Journal of Pharmaceutical Sciences 105 (2016) 205–211

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmaceutics, Drug Delivery and Pharmaceutical Technology

Improvement of the Antitumor Efficacy of Intratumoral Administration of Cucurbitacin Poly(Lactic-co-Glycolic Acid) Microspheres Incorporated in *In Situ*-Forming Sucrose Acetate Isobutyrate Depots

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ARTICLE INFO

Article history:
Received 9 June 2015
Revised 27 August 2015
Accepted 22 September 2015
Available online 13 November 2015

Keywords:
controlled release
poly(lactic/glycolic) acid (PLGA)
injectables
microspheres
targeted drug delivery
cancer
intratumoral injection
in situ-forming implant
antimelanoma
cucurbitacin

ABSTRACT

Localized drug delivery strategies for cancer therapy have been introduced for decades as a means of increasing drug concentration at tumor target site and minimizing systemic toxicities. In this paper, a combination of microspheres (MSs) and sucrose acetate isobutyrate (SAIB) *in situ*-forming implants (ISFIs) was evaluated for improving antitumor efficacy via intratumoral injection. Monodispersed cucurbitacin (Cuc)-loaded Poly (lactic-co-glycolic acid) (PLGA) MSs with mean diameter of about 5 μm were fabricated by Shirasu porous Glass (SPG) membrane emulsification technique, and their properties were investigated. The *in vitro* drug release pattern, antimelanoma efficiency, and drug distribution in tumor of three different intratumoral injection systems, that is, MSs, SAIB ISFIs, and combination of MSs and SAIB ISFIs (SAIB-MSs), was investigated. The Cuc-loaded MSs prepared by PLGA (LA/GA = 50:50, inherent viscosity = 0.87 dL/g), has an appropriate release pattern with lower initial burst and delayed drug release. SAIB-MSs have a much slower drug release rate than that of MSs or SAIB ISFIs. SAIB-MSs showed the best antitumor efficacy in melanoma-bearing mice model, and the results of drug distribution in tumor revealed that the incorporation MSs in SAIB solution obviously extended the residence of drug in tumor. The low Cuc concentration in tumor periphery region after intratumoral administration of SAIB-MSs demonstrated poor drug penetration of this system. For further improving the antitumor efficacy of intratumoral chemotherapy, elegant designing to carriers with both extended residency and wide drug distribution in tumor is needed.

Introduction

Cancer is a serious threat to human life and health, and 8.2 million people died of cancer in 2012 worldwide.[1] Although novel therapeutic strategies showed great potentials in the treatment of certain types of cancers, such as immunotherapy, gene therapy, and oncolytic virotherapy,[2,3] surgical resection is till now the first choice for most early-stage or intermediate-stage solid tumors in general,[4] and for the patients who are ineligible for surgical resection, systemic chemotherapy or radiation therapy is the common treatment. The toxicities of chemotherapeutic agents to normal cells and the complex biology of tumors often limited the application and the effectiveness of chemotherapy.[5] The intensively investigated nanomedicine or nanocarrier-based drug delivery systems (DDSs) that were designed for targeting drug delivery to tumor based on the enhanced permeability and retention effect or various active targeting mechanisms, failed to significantly improve the therapeutic benefits to solid tumors. One of the reasons for the limited efficiency of chemotherapy is attributed to the dense extracellular matrix (ECM) of the solid tumor that hindered the deep penetration and uniform distribution of the anticancer drugs in the overall tumor higher than the effective concentration.[6]

In order to increase drug concentration at the tumor target site and minimize systemic toxicities, regional tumor chemotherapeutic

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http://dx.doi.org/10.1002/jps.24695
0022-3549/

strategies were introduced. Local tumor administration, such as intratumoral or intracavitary administration, have gained clinical acceptance for the treatment of gliomas,[7-9] and especially the intratumoral route has attracted increasing attention.[10,11] In an efficacy and safety evaluation trail in patients, computed tomography–guided, percutaneous fine-needle 5% ethanol–cisplatin intratumoral injection combined with second-line chemotherapy was shown to improve the response rate and survival than second-line chemotherapy for patients with platinum-pretreated stage IV nonsmall cell lung cancer.[12] Intratumoral injection is also a widely used administration routes of gene therapy in patients.[13,14]

In recent years, different carriers have been designed for localized tumor drug delivery, including microparticles,[15] liposomes,[16] *in situ*-forming gels,[17] micelle gels,[18] and other types of DDSs.[19-21] Compared with free drugs (solution or suspension), higher local concentrations inside tumor and minimized systemic exposure to drugs were expected after intratumoral administration of these drug delivery carriers.[22-26] Because of the emerging disadvantages of localized tumor drug delivery carriers, for example, the initial burst release of *in situ*-forming gels and micelle gels,[18] and inflammatory response related to some polymer particles such as polyphosphoester particles,[27] more elegant delivery systems for intratumoral injection are needed to be developed.

