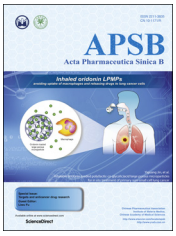
Acta Pharmaceutica Sinica B 2017;7(1):80–90

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



ORIGINAL ARTICLE

Inhalable oridonin-loaded poly(lactic-*co*-glycolic) acid large porous microparticles for *in situ* treatment of primary non-small cell lung cancer

Lifei Zhu[a,b,c,†], Miao Li[a,†], Xiaoyan Liu[a], Lina Du[a], Yiguang Jin[a,b,*]

[a]*Department of Pharmaceutical Sciences, Beijing Institute of Radiation Medicine, Beijing 100850, China*
[b]*Anhui Medical University, Hefei 230001, China*
[c]*Department of Pharmacy, Yangpu Hospital, Tongji University School of Medicine, Shanghai 200090, China*

Received 3 June 2016; revised 16 August 2016; accepted 23 August 2016

KEY WORDS

Large porous microparticle;
Non-small cell lung cancer;
Oridonin;
Poly(lactic-*co*-glycolic) acid;
Pulmonary delivery

Abstract Non-small cell lung cancer (NSCLC) accounts for about 85% of all lung cancers. Traditional chemotherapy for this disease leads to serious side effects. Here we prepared an inhalable oridonin-loaded poly(lactic-*co*-glycolic)acid (PLGA) large porous microparticle (LPMP) for *in situ* treatment of NSCLC with the emulsion/solvent evaporation/freeze-drying method. The LPMPs were smooth spheres with many internal pores. Despite a geometric diameter of ~10 μm, the aerodynamic diameter of the spheres was only 2.72 μm, leading to highly efficient lung deposition. *In vitro* studies showed that most of oridonin was released after 1 h, whereas the alveolar macrophage uptake of LPMPs occurred after 8 h, so that most of oridonin would enter the surroundings without undergoing phagocytosis. Rat primary NSCLC models were built and administered with saline, oridonin powder, gemcitabine, and oridonin-loaded LPMPs *via* airway, respectively. The LPMPs showed strong anticancer effects. Oridonin showed strong angiogenesis inhibition and apoptosis. Relevant mechanisms are thought to include oridonin-induced mitochondrial dysfunction accompanied by low mitochondrial membrane potentials, downregulation of BCL-2 expressions, upregulation of expressions of BAX, caspase-3 and caspase-9. The oridonin-loaded PLGA

Abbreviations: BSA, bovine serum albumin; DAB, 3,3′-diaminobenzidine; DAPI, 4′,6-diamidino-2-phenylindole; DPI, dry powder inhalation; EGFR, epidermal growth factor receptor; FPF, fine particle fraction; HPLC, high performance liquid chromatography; HRP, horseradish peroxidase; LPMP, large porous microparticle; NSCLC, non-small cell lung cancer; PLGA, poly(lactic-*co*-glycolic)acid; PVA, polyvinyl alcohol; qPCR, quantitative polymerase chain reaction; SEM, scanning electron microscopy; SLF, simulated lung fluid; TCM, traditional Chinese medicine; XRD, X-ray diffraction

*Corresponding author. Tel.: +86 10 88215159; fax: +86 10 68214653.
E-mail address: jinyg@bmi.ac.cn (Yiguang Jin).

†These authors made equal contributions to this work.

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

http://dx.doi.org/10.1016/j.apsb.2016.09.006

LPMPs showed high anti-NSCLC effects after pulmonary delivery. In conclusion, LPMPs are promising dry powder inhalations for *in situ* treatment of lung cancer.

1. Introduction

Lung cancer is a tumor with high mortality, is responsible for 23% of all cancer-related deaths, and poses a serious threat to human health. Lung cancer may be induced by sensitive gene mutations and/or environmental changes that include cigarette smoking, air pollution, and ionizing radiation[1]. Rapid industrialization of many developing countries is likely to lead to heavy air pollution which causes the incidence of lung cancer to increase faster than that of other malignant tumors[2,3]. Lung cancer is divided into two categories: small cell lung cancer and non-small cell lung cancer (NSCLC). NSCLC, accounting for about 80%–85% of all lung cancers, is readily transferred to other parts of the body and relatively poor in prognosis (approximately 85% mortality within 5 years)[4]. Moreover, the lung is also a major site of metastasis for other cancers including those of the breast, prostate, and colon[5].

