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A study of the synergistic effect of folate-decorated polymeric micelles incorporating Hydroxycamptothecin with radiotherapy on xenografted human cervical carcinoma

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ABSTRACT

In this study, Hydroxycamptothecin (HCPT)-loaded micelles were formed in water by the self-assembly of folate (FA)-decorated amphiphilic block copolymer, methoxy poly(ethylene glycol)-poly(ε-caprolactone) (MPEG-PCL), and achieved a hydrodynamic diameter about of 132 nm. HCPT release from the micelles exhibited no initial burst but showed a sustained release profile. The cytotoxicity and targeting ability of FA conjugated polymeric micelles was investigated by using methylthiazoletetrazolium (MTT) and fluorescence microscopy. We found that FA-conjugated micelles had superior cytotoxicity against HeLa cells compared to non-conjugated micelles, and that they exerted this effect by folate receptor (FR)-mediated endocytosis. In addition, HeLa cells were xenografted into nude mice and subjected to radiotherapy (RT) and/or HCPT-loaded micelle treatment. The antitumor efficacy was detected by analysis of tumor growth delay (TGD) and median survival time. Micro fluorine-18-deoxyglucose PET/computed tomography (^{18}F-FDG PET/CT) was performed to assess early tumor response to HCPT-loaded micelles in combination with RT. Analysis of cell cycle redistribution, apoptosis and expression of histone H2AX phosphorylation (λ-H2AX) was used to evaluate the mechanism by which HCPT loaded micelles led to radiosensitization. Taken together, the results showed that HCPT-loaded FA decorated micelles efficiently sensitized xenografts in mice to RT, and induced G2/M phase arrest, apoptosis and expression of λ-H2AX.

1. Introduction

Cervical cancer is the third most common malignant tumor in women worldwide and concurrent chemoradiation therapy has become a standard treatment [1,2]. However, traditional concurrent cisplatin-based chemoradiation therapy may lead to hematologic toxicity, as well as, gastrointestinal, or renal toxicity and neuropathy [3]. Thus, the development of agents and approaches to further improve the therapeutic index of chemoradiation therapy is a major research objective. It has been shown that camptothecin (CPT) and their derivatives enhance the lethal effects of ionizing radiation both *in vitro* and *in vivo* [4–6]. Consequently, we used HCPT, a natural analog of CPT, as a model drug in this study to evaluate the radiosensitizing effect on a xenografted human cervical carcinoma. However, several physical properties of HCPT, such as insolubility in water, instability at neutral pH and toxicity to normal tissues [7] greatly restrict its application. In recent years, with the development of nanobiotechnology, much effort has been directed toward the design and synthesis of high performance nanoscale HCPT delivery systems, such as magnetic nanohybrids [8], dendrimers [9], nanoparticles [10] and hydrogel [11].

Most recently, polymeric micelles such as biodegradable block copolymers with poly(ethylene glycol) (PEG) and aliphatic polyesters have been used as potential drug carriers, due to attractive properties, such as the ability to increase drug bioavailability, prolong blood circulation, enhance drug solubility, and control drug release [12,13]. It is well known that many malignant tumors, especially ovarian, nasopharyngeal, cervical and chorion carcinomas, express high levels of folate receptors (FR). Folate (FA)

