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Synergistic anti-tumor activity through combinational intratumoral injection of an in-situ injectable drug depot

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ABSTRACT

Here, we describe combinational chemotherapy via intratumoral injection of doxorubicin (Dox) and 5-fluorouracil (Fu) to enhance the efficacy and reduce the toxicity of systemically administered Fu and Dox in cancer patients. As the key concept in this work, mixture formulations of Dox-loaded microcapsules (Dox-M) and Fu-loaded Pluronic® hydrogels (Fu-HP) or Fu-loaded diblock copolymer hydrogels (Fu-HC) have been employed as drug depots. The *in vitro* and *in vivo* drug depot was designed as a formulation of Dox-M dispersed inside an outer shell of Fu-HP or Fu-HC after injection. The Dox-M/Fu-HP and Dox-M/Fu-HC formulations are free flowing at room temperature, indicating injectability, and formed a structural gelatinous depot *in vitro* and *in vivo* at body temperature. The Fu-HP, Fu-HC, Dox-M/Fu-HP, Dox-M/Fu-HC, and Dox-M formulations were easily injected into tumor centers in mice using a needle. Dox-M/Fu-HC produced more significant inhibitory effects against tumor growth than that by Dox-M/Fu-HP, while Fu-HP, Fu-HC and Dox-M had the weakest inhibitory effects of the tested treatments. The *in vivo* study of Dox and Fu biodistribution showed that high Dox and Fu concentrations were maintained in the target tumor only, while distribution to normal tissues was not observed, indicating that Dox and Fu concentrations below their toxic plasma concentrations should not cause significant systemic toxicity. The Dox-M/Fu-HP and Dox-M/Fu-HC drug depots described in this work showed excellent performance as chemotherapeutic delivery systems. The results reported here indicate that intratumoral injection using combination chemotherapy with Dox-M/Fu-HP or Dox-M/Fu-HC could be of translational research by enhancing the synergistic inhibitory effects of Dox and Fu on tumor growth, while reducing their systemic toxicity in cancer patients.

1. Introduction

As a first treatment, most cancer patients receive surgery to remove as much of the tumor as possible [1]. Thereafter, systemic chemotherapy or local radiotherapy is performed to eliminate residual cancer and prevent the recurrence of carcinogenesiss [2].

Many chemotherapeutic drugs have been used clinically. Doxorubicin (Dox) and 5-fluorouracil (Fu) have been extensively employed in individual and combinational chemotherapy for solid tumors [3–5]. Because both Dox and Fu can effectively treat many types of solid tumors, we hypothesized that cancer treatment with a combination of Dox and Fu could synergistically inhibit tumor growth *in vivo*.

However, it is difficult to achieve drug accumulation inside the target tumor with drug-only formulations of Dox and Fu owing to rapid clearance from tumor by the blood circulation [6,7]. Furthermore, reports have found that drug-only formulations of Dox and Fu showed *in vivo* stability for up to 1 day or a few days, respectively [8–10]. Thus, repeated administration of Dox and Fu is required to maintain therapeutic activity at target tumors [11]. However, numerous studies have found that administration of Dox or Fu can cause side effect owing to low selective chemotherapeutics through dose-limiting of the drugs [12].

Therefore, maintenance of therapeutic concentrations of Dox and Fu within target tumor tissues over a prolonged period with few systemic side effects has been the goal of most researches (Fig. 1).

Targeted drug delivery, such as direct intratumoral injection, can

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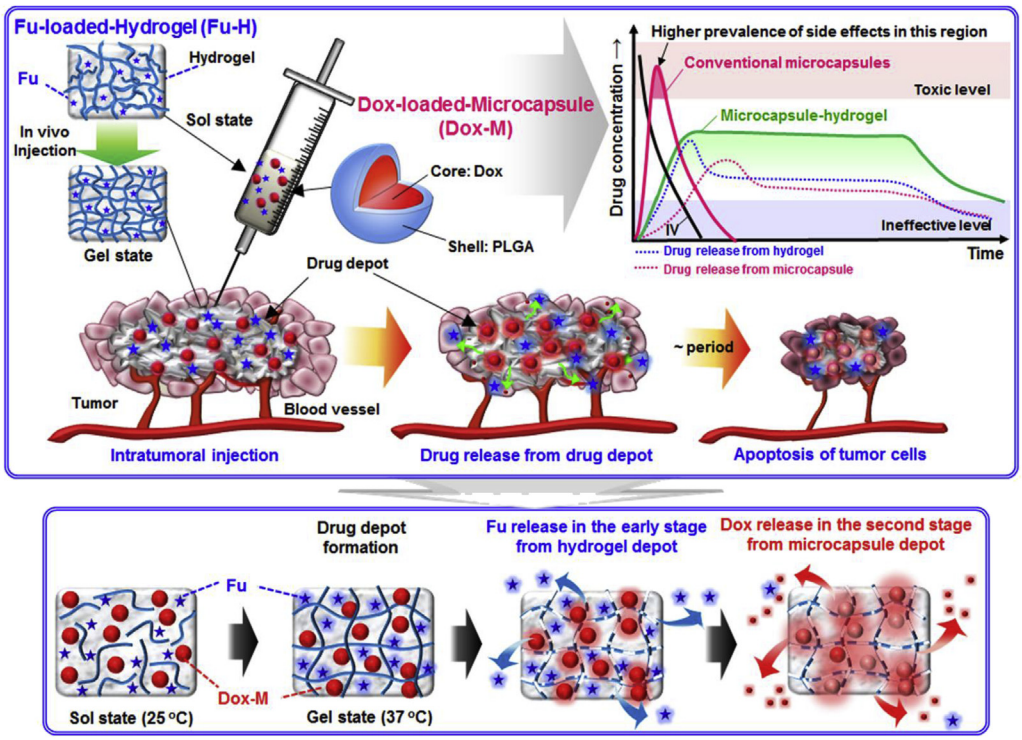

