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Hyaluronic acid-decorated reconstituted high density lipoprotein targeting atherosclerotic lesions

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ABSTRACT

The primary aim of our current study was to utilize hyaluronic acid (HA) to decorate reconstituted high density lipoprotein (rHDL) loaded with lovastatin (LT), termed as HA-LT-rHDL, in order to investigate whether coating HA could efficiently evade from the undesired uptake of LT-rHDL in liver mediated by scavenger receptor class B type I (SR-BI) and then greatly accumulate LT-rHDL in atherosclerotic lesions via strong HA affinity to CD44 up-regulated at inflammatory sites such as atherosclerotic lesions, thus exerting enhanced atheroprotective efficacy. *In vitro* characterizations indicated the successful HA decoration onto the surface of LT-rHDL, which could be indirectly verified by the increased particle size, enhanced negative surface charge and reduced *in vitro* drug release rate after HA decoration. Compared with rHDL without HA, HA decoration endowed rHDL with better atherosclerotic lesions targeting efficiency and lower liver accumulation, proved by results from *ex vivo* imaging and tissue distribution. Furthermore, atheroprotective efficacy in model animal showed that HA-LT-rHDL had the best potent efficacy than other LT preparations, which was demonstrated by the fewest atherosclerotic lesions sizes, the most minimum mean intima-media thickness (MIT), the lowest macrophage infiltration and expression of matrix metalloproteinase-9 (MMP-9), respectively. Above results demonstrated that the newly designed HA-LT-rHDL could decrease the non-targeted uptake by liver and deliver a large amount of drug into atherosclerotic lesions so as to efficiently suppress the advancement of atherosclerosis.

1. Introduction

Atherosclerosis is a cardiovascular disease mainly affecting arterial vessels and a chronic inflammatory response in the arteries' walls, primarily aroused by the constant accumulation of macrophage-derived foam cell [1]. Detection of atherosclerotic lesions as soon as possible and then promptly effective prevention on the deterioration of lesions had been generally put forward by pharmacologists in development of effective antiatherosclerotic regimen [2,3]. Among them, directly delivering drug into the specific target in atherosclerotic lesions, greatly inhibiting the progress of plaques and markedly improving the stability of plaques, would become one of the most promising treatment protocols in future. Recently, researchers have discovered multiple potential targets preferentially presented on atherosclerotic progress, including some specific inflammatory cells and a number of particular cell surface receptors, etc. Several atherosclerotic lesions-targeted ligands have been developed hitherto, which contain phage display technology-derived special peptides targeting for vascular cellular adhesion molecule-1 (VCAM-1) [4], dextran sulfate for scavenge receptor type-A (SR-A) [5], phosphatidylserine for cluster of differentiation 36 (CD36) [6] and LyP-1 peptide for cell face p32 abundant in foam cell [7], etc. However, those atherosclerotic lesions-targeted ligands were almost exploited in atherosclerotic lesions imaging, but not involved in the lesions-targeted drug delivery for efficient treatment of atherosclerosis yet.

High density lipoprotein (HDL) is a type of endogenous nanoparticles basically composed of multiple biological lipids and apolipoproteins (apos). It has been commonly acknowledged that plasma HDL concentration is inversely correlated with the incidence of cardiovascular disease and meanwhile HDL exerts a crucial role in protecting cardiovascular system on account of its peculiar protective effects such as removing excess cholesterol from peripheral tissues, improving endothelial dysfunction and exerting antioxidant effect [8]. Reconstituted HDL (rHDL), the synthetic form of endogenous HDL, is mostly composed of natural or synthetic phospholipids, apos, triglycerides, free cholesterol and cholesterol

