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Green design "bioinspired disassembly-reassembly strategy" applied for improved tumor-targeted anticancer drug delivery

Ruoning Wang [a], Xiaochen Gu [b], Jianping Zhou [a,*], Lingjia Shen [c], Lifang Yin [a], Peiying Hua [a], Yang Ding [a,*]

[a] State Key Laboratory of Natural Medicines, Department of Pharmaceutics, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China
[b] College of Pharmacy, University of Manitoba, 750 McDermot Avenue, Winnipeg, MB R3E 0T5, Canada
[c] National Engineering and Research Center for Target Drugs, Jiangsu, Hengrui Medicine CO., LTD, 7 Kunlun Shan Road, Lianyungang 222000, China

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ABSTRACT

In this study, a simple and green approach 'bioinspired disassembly-reassembly strategy' was employed to reconstitute lipoprotein nanoparticles (RLNs) using whole-components of endogenous ones (contained dehydrated human lipids and native apolipoproteins). These RLNs were engineered to mimic the configuration and properties of natural lipoproteins for efficient drug delivery. In testing therapeutic targeting to microtubules, paclitaxel (PTX) was reassembled into RLNs to achieve improved targeted anti-carcinoma treatment and minimize adverse effects, demonstrating ultimately more applicable than HDL-like particles which are based on exogenous lipid sources. We have characterized that apolipoprotein-decoration of PTX-loaded RLNs (RLNs-PTX) led to favoring uniformly dispersed distribution, increasing PTX-encapsulation with a sustained-release pattern, while enhancing biostability during blood circulation. The innate biological RLNs induced efficient intracellular trafficking of cargos *in situ via* multi-targeting mechanisms, including scavenger receptor class B type I (SR-BI)-mediated direct transmembrane delivery, as well as other lipoprotein-receptors associated endocytic pathways. The resulting anticancer treatment from RLNs-PTX was demonstrated a half-maximal inhibitory concentration of 0.20 μg/mL, cell apoptosis of 18.04% 24 h post-incubation mainly arresting G2/M cell cycle *in vitro*, and tumor weight inhibition of 70.51% *in vivo*. Collectively, green-step assembly-based RLNs provided an efficient strategy for mediating tumor-targeted accumulation of PTX and enhanced anticancer efficacy.

1. Introduction

Conventional chemotherapy has been faced wit, a variety of intrinsic deficiencies in terms of drug applications, ranging from low drug solubility, poor dosage stability, adverse drug effects or toxicity, to inability of molecules to cross cell membrane [1,2]. Various nano-carriers, including liposomes [3], micelles [4], polymeric nanoparticles [5], nanogels [6], and nanofibers [7], have all been investigated for their potentials as novel drug delivery systems for anti-cancer and imaging agents [8]. Several platforms have subsequently led to fundamental comprehension and appreciation of these preparations in innovative chemotherapy [9,10]. In particular, nanoparticles made of naturally occurring biomaterials have been regarded as one of the most efficient drug delivery devices due to their exceptional characteristics, such as biodegradability, biocompatibility, biorenewablity and superior binding capacity to multiple drugs [11–13].

Human endogenous lipoprotein systems, including classes of low-density lipoproteins (LDL) [14] and high-density lipoproteins (HDL) [15], are composed of lipids, apolipoproteins, cholesterol, and other specialized proteins. They form core-shell spherical or discoidal nanoparticles of 7–600 nm *in vivo* to transport water-insoluble lipids for peripheral usage [16,17]. Structurally, the amphiphilic nature of the lipids is capable of stabilizing the lipophilic cores, while the anchored apolipoproteins maintain the hydrodynamic size of the self-assembled natural nanoparticles [18,19]. Furthermore, lipoproteins possess specific affinity to their endogenous receptors *in vivo*. For example, scavenger receptor class B type I (SR-BI) facilitates HDL-cargo transfer [16,17]; LDL binds to corresponding high-affinity cell surfaces that contain LDL receptors (LDLR) and/or their related proteins (LRP). Study has also indicated increased presentation of SR-BI, LDLR and LRP in malignancies [20]. As such, endogenously created lipid-protein complexes have been investigated for drug-loading strategies [21,22]. This bioinspired drug delivery approach might eventually open an emerging avenue for innovative drug delivery and specific drug targeting, particularly, for cancer treatment and antitumor drug delivery [23].

