reserved, including those for text and data mining, AI training, and similar technologies. For all open access content, the Creative Commons licensing terms apply.

RELX™