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RGD peptide conjugated liposomal drug delivery system for enhance therapeutic efficacy in treating bone metastasis from prostate cancer

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ABSTRACT

Targeting αvβ3 integrin is particularly promising for the treatment of bone metastases by targeting integrin-rich tumor cells and by inhibiting integrin-involved bone metastases. In this work, a liposomal drug delivery system conjugated with cyclic arginine-glycine-aspartic acid-tyrosine-lysine peptide (cRGDyk) as αvβ3 integrin ligand was thus developed to improve therapeutic efficacy in a mice model of bone metastasis from prostate cancer. The resultant liposomes were characterized in terms of size, morphology, zeta potential, stability, drug encapsulation percentage and loading efficiency, and drug release. Compared with free cisplatin and cRGDyk-free liposomes, cRGDyk conjugated liposomes showed significantly higher cellular uptake and higher cytotoxicity of loaded cisplatin, as evidenced by in vitro cell experiments. In vivo results revealed that free cisplatin and free cRGDyk could relieve tumor-induced pain but had no contributions to tumor regression and overall survival improvement. cRGDyk-free liposomal drug system with prolonged blood circulation time could accumulated in the tumor sites in the bone through enhanced permeability and retention (EPR) effects and however, did not exhibit desirable therapeutic efficacy superior to free cisplatin and free cRGDyk. This strongly suggested that ERP effects were not effective in treating metastases. By taking advantages of targeted drug delivery and synergistic antitumor activity of cRGDyk and loaded cisplatin, cRGDyk conjugated liposomal drug system could inhibit osteoclastic and osteoblastic bone lesions, relieve pain, and improve overall survival. Inspired by their enhanced therapeutic efficacy and low organ toxicity, cRGDyk conjugated liposomes could serve as an effective drug system for targeted and synergistic therapy of bone metastases.

1. Introduction

The deadliest characteristic of a cancerous cell is its ability to metastasize, and metastasis is the fatal stage in all cancers. Bone is the most prone site for metastasis because of its physiological environment which supports tumor inoculation and progress [1]. Bone metastases are predominant in breast (65 — 75%), prostate (65 — 75%), thyroid (60%), lung (30 — 40%), and kidney cancer (20 — 25%) [2]. The patients with bone metastases suffer from high death rate, pain, and decreased quality of life, resulting from osteoclastic and/or osteoblastic bone lesions and its clinically important complications such as fracture, need for radiotherapy and/or chemotherapy, spinal cord compression, or hypercalcemia [3]. Patients who are diagnosed with bone metastases generally cannot be treated curatively in spite of extensive research, e.g., only 46% of patients with prostate cancer, 20% of patients with breast cancer, and less than 10% of patients with lung cancer are still alive five years after the diagnosis of bone metastases [2]. The management of bone metastases therefore represents a huge clinical burden.

The present approaches for treating bone metastases are surgery, radiotherapy, chemotherapy, and radiotherapy in combination with chemotherapy. However, these approaches are greatly limited due to presence of multiple bone metastatic nodules which are difficult to treat by localized treatment [4,5]. In the current regimens, achieving desired payload of therapeutic agents at the site of metastasis is still a major hurdle because of the presence of a blood-bone barrier that negatively affects the penetration efficiency of therapeutic agents [4]. For example, Brubaker et al. showed that docetaxel failed to inhibit growth of LuCaP 23.1 prostate cancer in the bone environment with the concentration which was effective for subcutaneous tumor [6]. Although traditional chemotherapeutic drugs are effective at high dosage and for prolonged period in treating bone metastases, they are toxic for bone marrow and cardiac tissues, resulting in acute side effects. Thus, an ideal therapeutic system for bone metastases should specifically target metastatic sites in the bone.