In our previous studies, cucurbitacin (Cuc)-loaded poly (lactic-co-glycolic acid) (PLGA) microspheres (MSs) with different particle sizes were prepared, and their antimelanoma efficiency after intratumoral injection was evaluated in animal model (mice).[28] The results revealed extended drug residence in tumor after administration of Cuc-loaded PLGA MSs, and compared with smaller or larger particles, PLGA MSs with a mean diameter of several microns showed steady drug release and low initial burst *in vitro*, and higher antimelanoma efficiency *in vivo*. But because of the obvious leakage of PLGA MSs from the injection site through the pin hole, only modest antitumor efficiency was observed. In our further studies, *in situ*-forming implants (ISFI) composed of PLGA and sucrose acetate isobutyrate (SAIB) were evaluated to extend the drug residence inside tumor.[29] Of these two ISFIs, SAIB ISFI displayed superior drug residence in tumor, thus lower plasma drug concentration and higher therapeutic index, which can be attributed to the rapid increase on viscosity of SAIB system after injection. On the basis of these results, a facial concept to further improve the efficacy of intratumoral-administered DDSs will be the combination of the MSs and ISFIs to overcome the problem of unsatisfied retention and drug release pattern inherent to both of the two systems.

In this paper, we fabricated monodispersed (mean diameter of about 5 μm) Cuc-loaded PLGA MSs by SPG membrane emulsification technique; the drug-loaded MSs was then incorporated into a SAIB–ethanol solution to form SAIB-MSs depots for intratumoral injection. The *in vitro* drug release property and the *in vivo* pharmacodynamics after intratumoral administration of SAIB-MSs to melanoma-bearing mice were investigated. Such intratumoral-injectable depots may have better performance of antimelanoma efficiency, which is expected to provide novel choice and play an integral role for cancer therapy.

Materials and Methods

Materials

Sucrose acetate isobutyrate (density of 1.146 g/mL at 25°C) was purchased from Sigma–Aldrich (St. Louis, MO). PLGA 50:50 (inherent viscosity = 0.87 dL/g in $CHCl_3$ at 25°C), PLGA 75:25 (inherent viscosity = 1.0 dL/g in $CHCl_3$ at 25°C), and PLGA 75:25 (inherent viscosity = 1.6 dL/g in $CHCl_3$ at 25°C) were kindly donated by Changchun SinoBiomaterials Company, Ltd. (Changchun, China). Cuc (Cuc-B content: 61.5%) was purchased from Tianjin Institute of Pharmaceutical Research (Tianjin, China). Dichloromethane and ethanol were obtained from Sinopharm Chemical Reagent Company, Ltd. (Shanghai, China). Polyvinyl acetate (PVA-217SB) was a kind gift from Kuraray Company, Ltd. (Osaka, Japan). RPMI 1640 was purchased from Invitrogen (Shanghai, China).

The B16 murine melanoma cell line was purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Cell line was cultured in RPMI 1640 supplemented with 10% fetal bovine serum. C57BL/6 male mice was obtained from the Experimental Animal Center of Shenyang Pharmaceutical University (Liaoning, China), and used at 6-8 weeks of age. All animal experiments were conducted in accordance with the ethical guidelines on animal experiments of Shenyang Pharmaceutical University.

Preparation and Characterization of PLGA MSs

Poly (lactic-co-glycolic acid) of various intrinsic viscosities was used to prepare the MSs by a modified solvent evaporation method and Shirasu porous glass (SPG) membrane emulsification technique. Briefly, 70 mg of PLGA and 30 mg of Cuc were dissolved in 1 mL dichloromethane to obtain the organic phase. The organic phase was quickly poured into 5 mL of 1% PVA aqueous solution; the mixture was kept in an ice-water bath and homogenized with a high-speed disperser (T18 Ul-tra Turrax®; IKA, Guangzhou, China) at 3000 rpm to form a primary emulsion. The primary emulsion was then introduced into the SPG membrane emulsification device (SPG Technology Company, Ltd. Miyazaki, Japan) and extruded through a 10-μm SPG membrane (SPG Technology Company, Ltd.) under nitrogen gas to form the uniform droplet emulsion. The organic solvent as then evaporated under vacuum using a rotary evaporator, then the resulted particles were collected by centrifugation, washed with purified water, and then freeze-dried to obtain the MSs samples A, B, and C (MSs-A, MSs-B, MSs-C; see Table 1).

The median diameter and particle size span of PLGA MSs were measured by Laser Particle Size Analyzer (BT-9300S; Bettersize Instrument Ltd., Dandon, China). PLGA MSs were sputtered with gold and then observed by scanning electron microscope (SEM) (Hitachi S-3400; Hitachi High Technologies, Kyoto, Japan).

To determine drug-loading (DL) efficiency, 10 mg of the MSs was dissolved in 1 mL of dichloromethane, and diluted to 25 mL with methanol, followed by sonication for 30 min in ice water bath. The solution was filtered, and the Cuc-B was assayed by high-performance liquid chromatography (Shimadzu LC-10A; Shimadzu, Kyoto, Japan) equipped with a C18 reverse-phase column and detected at 228 nm. The DL efficiency (%) and drug entrapment efficiency (EE, %) were calculated using the following equations, respectively:

$$\mathrm{DL}\ (\%) = \left(\frac{\text{amount of Cuc} - \text{B determined}}{\text{amount of PLGA microspheres}}\right) \times 100$$

$$\mathrm{EE}\ (\%) = \left(\frac{\text{Cuc} - \text{B content determined}}{\text{theoretical Cuc} - \text{B content}}\right) \times 100$$

Preparation of Cuc-Loaded SAIB ISFIs and SAIB-MSs-Injectable Depots

Sucrose acetate isobutyrate ISFIs was prepared by dissolving 30 mg Cuc in 10 mL of 80% SAIB solution in absolute ethanol. SAIB-MSs-injectable depot was obtained by dispersing 300 mg