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Ultrasound triggered drug delivery for mitochondria targeted sonodynamic therapy

MengJia Chen [a], Airen Xu [a], Wenyue He [a], Weicheng Ma [a, **], Song Shen [b, *]

[a] Clinical Pharmacology Research Center, Ningbo Urinary and Renal Diseases Hospital, Ningbo, 315000, China
[b] School of Pharmacy, Jiangsu University, Zhenjiang, 212013, China

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ABSTRACT

Mitochondria, which play an essential effect in cell apoptosis, are targets for cancer treatment. Here, mitochondria targeted liposomes loaded with sonosensitizer for sonodynamic therapy (SDT) of caner were studied. The (3-Carboxypropyl) triphenylphosphonium bromide (TPP), was grafted onto the liposomes using cholesterol (Chol) as anchor for mitochondria targeting. The hematoporphyrin monomethyl ether (HMME), a hydrophobic sonosensitizer, was loaded in the liposomes. The release of HMME from liposomes could be triggered by the irradiation of an extra ultrasound due to the oxidation of the lipid in liposomes. After incubation with cancer cells, the TPP modified liposomes (Lipo-TPP) could accumulate in the mitochondria and assist the HMME to achieve greater cancer cell inhibition effect in SDT. This work gives insights into the design of mitochondria-targeted carrier for SDT of cancer.

1. Introduction

Mitochondria have been described as “the powerhouse of the cell” because they generate most of the cell's supply of adenosine triphosphate (ATP) [1]. In addition to supplying cellular energy, mitochondria are involved in many other tasks, such as signaling, cellular differentiation, apoptosis, and cell death, as well as maintaining control of the cell cycle and cell growth [2]. The dysfunction of mitochondria will affect the catabolic processes of cells, including apoptosis, necrosis and autophagy [3]. Owing to their role in the regulation of fundamental cellular functions, mitochondrially-targeted compounds represent a promising approach to eradicate chemotherapy-refractory cancer cells [4–9]. Lipophilic cationic molecule such as triphenylphosphine (TPP) has the potential to promote endosomal escape and deliver chemotherapeutic drugs to the highly negatively charged mitochondria [4].

Reactive oxygen species (ROS) are chemically reactive molecules formed upon incomplete reduction of oxygen, including the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (HO·). ROS has been described as intracellular signaling molecules and mediate essential functions in living organisms. For cancer, ROS exerts both vital and lethal functions in physiological and pathological scenarios. On the one hand, moderate increase in ROS can promote cell proliferation and differentiation [10,11] and initiates the progression of cancer [12,13]. Many evidences suggest that different kinds of cancer cell have elevated levels of ROS compared with normal cells [14,15]. On the other hand, excessive amounts of ROS can cause oxidative damage to lipids, proteins and DNA [16–18] and operate as toxicant to damage the tumor. Many therapies based on ROS have been reported recently, including photodynamic therapy [19,20] and sonodynamic therapy [21,22]. Besides, some chemotherapy drugs kill cancer cells also by generating excessive amounts of ROS, such as doxorubicin [23], As_2O_3 [24], gadolinium texaphyrin [25], β-Lapachone [26], et al. Besides, ROS has recently been linked to the intrinsic chemotherapy resistance of cancer cells.

Sonodynamic therapy (SDT) is a form of therapy involving ultrasound and a sonosensitizing chemical substance to reduce tumor size and lessen recurrence without too much severe drug side effects. SDT has proven the ability to kill different kinds of cancer, including MCF-7, S180 and so on [21,22]. It is helpful in enhancing the therapeutic effect and minimizing side effect. Compared with photodynamic therapy (PDT) (using light as activation energy to generate ROS, which can only penetrate 1–2 cm in vivo), SDT

* Corresponding author.
** Corresponding author.
E-mail addresses: weichengma@126.com (W. Ma), jsdx.shensong@163.com (S. Shen).

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exhibits a more promising application in clinic because ultrasound can non-invasively transfer acoustic energy into deep tissues. In addition, the ultrasound can also be used for the triggered release of drug in a specific tumor location, thereby minimizing damage to surrounding normal tissues [27]. Hematoporphyrin monomethyl ether (HMME) is a derivative of protoporphyrin and usually used as photodynamic sensitizer. HMME could be activated by ultrasound to generate ROS effectively for cancer therapy [28]. In the present study, we fabricate mitochondria targeting liposomes for ultrasound stimulated drug release and SDT of cancer (Fig. 1).

2. Material and methods

2.1. Materials

Hematoporphyrin monomethyl ether (HMME, purity >98%) was purchased from Shanghai Dibo Chemical Technology Co. Ltd. Soybean phospholipid was purchased from Shanghai Taiwei Pharmaceutical Co. Ltd. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, 99%), *N*-hydroxysuccinimide (NHS, 98%), Cholesteryl chloroformate (Chol-chl), (3-Carboxypropyl) triphenylphosphonium bromide (TPP) was purchased from Aladdin Chemical Reagents Co. Ltd. 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) was purchased from Sigma Aldrich. Mitotracker green were purchased from Yeasen Biotechnology Company. DMEM cell culture medium, penicillin, streptomycin, fetal bovine serum (FBS), and heparin sodium were purchased from Gibco Invitrogen. All chemicals were used without further purification.