Recently, several targeted therapeutic agents that inhibit osteoclast-mediated bone resorption (Vacuolar H + -ATPase inhibitors, RANKL inhibitor, c-Src Inhibitors, αvβ3 integrin inhibitors, Cathepsin K inhibitors, bisphosphonates, etc) [3,7] and restore osteoblast functions (DKK-1 inhibitors, Activin A inhibitors, etc) [8] have been studied or evaluated in clinical trials. Although these do reduce some degree of metastatic

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growth, providing symptomatic relief and regression of bone disease, none of them have been proven to significantly control tumor progression or improve overall survival [9]. Thus, some drug analogues that combine bone-targeting molecules, such as bisphosphonates having high affinity for bone, with chemotherapeutic drugs, such as cisplatin and doxorubicin, have been designed for synergistic antitumor activity. Unfortunately, therapeutic efficacy of these smart drug analogues seems to be disappointing, although a bone targeting effect is obtained. Keppler et al. found that therapeutic efficacy of a bisphosphonate-cisplatin analogue was not superior to conventional cisplatin in a transplantable osteosarcoma model, even at a 28-fold higher dose [10]. No antitumor effect at all was obtained when amino group of doxorubicin was used to incorporate bisphosphonate when evaluated against human tumor xenografts [11].

Nanoparticles-based drug delivery system plays an important role in recent advancements in tumor theragnostics and has also been emerged as a promising alternative for treating bone metastases [12]. Especially, polymer microspheres or liposomes incorporated with targeted moieties have been extensively explored to deliver chemotherapeutic drugs to metastatic sites in the bone. However, in these cases targeted moieties are mainly limited to bisphosphonate or new-generation of bisphosphonate, such as zoledronate [13,14], alendronate [15,16], and so on. Furthermore, the present work focuses on targeting efficiency of drug delivery system, and its therapeutic efficacy in vivo is not well addressed [13–18].

Integrins are a family of cell surface receptors that primarily mediate interactions of cells with components of the extracellular matrix, consisting of noncovalently associated α and β subunits. It is well reported that αvβ3 integrin is usually expressed at low or undetectable levels in normal cells but can be highly upregulated in most of cancer cells, especially in prostate cancer cells [19,20], contributing to migration, proliferation, and survival of cancer cells. Moreover, αvβ3 integrin is also involved in the pathogenesis of bone metastases [21] and is crucial for bone metastases [22]. Thus, we hypothesize that therapeutic agents targeting αvβ3 integrin would be particularly promising for the treatment of bone metastases with improved therapeutic efficacy by targeting tumor cells and by inhibiting integrin-involved bone metastases. To date, there is preclinical evidence that RGD peptides as αvβ3 integrin antagonist can successfully block osteoclast-mediated osteolysis in animal models of bone metastases and some of them have advanced to clinic [21,23]. On the other hand, RGD peptides conjugated drug delivery system targeting αvβ3 integrin for targeted tumor theragnostics has been also well documented in animal models of primary tumors [24,25]. There is, however, lack of results concerning with therapeutic efficacy of such drug delivery system in treating bone metastases..

Herein, cyclic arginine-glycine-aspartic acid-tyrosine-lysine peptide (cRGDyk), a highly potent αvβ3 integrin ligand [26,27], conjugated and chemotherapeutic drug, cisplatin, loaded liposomal drug delivery system was designed for synergistic therapy in a mice model of bone metastasis from prostate cancer. The resultant liposomes were characterized and their targeting efficiency was studied both in vitro and in vivo using near-infrared-emitting (NIR) lead sulfide quantum dots (PbS QDs) as fluorescent probe. Importantly, enhanced therapeutic efficacy in vivo of cRGDyk conjugated liposomes including tumor regression, pain relief, and overall survival improvement was observed.