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esters, etc. Recently, rHDL has been not only demonstrated as similar physicochemical properties to native counterpart, but also extensively applied in pharmaceutical development as effective drug delivery system [9]. Among them, our previous studies have successfully constructed rHDL loaded with cardiovascular drug tanshinone IIA (TA) [10–12]. *In vitro* experiments manifested that TA-rHDL had similar micro-structure and several biological activities to natural HDL, meanwhile TA-rHDL possessed suitable particle size, high entrapment efficiency (EE) and comparable drug loading efficiency (DL). Moreover, cell experiments enlightened that TA-rHDL had high affinity to macrophage-derived foam cell over-expressing SR-BI and then delivered large amount of drug to foam cell through SR-BI-mediated endocytosis. Ultimately, *ex vivo* imaging and *in vivo* antiatherogenic efficacy on atherosclerotic model animal demonstrated that TA-rHDL could extremely improve pharmacokinetic profile of free TA, effectively deliver "cargo" to the atherosclerotic lesions and then exhibit potent atheroprotective efficacy, which indicated uptake of the TA incorporated in rHDL mediated via SR-BI abundant in macrophage-infiltrated atherosclerotic lesions. However, SR-BI is over-expressed not only in the atherosclerotic lesion's foam cell but also in the hepatic cells, inevitably resulting in the uptake of r-HDL by liver, so a large portion of injected rHDL loaded with drug was also detected in the liver because of enormous uptake by hepatic cell [13]. Thus, how to decrease the unintended drug encapsulated in rHDL uptake by liver and actively increase the drug accumulation in atherosclerotic lesions would be the urgent issues to figure out for enhancing atheroprotective efficacy.

Cell adhesion molecule CD44 is mainly up-regulated on the surface of injured endothelial cells at inflammatory sites such as various kinds of tumors and atherosclerotic lesions, etc. The principle endogenous ligand of CD44 is hyaluronic acid (HA), a negatively charged large linear mucopolysaccharide, which also is an important component of extracellular matrix in atherosclerotic lesions and has weak affinity to hepatic receptors [14,15]. It was also demonstrated that due to the similar molecular structure to widely used hydrophilic coating material - polyethylene glycol (PEG) and active targeting effect, HA-decorated nanocarriers could efficiently avoid recognition by reticuloendothelial system (RES) and circulate in the blood long enough to accumulate in CD44 over-expressed tissues [16,17]. In recent years, many researches had employed HA to decorate nanocarriers for anticancer drugs to improve the targeting efficiency to solid tumors via CD44-mediated uptake, while overlooking the valuable function of HA in diagnosis and therapy of atherosclerosis [18–21]. The up-regulated CD44 at atherosclerotic lesions coupled with the high affinity HA to CD44 at inflammatory sites render it attractive to utilize HA/CD44 interactions for effective plaque targeting [22,23]. Heretofore, barely any research was reported on HA-modified nanocarriers to achieve atherosclerotic lesions-targeted drug delivery.

Currently, we expected that rHDL coated with HA (HA-rHDL) could reduce the unwanted recognition by non-target tissues, especially by liver via SR-BI-mediated uptake, and then reinforce atherosclerotic lesions targeting efficiency by strongly adhering to the CD44 mostly located on the injured endothelial cells at atherosclerotic lesions. Specifically, at first, HA-rHDL loading drug could decrease the unintended recognition by liver due to shielding off SR-BI by HA coating, thus circulating for a long period in blood and then substantially accumulating in atherosclerotic lesions by active CD44-mediated targeting; moreover, once across the endothelium, HA-rHDL loading drug would expose naked rHDL loading drugs to foam cell after HA degradation by hyaluronidase (HAase) abundant intra plaque [24], then efficiently deliver the "cargo" into foam cell via SR-BI-mediated endocytosis.

In order to fulfill our propose mentioned above, we selected the classical anti-hyperlipidemia drug lovastatin (LT) as model drug and further developed a nanocarriers HA-rHDL loaded with LT (HA-LT-rHDL). In the present study, positively charged LT-rHDL was first constructed by sodium cholate mediation after thin film dispersion as described previously with minor revision, and then employed as the cationic core for HA coating through electrostatic adsorption to generate HA-LT-rHDL. *In vitro* studies, including physicochemical characterization, morphology and drug release of each preparation, were thoroughly investigated. *In vivo* studies, including pharmacokinetic behavior, atherosclerotic lesions targeting property and atheroprotective efficacy, were systematically examined in atherosclerotic New Zealand white (NZW) model rabbits. We hoped that the elucidated targeting of HA-LT-rHDL to atherosclerotic lesions could shed new lights for treatment of atherosclerosis in future. The main scheme of our present study was presented in Fig. 1.