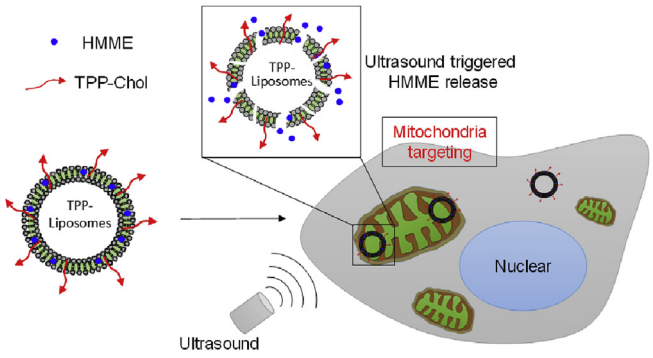

Fig. 1. A schematic diagram showing the construction of HMME-Lipo-TPP and the ultrasound triggered drug release from the liposomes.

2.2. Measurements and characterizations

The size and morphology of the particles were determined by transmission electron microscopy (TEM, Tecnai 12, Philips Company, Holland). The US apparatus was commercially available (838A-H-O-S, Shengxiang ultrasonic, China). The product was characterized using matrix-assisted laser desorption/ionization time of flight mass spectrometry (Ultraflex TOF/TOF, Bruker, Germen), fourier transform infrared spectroscope (Nicolet Avatar, Thermo, USA) and nuclear magnetic resonance spectroscopy (400 MHz ^{1}H NMR, Bruker AVANCE III 400, Germen). Laser confocal microscopy (TCS SP5 II, Leica, Germany) was used to visualize the uptake of the liposomes.

2.3. Synthesis and characterization of cholesterol-triphenylphosphonium

The cholesterol-triphenylphosphonium (Chol-TPP) was synthesized using hexamethylendiamine (HDA) as bridge, as shown in Fig. 2. 184 mg TPP was dissolved in 5 mL of chloroform, then 124 mg EDC and 74 mg NHS were added. After stirring in ice bath for 2 h, 49.7 mg HDA and 40 μL of triethylamine were added to the solution. The reaction was allowed to continue under the protection of nitrogen at room temperature for 2 h. The product was extracted by adding 5 mL deionized (DI) water and 20 mL chloroform. After evaporating the organic phase, triphenylphosphonium- hexamethylendiamine (TPP-HAD) was obtained. To synthesis Chol-TPP, 200 mg of as-prepared TPP-HAD and 50 μL of triethylamine were added to 7.5 mL of cholesteryl chloroformate solution (dissolved in chloroform, 25 mg mL^{-1}). The resulting product was obtained on a rotary evaporator. The raw product was further purified by silica gel column chromatography separation using the solvent (dichloromethane:methanol = 2:1) as eluent. The resulting solution was freeze-dried to obtain Chol-TPP.

2.4. Preparation of HMME loaded liposomes

TPP modified liposomes (donated as Lipo-TPP) were prepared by reverse evaporation method. Briefly, soybean lecithin, cholesterol and Chol-TPP (3/0.7/0.3, w/w) were dissolved in chloroform. The chloroform was removed by rotary evaporation to form lipid films. Then the film was dissolved in chloroform. Next, 5 mL of 0.01 M PBS (pH = 7.6) was added to the solution followed by sonication for 10 min to form homogenous W/O emulsion. After evaporating the organic solvent, liposomes were obtained. To construct HMME

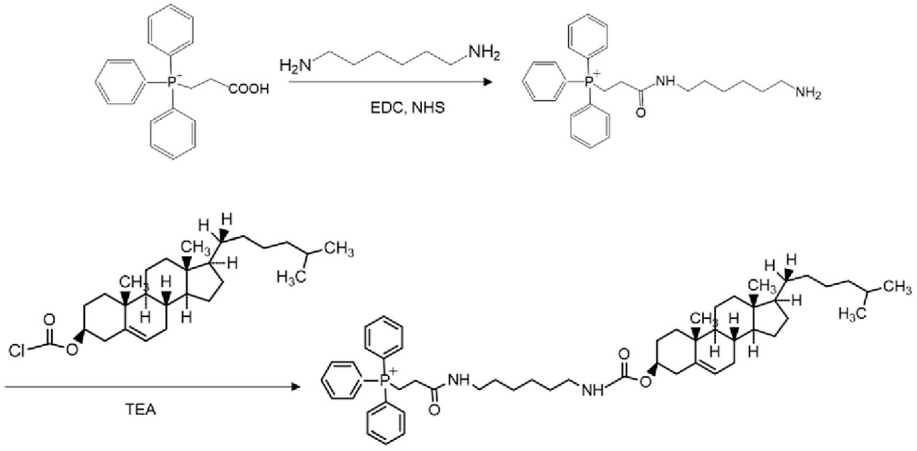

Fig. 2. Synthetic route of Chol-TPP.