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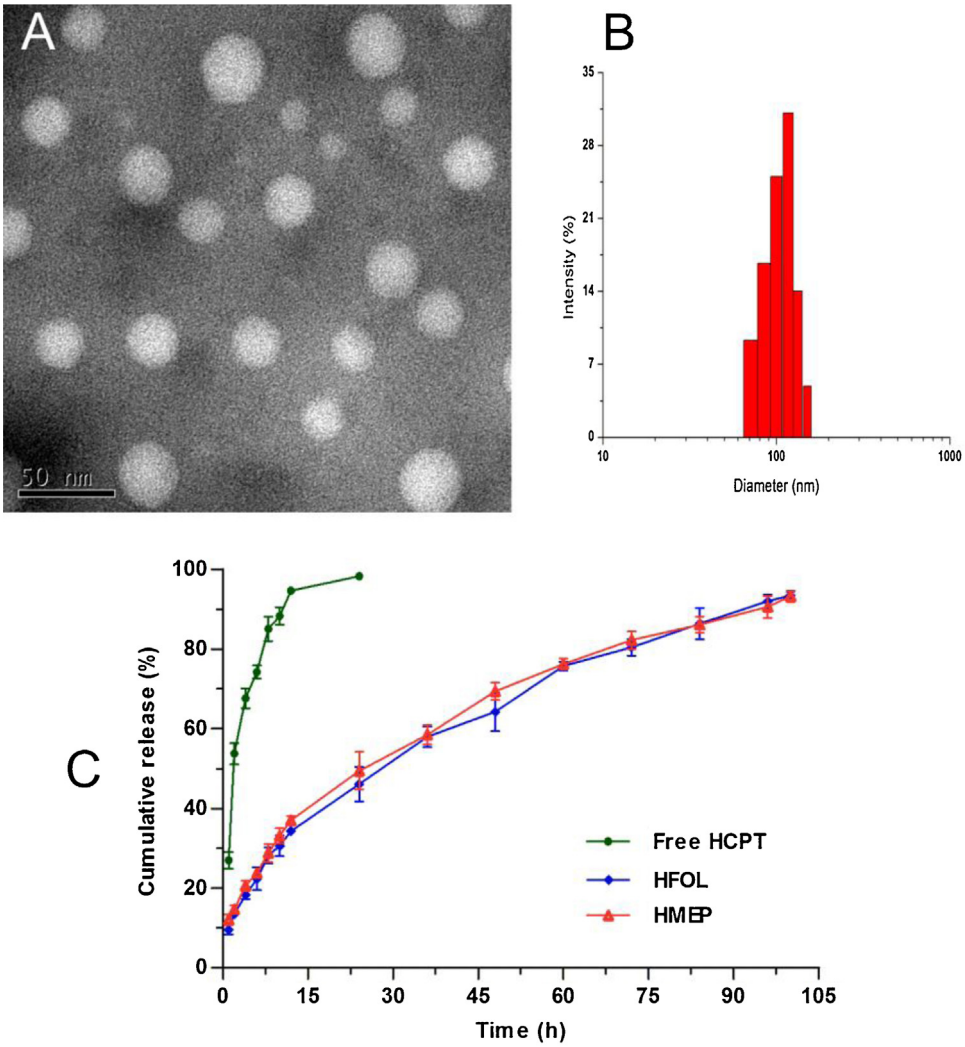

Fig. 1. Characterization of HFOL micelles. (A) TEM image of HFOL micelles; (B) Particle size distribution of HFOL micelles; (C) *In vitro* drug release.

has been widely used as a selective targeting moiety for various anticancer agents to specifically combine with the FR [14,15]. Studies have demonstrated that FA-functionalized polymeric micelle is a promising carrier for anticancer drugs, using FR-mediated endocytosis as a means by which to specifically target tumor cells [16].

Our previous work demonstrated that topotecan (TPT) enhances radiotherapy (RT) on human nasopharyngeal carcinoma [17]. To extend this finding, we investigated the radiosensitizing potential of HCPT-loaded FA decorated polymeric micelles (HFOL). We chose to administer the radiotherapy at 10 pm (15 h after light onset, 15HALO) and evaluated TGD and median survival time on xenografted human cervical carcinoma. In addition, micro ^{18}F-FDG PET/CT imaging was used to detect early tumor response to RT in combination with micelles alone, or with micelles conjugated with FA. The mechanism of radiosensitization was also evaluated by analysis of cell cycle redistribution, apoptosis and expression of γ-H2AX.

2. Materials and methods

2.1. Materials, cell lines, animals

10-Hydroxycamptothecin (HCPT, ≥99%) was purchased from Grandpharma Co. Ltd. (Wuhan, China). Hydroxylcamptothecine (HCPT) injection was obtained from Feiyun Pharmaceutic Co. Ltd. (Wuhan, China). Triethylamine and acryloyl chloride were provided by adamas-beta (Shanghai, China). Fluorescein isothiocyanate (FITC) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were provided by Sigma–Aldrich (USA). Histone H2AX (phospho-S139) polyclonal antibody was purchased from Bioworld technology Co. Ltd. (Nanjing, China). Folic acid (FA), dichloromethane (DCM), dimethyl sulfoxide (DMSO) and acetonitrile (HPLC grade) were all supplied by Kelong Co. Ltd. (Chengdu, China).

Human cervical carcinoma cells (HeLa) and human alveolar type II cells (AT2) were provided by the Experimental Medicine Center, at the First Affiliated Hospital of Sichuan Medical University (Luzhou, China). HeLa cells and AT2 cells were grown in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) at 37 °C in a humidified incubator containing 5% CO_2.

BALB/c Mice (female, nu/nu, 4 weeks old) were purchased from Chongqing Tengxin biotechnology Co. Ltd. (Chongqing, China) and were housed at a controlled temperature of 20–22 °C, with a relative humidity of 50–60% and 12 h light–dark cycles. Animals were provided with a diet of standard laboratory chow and tap water *ad libitum*. All animal procedures were performed following the protocol approved by the Institutional Animal Care and Treatment Committee of Sichuan Medical University (Luzhou, China) and all mice were treated humanely throughout the experimental period.