Biomaterials 51 (2015) 161–172

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Doxorubicin-loaded polysaccharide nanoparticles suppress the growth of murine colorectal carcinoma and inhibit the metastasis of murine mammary carcinoma in rodent models

Mingqiang Li [a, d], Zhaohui Tang [a], Dawei Zhang [a], Hai Sun [a], Huaiyu Liu [c], Ying Zhang [a], Yuanyuan Zhang [b, **], Xuesi Chen [a, *]

[a] Key Laboratory of Polymer Ecomaterials, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, PR China
[b] Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157, USA
[c] Laboratory Animal Center, Jilin University, Changchun 130012, PR China
[d] University of Chinese Academy of Sciences, Beijing 100049, PR China

ARTICLE INFO

Article history:
Received 27 August 2014
Received in revised form
30 January 2015
Accepted 1 February 2015
Available online 19 February 2015

Keywords:
Cancer model
Colorectal carcinoma
Mammary carcinoma
Metastasis
Doxorubicin
Chemotherapy

ABSTRACT

As a synergistic drug combination, doxorubicin-loaded cisplatin crosslinked polysaccharide-based nanoparticles (Dex-SA-DOX-CDDP) have demonstrated enhanced antitumor efficacy and reduced systemic toxicity *via* optimized biodistribution, controlled drug release, prolonged blood circulation, and improved tolerability, compared to the non-crosslinked nanoparticles or free doxorubicin. Herein, we apply the Dex-SA-DOX-CDDP nanoparticles as an efficient antitumor agent to treat colorectal and breast tumors in three different *in vivo* models, i.e. subcutaneously implanted colorectal carcinoma, dimethylhydrazine-induced autochthonous colorectal carcinoma, and metastatic mammary carcinoma, which more closely simulate the natural milieu of the original tumor with intact pathological and immunological responses. Based on the properties of this combination in higher tumor accumulation and penetrating efficiency, the Dex-SA-DOX-CDDP nanoparticles significantly decreased the tumor sizes in CT26 cell line xenograft tumors compared to control. In addition, the affected animals' lifespan was significantly extended after the Dex-SA-DOX-CDDP treatment, in the autochthonous colon cancer model. Moreover, with the aid of iRGD, Dex-SA-DOX-CDDP could effectively block primary tumor growth and prevent the metastasis of 4T1 murine mammary carcinoma. In conclusion, Dex-SA-DOX-CDDP nanoparticles remarkably inhibit growth of colorectal carcinoma and metastasis of mammary carcinoma *in vivo*, which provides potential application as a safe and efficient antitumor agent in treatment of these cancers.

1. Introduction

Both colorectal and mammary carcinoma are leading causes of cancer-related mortality worldwide [1–3]. Colorectal carcinoma causes approximately 50,000 deaths in the United States and 655,000 deaths throughout the world per year [4,5]. Breast cancer is the most common malignancy in women, with one in nine women developing breast cancer during their lifetime [6] and cancer death with more than a million newly diagnosed cases annually worldwide [3]. Surgical resection in combination with adjuvant therapy is efficient at the early stages of disease, but subsequent relapse and metastasis often occur. Tumor metastasis is a multistage process in the late stage of the cancers, which is defined as the ability of malignant tumor cells to invade local tissues at the primary site, traverse basement membranes as tissue barriers, migrate and re-establish at distant secondary sites [7]. More than 60% of the malignant tumors have been in progression of metastasis, when they are first diagnosed, while other tumor patients are also subject to tumor metastasis during treatment and even after first recovery for several years [7]. Tumor metastasis is responsible for as much as 90% of cancer-related deaths [8]. Therefore, inhibition of tumor metastasis is critical in cancer

* Corresponding author. Tel./fax: +86 431 85262112.
** Corresponding author.
E-mail addresses: yzhang@wakehealth.edu (Y. Zhang), xschen@ciac.ac.cn (X. Chen).

http://dx.doi.org/10.1016/j.biomaterials.2015.02.002
0142-9612/

therapy. Although significant progress has been made in the development of anticancer therapies, reduced toxicity and increased treatment efficiency of antimetastasis drugs are still much in demand.

Nanoparticle-mediated delivery of chemotherapeutic agents has demonstrated enhanced anticancer efficacy and reduced systemic toxicity in cancer treatment [9,10]. Nanomedicines with the proper sizes and surface properties provide a controlled release of anticancer drug system to retain optimal levels of drugs within solid tumors when injected into target tissues. In addition, this system may avoid the drugs rush into blood circulation, and enhances permeability and retention (EPR) effect of drugs in the host when systematically administrated [11]. Before EPR-based drug targeting technic can be translated to the clinic, a number of these therapeutics with nanotechnology (nanomedicines) for cancer treatment have been tested in animal experiments with promising results. Some are under clinical trials and some have been approved by the United States Food and Drug Administration (FDA) and other international equivalents for clinical use [11,12]. In particular, the success of polymeric micelles including Oncaspar (approved by FDA for acute lymphoblastic leukemia in 2006), Genexol-PM (approved by South-Korea for metastatic breast cancer in 2006), NK 911 (in phase II for pancreatic and colorectal cancers in Japan), NK 105 (in phase II for stomach cancer in Japan) and NK 012 (in phase II for breast cancer in Japan and USA) brings hope to the polymer-based nanomedicines for cancer therapy [13,14].

