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Transcription activator, hyaluronic acid and tocopheryl succinate multi-functionalized novel lipid carriers encapsulating etoposide for lymphoma therapy

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ABSTRACT

Delivery of chemotherapeutic drugs using nanocarriers is emerging as a promising approach for the treatment of cancer. The aim of this research was to develop a dual targeted D-α-tocopheryl succinate (TOS) functionalized nanostructured lipid carriers (NLCs), using etoposide (ETP) as a model drug to prove their *in vitro* and *in vivo* anti-tumor effects. Novel ETP loaded NLCs were constructed (ETP-NLCs). Hyaluronic acid (HA) and cell-penetrating peptide transcription activator (TAT) was applied for the surface decoration of ETP NLCs (HATOS/TATTOS-ETP-NLCs). The antitumor efficiency of HATOS/TATTOS-ETP-NLCs was evaluated in tumor bearing animal models. HATOS/TATTOS-ETP-NLCs displayed significantly higher transfection efficiency and better antitumor ability than undecorated ETP-NLCs in lymphoma cells bearing mice model. The newly constructed NLCs could successfully load drug and gene; and TAT could function as excellent targeting ligands to improve the cell targeting ability of the gene loaded nanocarriers. The resulting dual ligands decorated vectors could be a promising targeted gene delivery system for the lymphoma treatment.

1. Introduction

There are about 70,000 new cases of non-Hodgkin lymphoma each year in the United States [1]. Ninety percent are B-cell lymphomas, which cause an estimated 19,000 annual deaths [2]. Diffuse large B-cell lymphoma (DLBCL), derived from either germinal center B cells or activated B cells, is the most common type of B-cell lymphoma in developed and developing countries [3]. Although traditional chemical therapies and bone marrow transplantations can increase survival rates and even cure certain patients, relapse and drug resistance remain a clinical challenge [4]. Therefore, different drug delivery systems (DDS) targeting to tumor sites had been designed to overcome these problems [5]. To solve these drawbacks and increase the therapeutic outcome important progress has been achieved in the field of nanotechnology and offers a promising and effective alternative [6].

Nanostructured lipid carriers (NLCs) are one of the promising nanocarriers for chemotherapy for cancer [7,8]. NLCs, the second generation of solid lipid nanoparticles (SLNs), consist of an unstructured solid lipid matrix made of a mixture of blended solid and liquid lipids, and an aqueous phase containing a surfactant or a mixture of surfactants [9,10]. Because of the special structure of NLCs, they overcome SLNs drug expulsion or lower drug loading attributed to the high matrix crystallinity [11]. NLCs also exhibit many excellent characteristics for application as a drug carrier: controlled drug release; improved aqueous solubility of hydrophobic cancer therapeutics; high drug loading efficacy; improved physical and chemical long term stability [12]. Moreover, the grafting of targeting ligands to NLCs surface can achieve site specific targeting for better efficacy, reduce dose related toxicity, and overcome drug resistance [13].

Efficient delivery of drugs to the targeted cell surface and further into intracellular without apparent toxicity and side effects in cancer therapy requires a targeted delivery vector. Various targeting ligands have been applied to target drug or gene to tumor tissues or cells, such as folic acid, transferrin, bombesin, hyaluronic acid, *etc* [14–17]. Attachment of these targeting ligands to NLCs ensures selective cellular binding and enhanced cellular uptake *via* receptor-mediated endocytosis [18,19]. However, it should be

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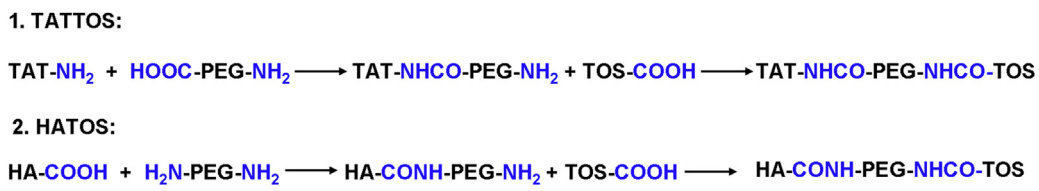

Fig. 1. Synthesis of TATTOS (1) and HATOS (2). TAT: transcription activator; HA: Hyaluronic acid; TOS D-α-tocopheryl succinate.

noted that in most cases the used ligand does not effectively improve the efficiency of intracellular transport of nanocarriers, probably due to its receptor saturation and lack of enough membrane penetrating ability [5,20,21]. To overcome this drawback, cell-penetrating peptides (CPPs) have received great attention as efficient cellular delivery vectors due to their intrinsic ability to enter cells and mediate the uptake of a wide range of macromolecular cargo such as drugs and nanocarriers [22]. Therefore, novel hyaluronic acid (the target ligand) and transcription activator (TAT, one of CPPs) dual targeted NLCs were designed by our group as the platform for the delivery of drugs.

Hyaluronic acid (HA) is a non-sulfated glycosaminoglycan composed of a relatively simple linear structure of alternating units of D-glucuronic acid and *N*-acetyl-D-glucosamine, linked *via* β-1,3- and β-1,4-glycosidic bonds [23]. HA can be bind to a cluster of differentiation-44 (CD44) and HAMM (CD168) receptors, which are over-expressed in a variety of solid tumors, such as pancreatic, lung, and breast cancer [24,25]. Moreover, HA is widely used in anticancer drug and nucleic acid delivery [26]. TAT peptide is a well-known CPP, which could promote efficient delivery of different molecules and even nanocarriers into mammalian cells [27,28]. Thus, simultaneous attachment of TAT peptide and HA specific ligand to NLCs using PEG as linker may take advantage of the respective merits of non-specific and specific ligands.