Gemcitabine, a nucleoside analogue of deoxycytidine, is the general treatment for non-small cell lung cancer. Gemcitabine requires intracellular phosphorylation mediated by deoxycytidine kinases (dCKs) to get converted into its triphosphate form (dFdC-TP). This metabolite exerts its cytotoxic effects by incorporation into DNA and inhibiting DNA synthesis[6]. Systemically administered drugs (the oral or injection routes) can produce serious toxic side effects with widespread damage due *in vivo* distribution[7], yet result in limited drug distribution into lung tissue[8]. Therefore, lung tumor–targeted drug delivery systems have become increasingly popular research topics despite the fact that they are only administered *via* intravenous injection and applied to the lung metastatic models, not primary NSCLC[9,10].

Pulmonary delivery of drugs is a non-invasive method for treatment of lung diseases, in which aerosols or dry powder inhalations (DPIs) are the common dosage forms[11,12]. In this way, the dose in the lung can be maximized because the lung tissue is directly exposed to the aerosols or dry powders delivered *via* the airways. Additionally, the delivery is non-invasive, and thus improves patient compliance *versus* intravenous injection[13]. It should be an ideal chemotherapeutic approach to lung cancer compared to the oral and intravenous routes[14]. So far, only a few local therapies of non-primary (transplanted or metastasis) NSCLC animal models have been reported[4,15–17], and *in situ* treatment of primary NSCLC has not been reported. Thus, there is a need to search for suitable drugs with weak toxicity to treat primary NSCLC, especially drugs capable of local or topical application.

The market and research of DPIs are increasing due to high drug loads, stability, user-friendliness, and patient compliance. For DPIs, the aerodynamic diameters of particles generally range from 1 to 5 μm[18,19]. In most cases, the range cannot be achieved so that some modifications are needed, such as the use of lactose as the supporter. Moreover, the particles of 1–5 μm tend to agglomerate due to van der Waals and electrostatic forces[18]. Another problem is that particles less than 10 μm are prone to phagocytosis by alveolar macrophages[20]. Therefore, the diameters of inhalable particles have become a dilemma. The only solution seems to lie in a novel strategy in which a large porous microparticle (LPMP) keeps a relatively apparent large diameter but with low density and small aerodynamic diameters[21,22]. LPMPs have been demonstrated to exhibit such ideal lung deposition profiles[23].

Oridonin is an active diterpenoid isolated from a traditional Chinese medicine (TCM) *Isodonrubescens* (*Hemsl*) *Hara* (Chinese: Dong Ling Cao) which mainly grows in the Henan and Shaanxi provinces of China. This compound has been tried as an anti-inflammatory, antibacterial, and anticancer agent. Since oridonin shows anticancer effects with little adverse reactions, it has attracted much attention from oncologists and pharmacologists[24]. The anticancer mechanism of oridonin may involve inhibition of NF-κB transferring from the cytoplasm to the nucleus in the localization of metastasis, activation of caspase-mediated apoptosis pathway, and induction of apoptosis mediated by blocking the epidermal growth factor receptor (EGFR) signaling pathway[25–27]. Although the effects of oridonin on different cancers have been explored, clinical utilization of this drug has been highly hindered due to poor water solubility and low bioavailability. Furthermore, little is known about the effects of oridonin on lung cancer.

Here, we present a novel LPMP loading oridonin for the direct *in situ* treatment of primary NSCLC with pulmonary delivery. The formulation and preparation methods of the microparticles were optimized and the characteristics and drug release of the microparticles were investigated. Finally, substantial anticancer effects of the microparticles were demonstrated on the rat primary NSCLC models and the relevant mechanisms were explored.

2. Materials and methods

2.1. Materials

Oridonin was obtained from the Shaanxi Huike Botanical Development Co., Ltd., Shaanxi, China. Poly(lactic-*co*-glycolic)acid (PLGA, lactide/glycolide, 50:50, mol/mol, MW 10 kDa) was produced by Jinan Daigang Biomaterial Co., Ltd., Shandong, China. Gemcitabine, used as a positive control drug, was purchased from Hansoh Pharmaceutical Co., Ltd., Jiangsu, China. Polyvinyl alcohol (PVA, 87%–89% alcoholysis, MW 75000 Da) was purchased from the Aladdin Industrial Corporation, Shanghai, China, and ammonium bicarbonate was purchased from the Sinopharm Chemical Reagent Co., Ltd., Beijing, China. Cy7 was purchased from Fanbo Biochemicals Co., Ltd., Beijing, China. 3-Methyl cholanthrene (MCA, Sigma, USA), diethyl nitrosamine (DEN, Tokyo Chemical Industry, Japan), and iodized oil (Guerbet, French) were used. Anti-BCL-2 and anti-BAX antibodies were from the Cell Signaling Technology Inc. (Danvers, USA). All other chemicals and solvents were of analytical grade or high performance liquid chromatographic (HPLC) grade.