The Anti-Melanoma Efficiency of the Intratumoral Injection of Cucurbitacin-Loaded Sustained-Release Carriers: A PLGA Particle System

GUO JIANBO,[1] LI XUE,[1,2] YU HONGDAN,[1] TANG ZHAOHUI,[2] TANG XING,[1] CAI CHENCHEN,[1] XU JINGHUA,[3] XU HUI[1]

[1]School of Pharmacy, Shenyang Pharmaceutical University, Shenyang, Liaoning 110016, China

[2]Key Laboratory of Polymer Ecomaterials, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, China

[3]School of Life Science and Biopharmaceutics, Shenyang Pharmaceutical University, Shenyang, Liaoning 110016, China

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ABSTRACT: A dilemma for current cancer therapy is surgical injury in addition to the toxicities and inefficiencies of systemic chemotherapy. Meanwhile, localized therapies have become a noticeable strategy. In this study, Cucurbitacin (Cuc)-loaded poly(lactic-co-glycolic acid) particles of different sizes (about 50 μm, 5 μm, and 270 nm, respectively) were prepared as the sustained-release system for intratumoral injection, and their physicochemical properties, *in vitro* cytotoxicity, particles–cells interactions, pharmacokinetics, and pharmacodynamics were systemically investigated for the first time. The results of cytotoxicity experiments and pharmacokinetic/pharmacodynamic studies indicated that the release patterns of the particles would strongly affect the biological efficiency not only at the cell level but also in two types of animal models. Cuc raw material and nanoparticles showed higher initial burst release *in vitro*, and higher drug concentration in tumor and plasma. Large particles (about 50 μm) showed lower initial burst and slow drug release, which would not supply enough amount of drug to inhibit the cancer cell growth during the whole treatment period. Particles with mean diameter of about 5 μm performed the best anti-melanoma efficiency both *in vitro* and *in vivo*. The release patterns, instead of the particles–cells interactions, would be the key factors to affect the biological effects of the particulate system for intratumoral injection. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:2550–2563, 2013

Keywords: Poly(lactic/glycolic) acid (PLGA); microparticles; particle sizing; injectables; release patterns; cytotoxicity; intratumoral; anti-melanoma efficiency; pharmacokinetics; Cucurbitacin

INTRODUCTION

A dilemma for current cancer therapy is surgical injury in addition to the toxicities and inefficiencies of systemic chemotherapy. Although surgical resection for solid tumors is the first treatment option, many patients are ineligible, and the residual cancer tissue is as dangerous as the site of cancer recurrence. Moreover, conventional intravenous chemo- and immuno-therapies are severely limited because of their systemic toxicities and inefficiencies. Systemic chemotherapy is evidently not efficient enough as the tumor tissues and anatomical characteristics for many solid tumors show an increased interstitial fluid pressure, which forms a barrier to transcapillary transport. This barrier is an obstacle in tumor treatment as it results in inefficient uptake of therapeutic agents.[1,2]

Regional chemotherapy via localized administration has long been recognized as a potential method for delivering high doses at the target site(s) while minimizing systemic exposure.[3] Much research has focused on local tumor administration for tumor treatment, for example, percutaneous ethanol or intra-arterial chemoembolization for the treatment of

Additional Supporting Information may be found in the online version of this article. Supporting Information

Correspondence to: Xu Hui (Telephone: +86-24-23986356; Fax: +86-24-23986356; E-mail: xuhui_lab@163.com); Tang Xing (Telephone: +86-24-23986343; Fax: +86-24-23986343; E-mail: tangpharm@yahoo.com.cn)

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hepatocellular carcinoma. In addition, intratumoral or intracavitary chemotherapy for the treatment of gliomas has gained clinical acceptance.[4–6]

Accordingly, several strategies of localized delivery have been used to optimize the drug delivery to the tumor site *in vivo*, such as peritumoral[7,8] or intratumoral[9,10] administration of antitumor drugs with minimal toxicity to the host. Different administration methods have also been investigated, comparing intratumoral, peritumoral, and subcutaneous injections in SKOV-3 ovarian tumor-xenograft-bearing mice to optimize the theoretical efficiency. Of these, the intratumoral treatment proved to be most effective.[11]

