RESEARCH ARTICLE

Preparation and *In Vitro* Characterization of SN-38-Loaded, Self-Forming Polymeric Depots as an Injectable Drug Delivery System

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ABSTRACT: This work describes the preparation and characterization of anticancer-loaded injectable polymeric depots that consisted of D,L-lactide (LA), ε-caprolactone (CL), and poly(ethylene glycol) (PEG) or [poly(ε-caprolactone)-random-poly(D,L-lactide)]-block-poly(ethylene glycol)-block-[poly(ε-caprolactone)-random-poly(D,L-lactide)] (PLEC) copolymers for malignant gliomas treatment. PLECs were polymerized with different percentages of LA to deliver 7-ethyl-10-hydroxycamptothecin (SN-38), a highly potent anticancer drug. SN-38-loaded depots could form directly in phosphate buffer saline with more than 98% encapsulation efficiency. The release rate of SN-38 from depots was found to depend on the amount of LA in PLECs, loading content of SN-38 in the depots, and depot weight. Encapsulation of SN-38 inside depots could enhance the stability of SN-38 where all of SN-38 released after 60 days was in an active form. Depots without SN-38 were evaluated as noncytotoxic against U-87MG, whereas SN-38-loaded depots showed cytotoxic effect as a function of concentration. © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

Keywords: biodegradable polymers; cancer; controlled release; depot; site-specific delivery; SN-38

INTRODUCTION

7-Ethyl-10-hydroxycamptothecin [SN-38; chemical formula, $C_{22}H_{20}N_2O_5$; molecular weight (MW) = 392.31 g/mol] is a potent anticancer drug for brain cancer[1,2] that integrates its structure to DNA or inhibits topoisomerase enzyme.[3] This results in the blockage of DNA synthesis and fragmentation of DNA, which is 100–1000-fold more active than irinotecan (CPT-11), a prodrug of SN-38.[4–6] However, SN-38 is a poorly soluble drug and has severe side effects that limit its clinical applications.[3] Moreover, SN-38 is unstable at physiological pH with half-life of approximately 12 min.[3] The deactivation of SN-38 occurs when an active form (lactone) of SN-38 convert to an inactive open ring structure (carboxylate).[7]

Drug delivery systems from biodegradable polymer become an alternative approach to solve these problems. Tremendous efforts have been made to develop drug delivery systems such as dendrimer[4], liposome,[8] and nanoparticles[9] or micelles as alternative strategies for SN-38 delivery.[10–12] Using biodegradable polymers as materials for drug delivery systems has the advantages of controlling drug release profiles, biodegradability, low toxicity, and good biocompatibility.[13,14] These polymers can be hydrolyzed and decomposed so the removal of these drug delivery systems from the body is unnecessary. Blood–brain barrier (BBB), however, remains the most challenging obstacle for brain cancer therapy.[15,16] Drug delivery systems that can bypass BBB and directly deliver therapeutic agents to brain tumors will therefore become a great approach to achieve this goal.[13]

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Advantages of implantable drug delivery systems are not only to establish the local treatment and improve a clinical efficacy of drugs but also to minimize drug's toxic effects. *In situ* forming polymer implants can be an alternative approach for drug delivery to the brains. This system offers tremendous advantages in preparation and administration because it can spontaneous solidify *in situ* via injection[17,18] and require only small opening for implantation, respectively.

Recently, we demonstrated the use of glycofurol (GF) and the formation of polymer implants from [poly(ε-caprolactone)-random-poly(D,L-lactide)]-block-poly(ethylene glycol)-block-[poly(ε-caprolactone)-random-poly(D,L-lactide)] (PLEC) in *in vitro* experiments and rat brains. For *in vitro* preparation, self-solidifying polymeric depots were successfully developed to encapsulate trypan blue, a hydrophilic dye, with more than 90% encapsulation efficiency.[19] Results from *in vivo* studies showed no neurologic or systemic toxicity, allowing the use of these depots for local delivery of a variety of therapeutic agents to treat neurological diseases.[20,21] Because both GF and PLEC could be used safely in rat brains, we therefore explored these depots as a drug delivery system. In this study, we described the *in vitro* preparation of SN-38-encapsulated PLEC depots. GF, a pharmaceutically accepted solvent, was chosen as a solvent for the preparation of this drug delivery system because it has been used clinically for parenteral delivery of drugs such as diazepam and phenytoin.[22,23] The effect of formulation and copolymer composition on encapsulation efficiency and *in vitro* release were evaluated. SN-38 was analyzed for its stability (active or inactive form) by high-performance liquid chromatography (HPLC). The cytotoxicity studies of depots against brain cancer cells, U-87MG cell line, were also carried out to evaluate the efficiency of these depots.

