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Review

Ursolic acid liposomes with chitosan modification: Promising antitumor drug delivery and efficacy

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ABSTRACT

There are tremendous challenges on antitumor and its therapeutic drugs, and preparation of highly efficient nano-vehicles represents one of the novel topics in antitumor pharmaceutical field. Herein, the novel chitosan-coated ursolic acid (UA) liposome (CS-UA-L) was efficiently prepared with highly tumor targeting, drug controlled release and low side-effect. The CS-UA-L was uniformly spherical particles with diameter of ~130 nm, and the size was more easily trapped into the tumor tissues. Chitosan modification can make liposomes carrying positive charges, which were inclined to combine with the negative charges on the surface of tumor cells, and then the CS-UA-L could release UA rapidly at pH 5.0 comparing with pH 7.4. Meanwhile, the CS-UA-L exhibited obvious anti-proliferative effect (76.46%) on HeLa cells and significantly antitumor activity (61.26%) in mice bearing U14 cervical cancer. The tumor tissues of CS-UA-L treated mice had enhanced cell apoptosis, extensive necrosis and low cell proliferation activity. These results demonstrated that the multifunctional CS-UA-L allowed a precision treatment for localized tumor, and reducing the total drug dose and side-effect, which hold a great promise in new safe and effective tumor therapy.

Contents

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1. Introduction

At present, the effective therapy of tumor is still a tremendous challenge for human beings. There are a number of critical drawbacks in traditional chemotherapeutics to overcome, such as harmful side-effects, low therapeutic efficiency, non-specific biodistribution, low circulation time and poor solubility [1,2]. This can be achieved by encapsulating the therapeutic agents in biocompatible and biodegradable nano-vehicles that can avoid the identification of the immune system and release drugs slowly [3]. Liposomes displayed excellent application prospect for advantageous drug transport, which are a self-assembling structure of a lipid dispersion in water and have been used to encapsulate both hydrophilic therapeutic compounds or lipophilic ones [4]. Liposomes have many advantages, such as increasing drug capacity, versatile structure and facile surface decoration, good biodegradability properties, and targeting and protection of entrapped agents [5,6]. In addition, as a drug delivery system, nanoparticle size would be a precondition and a crucial factor which decides the fate of drugs both in vivo and in vitro [7]. H. Maeda and co-workers have reported that most solid tumors possess unique pathophysiological characteristics, such as extensive angiogenesis, defective vascular architecture, and impaired lymphatic drainage/recovery system. Thus, tumor vessels were more permeable, so the molecules of certain sizes tend to accumulate in tumor tissue much more than they do in normal tissues. The phenomenon now known as the enhanced permeability and retention (EPR) effect [8]. The size between 100 and 200 nm was acknowledged as the best particle size for EPR effect, which is the main mechanism for passive targeting of nanoparticles to tumor site [9]. Therefore, appropriately sized liposomal carriers can be used to avoid drug extravasation through continuous capillaries of healthy tissues and provide a steric barrier to prevent exposure of the encapsulated drug to healthy cells [3]. So, the drugs with appropriately size distribution are beneficial to accumulate in tumor tissues.

However, targeting drug delivery and controlling drug release at the tumor site are still major challenges for efficient therapy of cancers. The selective and site-specific release drugs have the particular interest for reducing drug toxicity and improving overall therapeutic safety. The surface modification of liposomes has been performed to stabilize the liposomes in normal body environments and avoid the leakage of encapsulated compounds, meanwhile, promote drug rapid release from liposomes at target site [10]. Tumors exhibit a lower extracellular pH than normal tissues, as well as in their intracellular lysosomes and endosomes. Based on this property, a variety of materials have been functionalized to design pH-responsive delivery systems for the site-specific controlled release of payloads by simply exploiting the pH changes [11–13]. For example, the pH-controlled release of DOX from single-walled carbon nanotubes was successfully applied to in vivo cancer therapy [14]. The pH-responsive NPs of polyethylene glycosylated peptide dendron–doxorubicin conjugates were fabricated for cancer therapy [15]. Chitosan (CS), a natural polysaccharide, is derived from partial deacetylation of chitin of crustacean shell [16]. CS and its derivatives have been employed in many biomedical applications due to the polycation intrinsic properties, low toxicity and excellent biocompatibility [17]. In this study, CS was chosen to modify the liposomes surface, because CS is capable of opening the tight junctions of epithelial cells, resulting in a paracellular pathway through the epithelial barrier [18].

Ursolic acid (UA) is a natural pentacyclic triterpene of the cyclosqualenoid family, which is ubiquitous in the plant kingdom and found in many foods and herbs such as apple, cranberry, rosemary, and oregano [19]. UA, as a kind of bioactive natural compound, possesses a wide range of biological activities including anti-inflammatory, antibacterial, antiviral, anti-diabetes and immunomodulatory activity [1]. However, its most prominent function is anticancer effects [20], as it can influence many different cancer pathways including inhibition of tumor angiogenesis, promotion, proliferation, and metastasis [21]. Kassi et al. successfully used UA to treatment human prostate cancer [22]; UA could completely inhibit the appearance of a palpable tumor in a subset of mice, meanwhile, it would be capable of inhibiting cell proliferation, cell cycle distribution, and apoptosis of human lung cancer cell line A549 [23,24]. However, the clinical application of UA is limited as its poor water solubility, low bioavailability [25], and short plasma half-life [26]. Therefore, an efficient drug delivery system is desired to overcome the obstacles.

The main aim of the present work was to develop a CS modified drug delivery system to obtain the pH-responsive release and specifically drug accumulation in tumor cells (Scheme 1). The drug delivery system with smaller diameters of ~130 nm has been developed by loading of the natural antineoplastic drug UA, and CS was modified on the surface of liposomes. Through the surface modification of CS, the CS-UA-L could selectively target to the tumor site and be taken up by tumor cells. After encapsulating UA in CS-UA-L, its bioavailability could be effectively improved by release of drug slowly at tumor site. Such a pH-responsive delivery vehicle will provide a valuable approach to construct smart and biocompatible agents on tumor treatment.

2. Materials and methods

2.1. Materials and animals

All experimental protocols involving animals were approved by the Animal Subjects Committees at the Yanshan University, which were carried out in accordance to their guidelines and regulations. Ursolic acid was purchased from Hefei Hiromi Biological Technology Co., Ltd. (Shenyang, China); Soybean phosphatidylcholine (SPC) was purchased from Shenyang Tianfeng Biological Pharmaceutical Co., Ltd. (Shenyang, China); Cholesterol (CHOL), anhydrous ethanol and surfactant Tween-80 were purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China); Chitosan (degree of deacetylation: 92%) was purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China), Acetic acid glacial was obtained from Tianjin Fengchuan Chemical Reagent Technologies Co., Ltd. (Tianjin, China). Sephadex G-75® was obtained from Sigma Chemical Company (Henan, China). Hematoxylin and eosin were obtained from Beijing Biodee Biotechnology Co., Ltd. (Beijing, China). All reagents used were of at least analytical grade. HeLa cell was gifted from the first hospital of Qinhuangdao and U14 cervical carcinoma cell line was purchased from the Peking Union Medical College.

CD-1 female mice were purchased from Vital River Laboratory Animal Center (Beijing, China). The mice were maintained under standard conditions of temperature (22–25 °C), a humidity of 50–65% and a 12 h light/dark cycle. The animals were fed with a standard diet of mouse chow, and water was allowed ad libitum. All animal experiments were