Among the diverse approaches for manufacturing bio-derived nanoparticulates, those based on green technologies are gaining significant share rapidly. Green pharmaceutics aims at designing products and processes that significantly eliminate the use and generation of hazardous substances and prevent health impacts at source [24]. Nowadays, existing lipoprotein-based delivery platforms have so far been confined to single lipoprotein-like

* Corresponding authors.
E-mail addresses: zhoujianp60@163.com (J. Zhou), dydszyzf@163.com (Y. Ding).

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nanoparticles, such as conventionally reconstituted LDL (rLDL) [25] or reconstituted HDL (rHDL) [26], which were fabricated from isolated apolipoproteins and exogenous lipids. By mimicking the configuration of endogenous native lipoproteins, these recombinant LDL or HDL vehicles are capable of being retained in the systemic circulation for an extended period of time, largely evading the reticuloendothelial cells *in vivo*, and targeting cancer receptors (LDLR, LRP or SR-BI) to certain extents for enhanced therapeutic outcomes [18,27]. However, studies have also indicated that rLDL or rHDL mediated therapy might induce endogenous protein instability, compete with native lipoproteins, and potentiate risks for disturbing cholesterol homoeostasis *in vivo* [28,29]. Moreover, conventional rLDL or rHDL nanoparticles are normally reconstituted from exogenous lipid materials, either synthetic or from biological sources, and simplex apolipoproteins (*e.g.*, apoB-100, apoA-I), which are obtained from isolation of human serum, genetic engineering, or synthesis of mimetic peptides. These procedures in lipoprotein production may lead to defects in clinical applications, such as complex purification processing, introduction of immune disorders, and functional variation of synthetic lipoproteins [30].

In this study, we have proposed a novel green concept of 'bioinspired disassembly-reassembly strategy' to prepare nanoparticles of lipoproteins (Scheme 1). Instead of obtaining single rLDL or rHDL by reconstitution using purified apolipoprotein and commercial lipids, we utilized isolated, dehydrated human lipids and original proportions of endogenous apolipoproteins for reassembly. Furthermore, we chose identical composition ratio of native lipoprotein components in the preparation, hoping to restore natural structure of the endogenous lipoproteins. To evaluate the validity of these bioinspired reassembled nanoparticles, we prepared three different lipoprotein-like nanosystems based on the use of native components, *i.e.*, apoA-I-anchored nanoparticles (HDL-like or Type I), apoB-100-anchored nanoparticles (LDL-like or Type II), and mixed apolipoprotein-anchored nanoparticles (Type III). Antitumor compound paclitaxel (PTX) was incorporated into these nanoparticle preparations, and their antitumor behavior and efficacy were evaluated both *in vitro* and *in vivo*. The objectives of the study were to evaluate the cellular uptake mechanisms of the nanoparticles, in particular the multi-receptor mediated uptake mechanisms of the RLNs system, and to reduce drug side effect in comparison to commercial PTX preparation (Taxol®).