Fig. 1. Schematic representation of synergistic tumor suppression via intratumoral injections of dual-drug depots and controlled release of Dox and Fu in the first and second stage.

achieve a high local concentration inside the target tumor. In addition, injectable depot formulations of Dox or Fu can greatly increase desirable outcomes while minimizing negative side effects. It is therefore feasible that direct target delivery of injectable depot formulations of Dox or Fu could be achieved by intratumoral injection.

During the last decade, injectable in situ-forming hydrogels have attracted considerable attention, because they can exhibit solution-to-gel phase transition in response to changes in temperature [13,14]. Various anticancer drugs can be incorporated easily into the hydrogel solution at room temperature by simple mixing. Anticancer drug-loaded hydrogel (DH) solutions form hydrogel depots *in situ* at site-specific positions under physiological conditions, resulting in prolonged action periods for the loaded anticancer drugs.

Recently, our group reported a biodegradable poly(ethylene glycol)-*b*-poly(caprolactone-co-lactide) diblock copolymer (HC) as an intratumorally injectable drug depot with a biodegradation window adjustable from a few weeks to a few months [15–17]. Application of HC for intratumoral chemotherapy represents a promising approach to maintain therapeutic concentrations of Dox and Fu within the tumor through a single injection, as well as to induce biodegradation of the drug depot over a defined treatment period.

Microencapsulation has the capacity to selectively entrap a particular drug in a polymer matrix. Drug-loaded microcapsules (DM) can be subcutaneously or intratumorally injected to form a drug depot, where they slowly release their drug contents over time. Thus, DM depots can significantly reduce dosage frequency and improve drug efficacy without increasing the risk of toxicity. In previous studies, our group manufactured DM using a mono-axial nozzle ultrasonic atomizer [18–20]. Several drugs were encapsulated in the inner core of the microcapsules. Microcapsules with a round shape formed a DM depot following subcutaneous injection. In addition, *in vivo* drug release from the DM depot was maintained for at least 4 weeks in rats.

Because both DH and DM formulations can be easily prepared as solution and then can form drug depot through minimally invasive chemotherapeutic administration, thus we believed that intratumoral injection of a combination of anticancer DH and DM would effectively inhibit tumor growth.

To the best of our knowledge, intratumoral injection of combinations of DH and DM with anticancer drugs into animals has received little study for *in vivo* cancer treatment. Thus, in this work developing an efficient *in vivo* delivery strategy of combination of drug depot can achieve an unmet need for cancer treatment.

The *first aim of this work* was to prepare combination formulation of DH and DM with Dox and Fu (Fig. 1). Based on the stability of Dox and Fu, Dox (with 1 week of stability) was encapsulated inside the inner core of the Dox microcapsules (Dox-M) to prolong its action period, while Fu (with a few weeks of stability) was loaded in the in-situ hydrogels (Fu-H). Because the Fu-H was a viscous solution at room temperature, the easiest method of preparing the injectable Dox-M/Fu-H depot was to simply mix the Dox-M into the Fu-H solution. Thus, the Dox-M/Fu-H drug depot could be formulated with Dox-M dispersed inside an outer shell of Fu-H. It was expected that utilizing the microcapsule and hydrogel forms of Dox and Fu, respectively, would control their release. In addition, the hydrogel acts as an outer shell for the Dox-M, resulting in sustained Dox release from the microcapsules.

Reports have shown that the properties of hydrogels control the release of encapsulated drugs [18,21]. Recently, our group reported that Pluronic® and HC hydrogels showed a gel persistence of 2 days and a few weeks, respectively, and accordingly showed different drug release patterns [22]. Thus, in this work, we assessed the release of Fu and Dox from Dox-M/Fu-HP and Dox-M/Fu-HC prepared by using Pluronic® and HC with different gel persistence times, respectively, as additional outer shells. The *second aim of this work* was to compare the effects of Dox-M/Fu-HP and Dox-M/Fu-HC on tumor growth.