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Sustained delivery of cytarabine-loaded vesicular phospholipid gels for treatment of xenografted glioma

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ABSTRACT

This study described the development of vesicular phospholipid gels (VPGs) for sustained delivery of cytarabine (Ara-C) for the treatment of xenografted glioma. Ara-C-loaded VPGs in the state of a semisolid phospholipid dispersion looked like numerous vesicles tightly packing together under the freeze-fracture electron microscopy (FF-TEM), their release profiles displayed sustained drug release up to 384 h *in vitro*. The biodistribution of Ara-C in the rat brain showed that Ara-C-loaded VPGs could maintain therapeutic concentrations up to 5 mm distance from the implantation site in brain tissue within 28 days. At the same time, fluorescence micrograph confirmed drug distribution in brain tissue visually. Furthermore, after single administration, Ara-C-loaded VPGs group significantly inhibited the U87-MG glioma growth in right flank in comparison with Ara-C solution ($p < 0.01$). It was explained that the entrapped drug in VPGs could avoid degradation from cytidine deaminase and sustained release of drug from Ara-C-loaded VPGs could maintain the effective therapeutic levels for a long time around the tumor. In conclusion, Ara-C-loaded VPGs, with the properties of sustained release, high penetration capacity, nontoxicity and no shape restriction of the surgical cavity, are promising local delivery systems for post-surgical sustained chemotherapy against glioma.

1. Introduction

Gliomas are primary central nervous system tumors, with an incidence of approximately 80% in brain tumors, and 5–6 new cases of brain tumors per 100,000 people per year are diagnosed worldwide (Eckley and Wargo, 2010). The typical treatment strategy involves maximal surgical resection followed by post-operative radiotherapy and chemotherapy. However, radiation therapy could have adverse effects on brain function and, in particular, could increase the risk of growth delay in children. After surgical resection, chemotherapy is often recommended as first-line therapy for brain tumors. Systemic delivery of chemotherapeutic drugs can extend the patient's lifetime, although it may be accompanied by serious systemic toxic effects (Brandes et al., 2001). Due to the protective effect of the blood brain barrier (BBB), tumor cells in the brain may not be exposed to high enough concentrations of chemotherapeutic drugs. Usually, the recurrent gliomas were seen within 2 cm of the border of the original tumor site (Hochberg and Pruitt, 1980). In view of the above factors, it is highly desirable to develop new treatment strategies that bypass BBB and maintain effective therapeutic drug concentrations at the original brain tumor site. As a result, post-operative local chemotherapy was considered as a good choice to improve the therapeutic effects on brain tumors.

Recently, convection-enhanced delivery (CED), as a delivery technique, employs a catheter and a pump to deliver therapeutic agents into the brain under a simple pressure gradient. Compared with systemic administration, CED can significantly increase the *in situ* drug concentration. And this technique allows the delivery of a broad spectrum of substances, such as small molecules, macromolecules, and nanocarriers. However, this infusion approach associates with unpredictable drug distribution. During a long-time infusion, it would increase intracranial infection and edema (Allard et al., 2009; Allhenn et al., 2012).

Gliadel® wafers were developed to release carmustine slowly over two to three weeks after they had been placed up to the

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surface of the resected tumor beds. They had been successful in improving patient survival rates and prolonging survival time. Despite this success, cerebral edema and infectious complications had increased the risk of their clinical application (Weber and Goebel, 2005). In addition, low drug penetration (1–2 mm) into regions of the brain tissue had limited its effectiveness (Fung et al., 1996). In order to overcome such drawbacks, several studies on sustained local delivery systems had been carried out, including biodegradable implants, nanoparticles, nanofibers, films, disk, foams, and gels (Ong et al., 2009; Sawyer et al., 2011). Some progress had been made regarding increasing the surface area of polymer implants and improving drug penetration. However, the therapeutic effects of malignant glioma remained unsatisfactory. As expected, ideal local delivery systems would be developed for the treatment of glioma.

Ara-C, a member of the antimetabolite family with low toxicity, has been reported to be effective in the treatment of glioma (Anders et al., 2000). DepotCyt® is a sustained-release formulation of Ara-C using multivesicular liposome technology. It is indicated for the intrathecal treatment of lymphomatous meningitis. A single intrathecal injection of DepotCyt® maintains cytotoxic concentrations in the cerebrospinal fluid (CSF) for more than two weeks. Yet for post-operative chemotherapy of brain tumors, frequent intrathecal injection are undesirable for physician and patient (Angst and Drover, 2006; Glantz et al., 1999).

Vesicular phospholipid gels (VPGs), semisolid phospholipid dispersions, are made of numerous nanoscale vesicles and are different from some solid implants in having a high drug-loading capacity, sustained release properties, good biocompatibility, and lack of toxic effects (Brandl, 2007). VPGs are intended to implant or inject into the intratumor region. There have been reports in the literature regarding their use in cancer treatment (Grohganz et al., 2005; Moog et al., 2002).