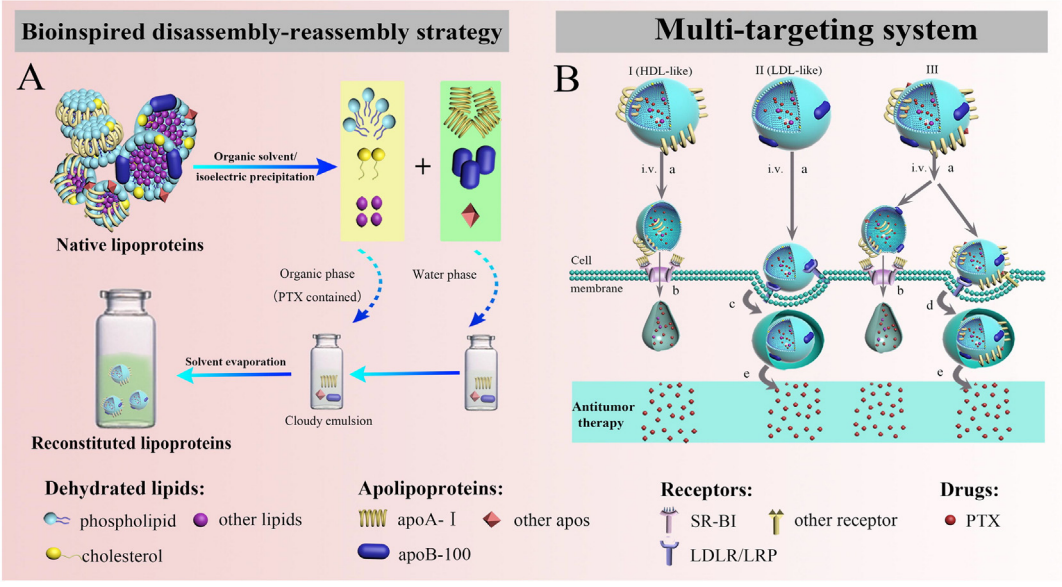
2. Materials and methods

2.1. Materials

Human plasma sample (precipitate IV) was generously donated by Tonrol Bio-Pharmaceutical Co., Ltd. (Hefei, Anhui, China). Paclitaxel (PTX) was purchased from Shanghai Zhongxi Pharmaceutical (Group) Co., Ltd. (Shanghai, China). Apolipoprotein A-I (apoA-I), high density lipoprotein (HDL), low density lipoprotein (LDL), coumarin-6 (C6) and 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl tetrazolium bromide (MTT) dye were purchased from Sigma Aldrich (St Louis, MO, USA). Soybean phospholipids (PC, purity 90%) were obtained from Evonik Degussa China Co., Ltd. (Shanghai, China). Cholesterol (Chol) and cholesteryl esters (CE) were purchased from Huixing Biochemical Reagent Co., Ltd. (Shanghai, China). Sodium cholate was offered by Aladdin Reagent Database Inc. (Shanghai, China). ELISA Kit for apoA-I, apolioprotein B-100 (apoB-100) and apolipoprotein E (apoE) were purchased from USCN Life Science Inc. (Wuhan, Hubei, China). LysoTracker Red was purchased from Invitrogen Corporation (Carlsbad, CA, USA). AnnexinV-FITC/PI Apoptosis Detection Kit was obtained from Sunshine Biotechnology Co., Ltd. (Nanjing, Jiangsu, China). BD PI/RNase Staining Buffer was obtained from BD Biosciences (San Diego, CA, USA). 1, 1′-dioctadecyl-3, 3, 3′, 3-tetramethylindotricarbocyanine iodide (DiR) was obtained from Fanbo Biochemicals Co. Ltd. (Beijing, China). All chemicals and reagents were of HPLC or analytical grade.

2.2. Disassembly of endogenous apolipoproteins and lipids

Green designed lipoprotein-inspired nanoparticles consisted of endogenous apolipoproteins and dehydrated lipids, which were isolated and purified from human plasma sample (precipitate IV) using an organic solvent/isoelectric precipitation method previously described with some modifications [31]. Briefly, precipitate IV was suspended; after centrifugation, the pH of the supernatant was adjusted to 5.5 and the resulting precipitate was collected by further centrifugation. The



Scheme 1. (A) 'Bioinspired disassembly-reassembly strategy' was applied for RLNs-PTX preparation *via* an emulsion-evaporation method by using the isolated, dehydrated human lipids and endogenous apolipoproteins. (B) Schematic cross-section of RLNs-PTX incubated with apoA-I only (I, HDL-like), apoB-100 only (II, LDL-like), or mixed apolipoproteins (III), respectively; proposed mechanism for multiple receptor-mediated PTX delivery. a) Enhanced permeation and retention effect. b) SR-BI-mediated direct transmembrane mechanism. c) LDLR or LRP mediated endocytosis. d) Other lipoprotein-receptors-associated endocytic pathway. e) Cytoplasmic PTX release for antitumor therapy.