2. Materials and methods

2.1. Materials

Lovastatin was kindly donated by Jiangsu Yangzi River Pharmacy Company (Jiangsu, China). Egg phospholipid (PC, Lipoid S100) was purchased from Lipoid GmbH (Germany). Octadecylamine (OL) and cholesterol were obtained from Sigma–Aldrich chemie GmbH (USA). Cholesteryl oleate was purchased from AlfaAesar a Johnson Matthey Co., Ltd. (USA). Glycerol trioleate was obtained from Tokyo Kasei Kogyo Co. Ltd. (Japan). Sodium hyaluronic acid (HA, the molecular weight of 200–400 kDa) was kindly provided by Freda Biochem Co., Ltd. (Shandong, China). The apos (97% purity) was isolated from the albumin waste as depicted previously. Bovine serum albumin (BSA) was purchased from Sun shine Biotech Co., Ltd. (Shanghai, China). Lovastatin capsule was bought from Chengdu Yongkang Pharmacy Co., Ltd. (Chengdu, China). Near infrared fluorescence dye DiR was purchased from Fanbo Biochemicals Co., Ltd. (Beijing, China). All other reagents were of analytical or chromatographic grade. Distilled and deionized water were used in all experiments.

2.2. Preparation of HA-LT-rHDL

Preparation procedure of HA-LT-rHDL was composed of constructing the cationic lipid cores of HA-LT-rHDL and subsequently formation of HA-LT-rHDL.

The cationic lipid cores of HA-LT-rHDL, namely LT-rHDL, were prepared by thin film dispersion and ensuing sodium cholate mediation as previously described with minor revision [25,26]. Specifically, LT with a lipid mixtures (phospholipids (PC:OL mole ratio, 1:0.125), cholesterol, cholesteryl oleate and glycerol trioleate) were dissolved in appropriate volume of methanol/chloroform (1:1, v/v) and dried under vacuum in an egg-plant flask at 50 °C so as to form dry film and remove the organic solvent. Then, 15 mL of 0.02 M Tris buffer (pH8.0) was added to hydrate the dried film for 1 h, and sodium cholate was added for later incubation with apos. After that, the hybrid suspension was vortexed thoroughly for 15 min followed by the ultrasonication in ice bath at 300 W. Then, the dispersion was extruded successively through 0.45 μm and 0.22 μm filters to obtain LT-loading nanostructured lipid carriers (LT-NLC). Next, 15 mL of LT-NLC was incubated with equivalent volume 0.02 M Tris buffer (pH8.0) containing 15 mg apos at 37 °C under 600 rpm for 8 h to obtain LT-loading rHDL (LT-rHDL) and then dialyzed to remove residual free drug and sodium cholate.

Furthermore, HA-coated LT-rHDL (HA-LT-rHDL) was prepared via electrostatic absorption reported by Almalik et al. and Jiang et al. [14,27]. Briefly, the LT-rHDL dispersion obtained above was dropwise added to 0.1% (w/v) HA solution at a volume ratio of 1:2 (v/v) under vigorous stirring for 1 h. The mixture was incubated at 37 °C for 1 h with gentle agitation to obtain the HA-LT-rHDL afterwards.

2.3. In vitro characterizations

2.3.1. Mean size, zeta potential, EE and DL

Mean size and zeta potential of LT-NLC, LT-rHDL and HA-LT-rHDL were determined by Dynamic Light Scattering (DLS) Analyzer (Zetasizer 3000 HAS, Malvern, UK), respectively. Measurements after appropriate dilution were carried out in triplicate at 25 °C on three independent preparations.

The EE and DL of LT-NLC, LT-rHDL and HA-LT-rHDL were measured after removal of non-entrapped drug through micro-column centrifugation as previously described, respectively [26]. Then, EE and DL of three different preparations were determined by an RP-HPLC method using Agilent 1200 series (Palo Alto, CA, USA) coupled with a UV detector set at 238 nm and a shim-pack VP-ODS column (150 × 4.6 mm, 5 μm) at 30 °C. The mobile phase consisted of methanol and water (80:20, v/v) and the flow rate was maintained at 1.0 mL/min.