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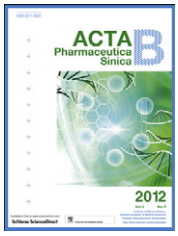

ORIGINAL ARTICLE

Doxorubicin-loaded zein *in situ* gel for interstitial chemotherapy of colorectal cancer

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KEY WORDS

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Abstract The aim of this research was to evaluate doxorubicin (DOX)-loaded zein *in situ* gels, a new drug delivery system in which a liquid state drug can be transformed into semi-solid after intratumoral injection. *In vitro* release of DOX-loaded zein was investigated and the pharmacokinetics, biodistribution and therapeutic efficacy of these DOX-loaded zein formulations were investigated using BALB/c nude tumor-bearing mice. *In vitro* release of DOX from the gels extended up to 7 days. Efficient accumulation of DOX in the tumor with lower drug concentration in blood and normal organs was obtained resulting in effective inhibition of tumor growth and fewer off-target side effects. In conclusion, a DOX-loaded *in situ* gel was developed with sustained release, enhanced anti-cancer efficacy for colorectal cancer *in vivo*, and especially with reduced off-target side effects.

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1. Introduction

Colorectal cancer (CRC) is one of the most frequent cancers worldwide and is a leading cause of cancer mortality[1]. Doxorubicin (DOX) is widely used for the treatment of a number of cancers, but its usage has been limited due to cardiotoxic effects[2]. Considerable effort has been put forth in the development of drug delivery systems (DDS) for DOX[3,4] to change tissue selectivity and improve the toxicity profile.

The increased permeability of blood vessels and the lack of lymphatic vessels in tumors leads to high hydrostatic pressure in tumor stroma. Therefore, drugs cannot be effectively delivered to the tumor tissues by systemic administration[5,6]. In order to eliminate these problems, efforts have been focused on development of the targeting DDS that achieves site-specific delivery and prolongs the exposure[7,8]. During the last decade, injectable *in situ* forming gels, as polymeric drug carriers, have attracted considerable attention[9]. For CRC, local injection of *in situ* gels can be applied by a Sigmoidoscope. Applications of the *in situ* gels provide a number of advantages, including easy application, localized delivery, prolonged delivery periods, decreased body drug dosage and improved patient compliance[10–12]; however, the great majority of the polymers require artificial synthesis.

Zein, a major storage protein comprising about 45–50% of total protein in corn[13], was a promising biomaterial with good biocompatibility for tissue engineering[14]. As indicated by SDS-PAGE in the present study, biochemically pure zein is mainly composed of two distinct bands with molecular weights of 23 and 21 kDa, with minor bands at 13 and 9.6 kDa. The high proportion of non-polar amino acids in zein determines its solubility behavior. The molecular structure is a helical wheel conformation where nine homologous repeating units are arranged in an anti-parallel form stabilized by hydrogen bonds[15]. Zein has been used to form microspheres by cross linking a zein solution containing the drug[16]. In order to increase the drug concentration in tumor and hence to improve the antitumor potency of DOX, DOX-loaded zein *in situ* gel was developed in this study. *In vitro* release of DOX-loaded zein was investigated and the pharmacokinetics, biodistribution and therapeutic efficacy of these DOX-loaded zein formulations were investigated using BALB/c nude tumor-bearing mice.

2. Materials and methods

2.1. Materials and chemicals

DOX hydrochloride was obtained from Lingnan Pharmaceutical, Ltd. (Guangzhou, China). Zein was from Rixing Pharmaceutical Adjuvant Factory (Gaoyou, China). Glycerol formal (GF) was purchased from AcrosOrganics (Geel, Belgium). All other chemicals were purchased from Tedia company (Fairfield, USA) and of HPLC or analytical grade.

2.2. Sample preparation

Zein was dissolved mixed with GF and 70% ethanol-water (3:7, *v*/*v*). The concentration of Zein was 15%, 20%, 25% for *in vitro* release and 20% for *in vivo* study. It was left overnight at room temperature to form a clear solution. DOX was added into the above solution and dissolved by stirring.

2.3. Tumor homograft

The HT-29 cells (human colorectal cancer cell line, Cell Bank of Peking Union Medical College) were harvested with 0.25% trypsin and made into suspension (1.0×10^7 cells/mL). The cell suspension was implanted subcutaneously into one forefoot armpit of immunodeficient mice (BALB/c nude mice) (Vital River, China).

2.4. In vitro DOX release

Half a milliliter of the sample was added to 10 mL PBS (pH 7.4) containing NaN_3 (0.05%, *w*/*v*) and trypsin 1:250 (1.0%, *w*/*v*)[17,18]. The gels formed were shaken in a constant temperature oscillator at 50 rpm and 37 °C. 5 mm of the buffer was collected at predetermined time intervals and replaced with fresh buffer. Drug concentration was analyzed by HPLC.

2.5. In vivo antitumor efficacy test

When the tumor volume reached approximately 100 mm^3 on the 7th day after cell injection, the mice were randomly divided into 4 groups ($n=6$): (1) normal saline (control), (2) aqueous solution of DOX, (3) blank gel only, (4) doxorubicin-loaded zein *in situ* gels (DOX-zein). The dosages of DOX for a mouse were set at 5 mg/kg. Gels or solutions were administered using a 22 G needle. The tumor diameters were measured in two dimensions every other day with the Somatom sensation 64 CT machine (Siemens, Germany) with automatic 3D reconstructions.

2.6. Plasma concentration and tissue distribution

When the tumor volume had reached about 100 mm^3, the mice were divided into two groups: (1) aqueous solution of DOX (*i.v.*) and (2) DOX-zein (intratumoral injection). The same dose of 5 mg/kg DOX was injected. At 1, 3, 5, 12, 24, 48, 72 h after injection the mice were killed. The heart, liver, spleen, lungs, kidneys and tumors were immediately removed, cleaned, weighed and homogenized with citric acid-buffered saline (CBS, pH 8.0, 0.5 mL/g tissue).

The drug was extracted with chloroform-methanol (4:1, *v*/*v*) from plasma diluted with CBS (pH 8.0) and tissues homogenized with the same buffer. The organic phase was dried under N_2 at room temperature. The residues were dissolved in methanol for HPLC analysis (Hitachi, L-2000, Japan). The AUCs of DOX in the plasma and tissues were calculated by DAS Ver1.0. The *in vivo* data was evaluated using Student's *t*-test and $P<0.05$ was considered to be statistically significant.

2.7. HPLC method

The HPLC analysis was performed under the same conditions as for the release test and tissue and plasma concentration tests on a Hitachi HPLC system (L-2000, Japan) controlled by D-2000 workstation using a C18 column (Thermo Hypersil ODS, 5 μm, 200 mm × 4.6 mm). The mobile phase consisting of 0.01 M ammonium dihydrogen phosphate, methanol,

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