Compared with intratumoral therapy with free drugs, administration of a prolonged biodegradable polymer depot would release cytotoxic drugs into the local tumor mass, providing very high local concentrations and minimizing the systemic exposure. The drug delivery systems used for localized tumor therapy in preclinical/clinical stages were mainly based on implants[12] or particulates, such as microparticles,[13] liposomes,[14,15] and lipid nanoparticles (NPs).[16,17] Localized biodegradable polymer depot delivery systems have been intensively investigated, and poly(lactic-co-glycolic acid) (PLGA) was a noticeable matrix material for its great physicochemical and biocompatible properties.[18] A surprisingly small subset of these technologies has demonstrated potentially curative preclinical results for cancer applications, and fewer have progressed toward commercialization.[12] Chakravarthi and cowokers[13,19] studied the paclitaxel-loaded PLGA particles of different sizes to treat the breast cancer, and the particles of about 1 μm showed better efficiencies after intratumoral injection to 4T1 cell-bearing mouse, which was suggested to be mainly attributed to the particles–cells interactions.[20]

Melanoma is a malignant tumor of melanocytes, which is less common than other skin cancers. However, melanoma is much more dangerous than other skin cancers if it is not found early. It causes the majority (~75%) of deaths related to skin cancer.[21] Melanoma treatment includes the surgical removal of the tumor at early stages and chemoimmunotherapy or radiation therapy at later stages. Although early stage surgery may remove the tumor, further surgery is often necessary to reduce the risk of recurrence.[22] For the later stages, melanomas are often metastatic; the overall success rate in metastatic melanoma is quite limited.[22,23] Intratumoral administration to melanomas has been performed clinically. However, after treatment with a low molecular weight drug, which is rapidly passed into the blood circulation, the time of retention in the tumor is very short. To overcome this problem, drug carrier systems such as liposomes are being investigated. This strategy is especially important for melanomas as this type of tumor is highly resistant to chemotherapy and radiation therapy, and therapies for melanomas have generally been ineffective and have rarely resulted in sustained responses.[24,25]

Cucurbitacin (Cuc, the main component is Cuc-B) is a type of triterpenoid substance isolated from the members of the Cucurbitaceae family of plants, which presented widely activities, such as hepatoprotective, anti-inflammation, antimicrobial, anthelmintic, cardiovascular, and anti-diabetic effects,[26] and the oral tablet containing Cuc has been clinically used for adjuvant treatment of hepatitis in China. Recently, much research has indicated that Cuc could inhibit the growth of a wide spectrum of human malignant cells, both *in vitro* and in xenografted tumor models, including breast cancer, glioblastoma multiforme, myeloid leukemia, pancreatic cancer, laryngeal cancer, and melanoma.[27–32] The studies of Cuc were mainly focused on its antitumor targets, and only few were involved in the drug delivery systems. Cuc encapsulated in liposomes[33] and polymeric micelles containing Cuc-B and Cuc-E[34] would display potential antitumor efficiencies in different tumor models, both *in vitro* and *in vivo*.

In our recent research, Cuc encapsulated in PLGA micro-/nanoparticles was investigated for the feasibility of localized treatment of murine and human melanoma. As no apparent particles–cells interaction was observed between the microsized PLGA particles and cells, the initial burst release of the particles were believed to be the crucial factor to the notable different biological effects of micro/nano Cuc particles. In this paper, the physicochemical properties, *in vitro* cytotoxicity, particles–cells interactions, pharmacokinetics, and pharmacodynamics of PLGA particles of different sizes were systemically investigated for the first time, to elucidate the effect of drug release patterns of such particulate system on their *in vitro* and *in vivo* biological effects.

MATERIALS AND METHODS

Materials

Poly(lactic-co-glycolic acid) 50/50 (inherent viscosity was 0.50 dl/g in $CHCl_3$ at 25°C) was a kind gift from Changchun SinoBiomaterials Company, Ltd. (Changchun, China). Cuc (Cuc-B content: 61.5%) was purchased from the Tianjin Institute of Pharmaceutical Research (Tianjin, China). The fluorescence probe 7-acetoxy-4-methylcoumarin was purchased from Tokyo Chemical Industry Company, Ltd. (Tokyo, Japan). CH_2Cl_2 was obtained from the Sinopharm Chemical Reagent Company, Ltd. (Shanghai, China). PVA-217SB was a kind gift from Kuraray Company,