In a previous study, we successfully prepared and characterized the local delivery depot of VPGs loaded with Ara-C (Qi et al., 2012). Here, Ara-C-loaded VPGs were developed for the purpose of prolonging the sustained release duration of the drug, and maintaining effective cytotoxic concentrations for a long-time to produce maximum antitumor efficacy. In this study, we investigated the *in vitro* release properties and biodistribution in the rat brain of Ara-C-loaded VPGs. This was followed by exploration of the *in vivo* antitumor efficacy of local therapies in nude mice with right flank U87-MG glioma model.

2. Materials and methods

2.1. Materials and animals

Cytarabine (purity 99.6%) was purchased from Peking University Pharmaceutical Co., Ltd., (Beijing, China). Egg lecithin (E80) was obtained from Lipoid GmbH (Ludwigshafen, Germany). Chromatographic grade ammonium acetate was purchased from Dikma Co., Inc., (Richmond Hill, NY, USA). Chromatographic grade acetonitrile was obtained from Fisher Co., Inc., (Fisher Scientific, USA). Chromatographic grade methanol was purchased from Dikma Co., Inc., (Richmond Hill, NY, USA). Sodium sulfite was supplied by Nanjing Longyan Chemical Co., Ltd. FITC was obtained from Sigma International, Inc. Paraformaldehyde was purchased from Guangzhou Chemical Reagent Factory. Water was purified using a Barnstead EASYpure® II RF/UV ultrapure water system (Dubuque, Iowa, USA). All other materials were of analytical grade. Wistar rats were provided by the Experimental Animal Center of Shenyang Pharmaceutical University. BALB/c nude mice were purchased from Beijing FHK Bioscience Co., Ltd. All the animal experiments were approved by the University Ethics Committee.

2.2. Preparation of VPGs by dual asymmetric centrifugation

VPGs were prepared by dual asymmetric centrifugation (DAC) as reported in the literature (Massing et al., 2008). 10 mg Ara-C/g VPGs, 400 mg phospholipids/g VPGs, and 0.2 mg sodium sulfite/g VPGs were added to a cylindrical PVC container, then 50 mM pH 7.4 phosphate buffered saline (PBS) was 60% of the VPGs was added in PVC container with a total volume of 20 ml, and the same mass of zirconia beads (Sartorius GmbH, Göttingen, Germany, ø 3 mm) was added. After closing the container tightly, the homogenization was performed in the dual asymmetric centrifuge (DAC150 FVZ, Hauschild GmbH&CoKG, Hamm, Germany) at a maximum speed of 3540 rpm in two 5-minute runs (5 min is the maximum run time that can be selected for the DAC). VPGs were sealed in glass vials under nitrogen and autoclaved for 15 min at 121 °C in an autoclave (Nanjing Astent Technology Development Co., Ltd.), then stored at 4–8 °C. Blank VPGs (without drug) were also prepared by the same method and FITC-loaded VPGs were also prepared in this way with drug replaced by FITC.

2.3. Physicochemical characterization of VPGs

The morphology of VPGs was examined by freeze-fracture electron microscopy (FF-TEM). Briefly, small portions of VPGs were mounted on a gold specimen holder and quick-frozen by plunging them into liquid nitrogen, then specimens were inserted into the pre-cooled freeze fracture unit. Fracturing was carried out at 173 K and between 3.5×10^6 and 1.4×10^5 Pa. The fracture faces were etched for 30 s at 173 K. Subsequently, the etched surface was vacuum deposited unidirectionally with platinum. Traces of lipid were removed by repeated flushing with ethanol: chloroform mixtures. Visualization was performed using a Hitachi H-600 transmission electron microscope operated at 100 kV.

The particle size was determined with a Nicomp™ 380 Particle Sizer/Zeta Potential (Particle Sizing System, Santa Barbara, USA) at 25 °C. Samples were redispersed in 50 mM pH7.4 PBS and diluted to a light intensity near to 300 kHz. The entrapment efficiency (EE) of the Ara-C-loaded VPGs was evaluated by the ultrafiltration (UF) method and subsequently analyzed using HPLC (HITACHI Company, Japan) as described in the literature (Grohganz et al., 2005). All the experiments were carried out in triplicate.

2.4. In vitro *erosion and release testing*

A horizontal type diffusion cell (TM-1, Shenyang Tianmeida Scientific Instrument Co., Ltd., China) was used for studying the *in vitro* drug release of VPGs. Ara-C-loaded VPGs (0.5 g) were placed in the donor compartment of the apparatus, with pH 7.4 PBS as release medium, and stirred with a magnetic bar at 37 °C in the acceptor compartment. The donor and acceptor compartments were separated by a nylon sieve with the sieve size of 0.074 mm. The erosion fragments falling off from the VPGs passed through the nylon sieve. The medium in the acceptor compartments was removed at scheduled time intervals and replaced with the same volume of PBS (pH 7.4). Free drug was separated from the release fractions by ultrafiltration. Total drug and free drug from the release fractions were determined by HPLC as described in the literature (Qi et al., 2012). The amount of eroded phospholipids was analyzed by a colorimetric method using ammonium ferrothiocyanate (Stewart, 1980). All data were obtained in triplicate.

2.5. In vivo *drug release into rat plasma and bio-distribution in rat brain*

Rats were anesthetized with 7% chloral hydrate (0.5 ml per 100 mg body weight) and immobilized on a head stereotaxic

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