2. Materials and methods

2.1. Materials

Lead (II) acetate trihydrate, sodium sulfide nonahydrate, oleic acid, decane, diethyl dithiocarbamate (DDTC), and nickel chloride were purchased from Alfa Aesar (MA, USA). 4', 6-diamidino-2-phenylindole (DAPI) and 3-(4,5)-dimethylthiahiazo(-z-y1)-3,5-di-phenytetrazoliumromide (MTT) were purchased from Sigma-Aldrich (MO, USA). Phosphatidylcholine (PC) from eggs, cholesterol (Chol), and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[maleimide (polyethylene glycol)-2000] (DSPE-PEG-mal) were purchased from Advanced Vehicle Technology Pharmaceutical L.T.D. (Shanghai, China). Cyclic arginine-glycine-aspartic acid-tyrosine-lysine peptide (cRGDyk, MW = 617.6) was purchased from Apeptide Co. Ltd (Shanghai, China). The other main chemicals were from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Cisplatin was obtained from Shandong Qilu Pharmaceutical Co., Ltd. (Shandong, China). Sephadex G-50 was obtained from GE Healthcare Life Sciences (WI, USA). Fetal bovine serum (FBS) was purchased from Gibco Life Technologies (AG, Switzerland). Dulbecco's Modified Eagle's Medium (DMEM) was from Invitrogen Corporation (CA, USA). All chemicals were analytical or HPLC grade and used without further purification. Mouse prostate cancer cells (RM-1) line was obtained from Insitute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). C57BL/6 mice (aged 8-10 weeks, 18-22 g in body weight, female) were obtained from Animal Center of Nantong University and used in accordance with the guidelines approved by Animal Care and Use Committee of Nantong University.

2.2. Preparation of multifunctional liposomes

2.2.1. Preparation of oleic acid capped PbS QDs

Oleic acid capped PbS QDs were synthesized according to a previous report: Briefly, 0.038 g of lead (II) acetate trihydrate (0.1 mmol), 0.13 mL of oleic acid (0.4 mmol), and 10 mL of decane were mixed in a sealed flask at room temperature. The mixture was heated up to 130 °C for 20 min under nitrogen atmosphere to form lead oleate precursor solution. Afterwards, the flask was cooled to 40 °C, followed by dropwise injection of 5 mL of Na_2S (6 M). The mixture was continuously stirred for 5 min, and then the reaction was cooled to room temperature. The organic phase was separated from the crude solution and oleic acid capped PbS QDs was obtained. Such PbS QDs could be readily dispersed in a nonpolar solvent such as toluene or hexane.

2.2.2. Preparation of cRGDyk and DSPE-PEG-mal conjugate (DSPE-PEG-RGD)

The conjugation of cRGDyk to DSPE-PEG-mal was performed by modifying a method previously reported [28]. Briefly, DSPE-PEG-mal (2 mM) and cRGDyk (4 mM) were dissolved in PBS buffer solution (pH = 7.4). The mixture was gently shaken overnight in a glass bottle. The synthesized product was identified by high-performance liquid chromatography (HPLC, CTO-10AS, Shimadzu) with water containing 0.1% triethanolamine and acetonitrile containing 0.1% triethanolamine (1:1,v/v) as mobile phase at 40 °C at a flow-rate of 1.0 mL/min [29].

2.2.3. Preparation of cRGDyk conjugated liposomes loaded with cisplatin (RGD-CIS-liposomes) or PbS QDs (RGD-PbS-liposomes)

Thin film evaporation-sonication method, in which the phospholipid vesicles were prepared by extrusion of suspensions obtained by hydration of thin and dried lipid films, was used in the preparation of liposomes [30]. In a typical experiment, PC, Chol, and DSPE-PEG-RGD in weight ratio of 4:1:1 (75 mg in total) were dissolved in 15 mL of chloroform/methanol (4:1, v/v) in a round-bottomed flask. The mixture was dried to form a thin film in the rotary evaporation apparatus under vacuum in water bath at room temperature. The resultant film was left overnight and then hydrated with 15 mL of PBS while sonication for 10 min at 55 °C.

Various combinations of mixing ratio were considered to find the optimum condition for liposome formation (Table 1). Hydrophobic PbS QDs or hydrophilic cisplatin were added in the process of thin film formation or hydration of film by bath sonication, forming RGD-PbS-liposomes or RGD-CIS-liposomes, respectively. Various amount of cisplatin was employed to determine the influence of cisplatin concentration on encapsulation percentage and loading efficiency of