Animal models have been used as the front line in predicting efficacy and finding toxicities for cancer chemotherapeutic agents before entering the clinic [15]. Currently, the most common animal models used for the development of potential antitumor agents involve subcutaneous implantation of cultured murine tumor cell lines into syngenic mouse strains or injection of human tumor cell lines into immunocompromised mice [16,17]. Subcutaneous tumor models are widely used because of their simplicity. In addition, the primary tumor growth can be conveniently monitored by periodic caliper measurements to evaluate the therapeutic efficacy. Nevertheless, the ectopic subcutaneous models possess limited pathophysiological relevance, heterogeneity and clinical predictability[18,19]. Compared to the ectopic subcutaneous tumor-implantation model, carcinogen-induced models more closely simulate the natural milieu of the original tumor, with intact pathological and immunological responses. Especially, carcinogen-induced models can effectively recapitulate the time-dependent and multistage progression of tumor pathogenesis in response to environmental carcinogens and tumor-promoting agents [17]. Their molecular, biochemical, and histopathological characteristics are similar to the developmental consequences of specific human cancers, from hyperplasias, pre-malignant lesions, low grade well-differentiated carcinomas, and ultimately to invasive and more poorly differentiated carcinomas [20,21]. Consequently, carcinogen-induced primary tumor models have been used as a general class of preclinical cancer models to offer various distinct advantages and clinical relevance to human cancers. Orthotopic tumor models more closely mimic the human clinical course of metastatic disease. Generally, they involve the implantation of cultured tumor cells or primary tumor tissue explants into the originating tissue site of the cancer in rodents, resulting in much higher metastatic rates and a pathological phenotype [17,22]. Multiple preclinical models of cancers are indispensable in the drug discovery and development process for new cancer drugs. Moreover, evaluating the efficacy of novel therapeutic agents in a variety of preclinical models can better mimic the heterogeneity of human cancers and also assist in establishing dose levels, dose regimens and drug combinations for use in clinical trials [17]. At present, the therapeutic effects of most of the reported nanomedicines have been just evaluated in one subcutaneous tumor model with lack of continuous and intensive investigations.

We have demonstrated that, by favored biochemical property of cisplatin (CDDP) pro-drug as the cross-linker for doxorubicin (DOX) delivery, the rationally-designed polysaccharide-based drug delivery system (doxorubicin-loaded cisplatin crosslinked dextran-based nanoparticles, Dex-SA-DOX-CDDP, Scheme 1A) facilitated intracellular drug delivery and inhibited tumor growth in A549 xenograft murine model [23]. In addition, antitumor drug loading procedure can be efficiently performed in aqueous medium with non-toxic reagents or organic solvents, thus representing a safe approach with green chemistry action. Importantly, *in situ* crosslinking of the DOX-loaded polysaccharide nanoparticles by introducing a small amount of cisplatin as the crosslinker significantly increase the surface charge and stability. This would further improve the tolerability, *in vivo* pharmacokinetics, bio-distribution, and antitumor efficacy, and reduce drug-related multiorgan toxicity side-effect. Despite the great potential of Dex-SA-DOX-CDDP nanoparticles for cancer therapy, the detailed application and more comprehensive study of such nanoparticle system for other solid tumor models, especially for orthotopic and metastatic models, have not been explored.

In the present study, for the first time, we aim to evaluate the therapeutic efficacy of Dex-SA-DOX-CDDP nanoparticles in three solid tumor animal models, including subcutaneous colorectal carcinoma, primary colorectal carcinoma and metastatic mammary carcinoma. With the reinforced stability, prolonged blood circulation and efficient delivery of drug into the tumor tissues, we propose that Dex-SA-DOX-CDDP can efficiently suppress the growth of murine colorectal carcinoma. Furthermore, it has been reported that co-administration of nanomedicines with internalizing arginine–glycine–aspartic acid (iRGD), a tumor specific vascular-extravasation and tissue-penetration peptide that combines RGD with a tumor-penetrating CendR motif (RGDK/R), exhibits enhanced tumor targeting, penetration, and therapeutic effect compared to the single nanomedicines [24–27]. Currently, clinical trials are under way in the United States to evaluate the effect of iRGD on tumor vasculature [28]. In this study, we also demonstrate for the first time that co-administration of iRGD and Dex-SA-DOX-CDDP may not only effectively block primary tumor growth, but also prevent the metastasis of murine mammary carcinoma (Scheme. 1B), which is significantly important for the development of new approaches to metastatic cancer treatment. We believe that the therapeutic evaluation of Dex-SA-DOX-CDDP nanomedicine in these specific types of preclinical tumor models is crucial and necessary for its further development in clinical applications.

2. Materials and methods

2.1. Materials

Dex-SA-DOX-CDDP nanoparticles were synthesized according to the previous report [23]. 3-(4,5-Dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma), 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI, Sigma), ethylenediaminetetraacetic acid disodium salt (EDTA-Na_2, Tianjin Guangfu Fine Chemical Research Institute, China) and dimethylhydrazine (DMH, TCI, Tokyo, Japan) were used without further purification. Cyclic iRGD (CRGDKGPDC) was customized from Apeptide Co. Ltd. (Shanghai, China). All other reagents and solvents were of analytical grade and used without further purification.

2.2. Animals

Male or female Balb/c mice at 5–6 weeks of age were obtained from Beijing HFK Bioscience Co., Ltd. All animals received care in compliance with the guidelines outlined in the Guide for the Care and Use of Laboratory Animals and all procedures were approved by the Animal Care and Use Committee of Jilin University.