Nanocarriers functionalized with biological molecules may trigger additive therapeutic effect with the delivered drugs after intracellular uptake [29]. D-α-Tocopheryl polyethylene glycol 1000 succinate (TPGS) and D-α-tocopheryl succinate (TOS) are capable of improving drug permeability by inhibiting the P-glycoprotein (P-gp) pumps thus increasing the absorption of drug and reducing multidrug resistance [30]. Moreover, TPGS or TOS exert antitumor effect, which has synergistic anticancer effects with other anticancer agents [31,32].

In the present study, HA and TAT dual targeted, TOS functionalized NLCs was designed using etoposide (ETP) as a model drug to prove their *in vitro* and *in vivo* anti-tumor effects. Firstly, we synthesized the two amphiphilic targeting molecules, HA-PEG-TOS (HATOS) and TAT-PEG-TOS (TATTOS), by chemically linking the TOS to HA and TAT respectively *via* the PEG chain. Further, ETP NLCs (ETP-NLCs), HATOS decorated ETP-NLCs (HATOS-ETP-NLCs), TATTOS decorated ETP-NLCs (TATTOS-ETP-NLCs), HATOS and TATTOS dual targeted ETP-NLCs (HATOS/TATTOS-ETP-NLCs) were engineered with HA and/or TAT on the outer layer of ETP-NLCs. NLCs were then characterized for their particle size and size distribution, plasma stability, drug encapsulation efficiency, drug loading, drug release profile, *in vitro* cytotoxicity, and *in vivo* antitumor effect.

2. Materials and methods

2.1. Materials

Etoposide, chloroform, acetonitrile, dimethyl sulfoxide (DMSO), *N*-(3-Dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC), 4-dimethylaminopyridine (DMAP), *N*-hydroxysuccinimide (NHS), glyceryl monostearate (GM), cetyltrimethyl ammonium bromide (CTAB), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Miglyol 812N was purchased from Peter Cremer GmbH (Hamburg, Germany). TAT peptide with terminal Cysteine (Cys-AYGRKKRRQRRR) was purchased from NJ Peptide Biological Technology Co., Ltd. (Nanjing, China). Hyaluronic acid (1600 kDa, purity of 95%) was provided by Shandong Freda Pharmaceutical Group Co., Ltd. (Jinan, China). H_2N-PEG_{2000}-NH_2 and HOOC-PEG_{2000}-NH_2 were obtained from Shanghai Ponsure Biotechnology Co., Ltd (Shanghai, China). Poloxamer 188 was kindly provided by BASF Corporation (Ludwigshafen, Germany). RPMI 1640 cell culture medium, fetal bovine serum (FBS), penicillin and streptomycin were supplied by Gibco BRL (Gaithersburg, MD, USA). All other chemicals used in this study were of analytical grade.

2.2. Synthesis of TATTOS and HATOS

The amino group of TAT-NH_2 was conjugated to the carboxyl group of HOOC-PEG_{2000}-NH_2 with the help of EDC and NHS (molar equivalents of EDC/NHS = 1/1) in sodium borate buffer (pH 8.2) (Fig. 1-1) [33]. In brief, HOOC-PEG_{2000}-NH_2, EDC, and NHS were mixed in 2 mL of sodium borate buffer (pH 8.2) at 25 °C for 5 h, stored in a refrigerator at 4 °C over 24 h, mixed with 1 mL of 1% (w/v) TAT-NH_2 and stirred at 4 °C over 8 h to get TAT-NHCO-PEG-NH_2. TOS-COOH, EDC, and NHS were mixed in 2 mL of sodium borate buffer (pH 8.2) at 25 °C for 5 h, stored in a refrigerator at 4 °C over 24 h, mixed with 1 mL of 1% (w/v) TAT-NHCO-PEG-NH_2 and stirred at 4 °C over 8 h to get TATTOS. The resulted product was then dialyzed using a dialyzing membrane (MWCO: 2 kDa) against deionized water for 48 h in order to remove excess TAT-NH_2, TOS-COOH, NHS and EDC.

HATOS was prepared by the same method above, using HA-COOH instead of TAT-NH_2, H_2N-PEG_{2000}-NH_2 instead of HOOC-PEG_{2000}-NH_2 (Fig. 1-2).

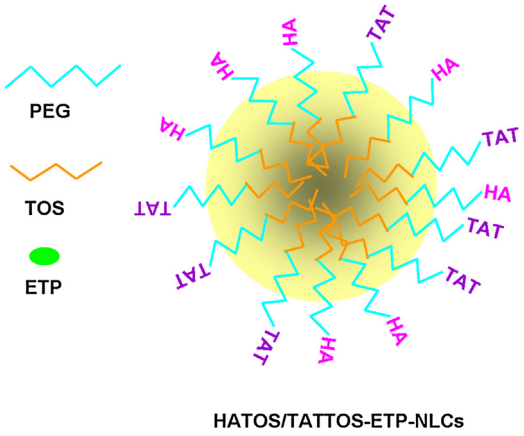

Fig. 2. Schematic diagram of HATOS/TATTOS-ETP-NLCs. TAT: transcription activator; HA: Hyaluronic acid; TOS D-α-tocopheryl succinate; ETP: etoposide; NLCs: nanostructured lipid carriers.