MATERIALS AND METHODS

Materials

7-Ethyl-10-hydroxycamptothecin was obtained from Abatra Technology Company Ltd. (Xi'an, Shaanxi, China). GF and tin (II) 2-ethylhexanoate were obtained from Sigma–Aldrich (St. Louis, Missouri). D,L-Lactide (LA), ε-caprolactone (CL), and poly(ethylene glycol) (PEG; $M_n = 1000$ Da) were obtained from Acros Organics (Morris Planis, New Jersey). Monomers (LA, CL, and PEG) were prepared by the following procedure: LA was purified by recrystallization with ethyl acetate, and CL was purified by distillation over calcium hydride (CaH_2). Dimethyl sulfoxide and tetrahydrofuran (THF; HPLC grade) were obtained from RCI Lab-scan Ltd. (Milwaukee, Wisconsin).

Human glioblastoma cell, U-87MG, obtained from the American Type Culture Collection (Manassas, Virginia) was used in the cytotoxicity study. Cells were cultured in Eagle's minimum essential medium (EMEM; Gibco, Grand Island, New York) supplemented with 10% fetal bovine serum (JR Scientific, Inc., Woodland, California), 110 mg/mL of sodium pyruvate (Gibco, Grand Island, New York), 100 U/mL of penicillin, 100 μg/mL of streptomycin, and 25 μg/mL of amphotericin B (JR Scientific, Inc., Woodland, California). The culture was incubated at 5% CO_2 in a humidified atmosphere at 37°C.

Polymerizations

[Poly(ε-caprolactone)-random-poly(D,L-lactide)]-block-poly(ethylene glycol)-block-[poly(ε-caprolactone)-random-poly(D,L-lactide)]s were synthesized as previously reported.[19,21,24] The mixture was mixed by various ratios of CL and LA in the dried round-bottomed flask under dried argon and reduced pressure for 6 h using toluene as a solvent. Tin (II) 2-ethylhexanoate, a catalyst, was added to catalyze the polymerization, then the mixture was heated at 130°C for 48 h. PLECs were precipitated in cold methanol and dried in vacuum, then they were analyzed by nuclear magnetic resonance (NMR) spectroscopy and gel permeation chromatography (GPC).

Preparation of Polymer Depots/Loaded Polymer Depots

7-Ethyl-10-hydroxycamptothecin and copolymer were dissolved by mixing vigorously in GF at the ratio of 30% (w/v). It should be noted that 30% (w/v) comprised 30 mg of copolymer in 100 μL of GF. Loading of SN-38 was varied from 0% to 30% (w/w) as compared with PLEC weight. PLEC solution and SN-38-loaded PLEC solution were then injected into a vial containing 10 mL of phosphate buffer saline (PBS; pH 7.4) where PLEC depots were immediately formed. Depots were incubated at 37°C and stirred by orbital shaker at 90 rpm.[19,21] To harvest PLEC depots, PBS buffer was removed by pipette then depots were rinsed with deionized (DI) water, followed by freeze-drying (model: FDU-1200; EYELA, Bunkyo-ku, Tokyo, Japan). The morphology of depot surface was characterized by scanning electron microscopy (SEM). Unencapsulated SN-38 in the first suspension buffer was measured spectrophotometrically at 384 nm. Encapsulated SN-38 was presented as encapsulation efficiency (%).

In Vitro SN-38 Release Studies from Depots

After depot formation in PBS as mentioned in the section *Preparation of Polymer Depots/Loaded Polymer Depots*, they were incubated in 10 mL of PBS (pH 7.4) at 37°C and orbital shaking at 90 rpm.[19,21] At the predetermined time, PBS buffer was removed periodically, and the amount of SN-38 was determined

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