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Research paper

Antitumor effect of an injectable in-situ forming drug delivery system composed of a novel tissue adhesive containing doxorubicin hydrochloride

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Abstract

Our group has developed a novel tissue adhesive composed of biomacromolecules and organic acid derivatives which have good biocompatibility and exhibit high bonding strength to living tissues. We propose to use this tissue adhesive for in-situ forming drug delivery system (DDS) for cancer chemotherapy. In a previous work, we had prepared a novel in-situ forming DDS composed of human serum albumin (HSA) and tartaric acid derivative (TAD) containing doxorubicin hydrochloride (DOX), and we had demonstrated an in vitro release profile of DOX from HSA–TAD gel for approximately up to 100 h. Here, we report on antitumor effect of this injectable in-situ forming DDS. Local injection of DOX by the HSA–TAD was administered to human colon carcinoma (WiDr) implanted subcutaneously onto the immunodeficient mouse. The results of the in vivo experiments showed that the presence of DOX in blood of mice was detectable for up to 3 days, and that the tumor volume was effectively minimized with injection of HSA–TAD containing DOX. The in-situ forming DDS with the novel tissue adhesive containing DOX, therefore, is a useful technique for cancer chemotherapy.

Keywords: Tissue adhesive; Hydrogel; Drug delivery system; Human serum albumin; Tartaric acid; Injectable; in-situ forming; *N*-Hydroxysuccinimide; Doxorubicin; Antitumor effect

1. Introduction

Injectable polymers have drawn considerable attention as promising biomaterial for drug delivery and regenerative medicine. Multiple biocompatible and biodegradable polymers are routinely employed as carriers of injectable DDS in order to diminish the drug side-effect, especially for local administration and delivery when used for anticancer chemotherapy. Many polymeric materials for injectable DDS, such as nanoparticle [1–3], microsphere [4,5], polymeric micelles [6–8], liposomes [9–12], and hydrogel system [13,14], have been investigated and developed. Although some formations of them have succeeded in clinical applications there still remain many problems that need to be addressed. One of the recent manifestations of stimuli-responsive polymers lies in in-situ forming DDS by sol–gel transition for in-situ forming hydrogel because it is feasible to use them as carrier for local administration [15]. As representatives of stimuli-responsive polymer for in-situ forming hydrogel, there are several candidates that include thermoresponsive polymers such as *N*-isopropyl acrylamide copolymer [16], polyethylene glycol–polypropylene glycol–polyethylene glycol (PEG–PPG–PEG) triblock copolymer [17], and polyethylene glycol–poly L-lactic acid–polyethylene glycol (PEG–PLLA–PEG) triblock copolymer [18]. These thermoresponsive polymers exhibit thermo-dependent sol–gel transition in aqueous solution via hydrophobic interaction. The advantage of these kinds of polymers is in their ability to avoid toxic cross-linkers which are usually employed to form hydrogel. However, local injection of thermoresponsive polymers is

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operationally difficult. Their thermoresponsiveness is too sensitive for injection by syringe pump and for this reason they must be cooled down to below the transition temperature before they can be injected. Furthermore, ion-mediated cross-linked hydrogels, such as alginates, which form a gel upon contact with divalent cations, have been widely researched as injectable in-situ forming DDS and tissue engineering because of their biocompatibility [19,20]. Many alginate derivatives such as lectin-modified alginate [21] and RGD containing alginate [22] have also been synthesized. Despite many of their applications, alginate hydrogels have limited use because of their low shelf lives.

Recently, we have developed a novel tissue adhesive consisting of biomacromolecules such as collagen, gelatin and human serum albumin, and organic acid derivatives with active ester groups [23–27]. The bonding strength and biocompatibility of these adhesives to soft tissues were found to be superior to the ones obtained from the commercially available surgical glues such as cyanoacrylate derivatives [28,29], fibrin glue [30] and biomolecules-aldehydes glue [31]. The ability of an adhesive to bind to living tissue is very important in order to immobilize the tissue at some local site after administer drugs into a subject's body. The novel tissue adhesive, therefore, has high potential for use as injectable in-situ forming DDS (Fig. 1). The work on injectable in-situ forming DDS using fibrin glue for cancer chemotherapy has already been reported, however, desirable results were not achieved during the long-term release of carcinostatics [32–34]. In an earlier work, we prepared an injectable in-situ forming DDS that was composed of human serum albumin (HSA) and tartaric acid derivative (TAD) (Fig. 2). And there it was also shown that physicochemical properties such as gelation time, gel strength and bonding strength of HSA–TAD gel could be controlled with material composition [35]. Furthermore, we demonstrated long-term release of doxorubicin hydrochloride (DOX) from HSA–TAD gel for approximately up to 100 h in the in vitro.

Their antitumor effect was evaluated using human colon carcinoma (WiDr) that was implanted subcutaneously onto immunodeficient mouse. Tumor volume and blood plasma level of DOX were then evaluated.

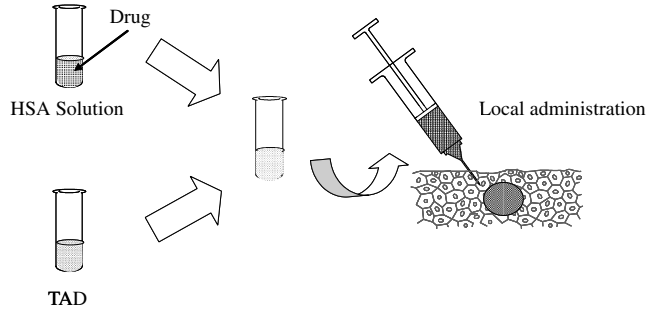

Fig. 1. Injectable in-situ forming drug delivery system with HSA–TAD adhesive.

2. Materials

Human serum albumin was obtained from Sigma–Aldrich Co. (St. Louis, MO, USA). Tartaric acid, *N*-hydroxysuccinimide (HOSu) and doxorubicin hydrochloride (DOX) were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Dicyclohexylcarbodiimide (DCC) was purchased from Kokusan Chemical Co., Ltd. (Tokyo, Japan). All other reagents used were HPLC or analytical grade without further purification.

3. Methods

3.1. Synthesis of TAD

TAD was prepared by a similar procedure previously reported [24]. Briefly, tartaric acid (5 g) was first dissolved in THF (200 ml), and then HOSu (9.58 g) and DCC were added. After mixing for 30 min, the mixture was concentrated with rotary evaporation under a reduced pressure to remove THF. The resulting mixture was recrystallized to yield pure TAD. The resulting TAD was confirmed by ^{1}H NMR spectroscopy and elemental analysis, as well as by a previous work [35].

3.2. Determination of gelation time

HSA was dissolved in PBS (0.1 M, pH 7.4) at various concentrations. HSA solutions were weighed for 0.5 g in a polypropylene tube, and then different amounts of TAD (0.05, 0.075, and 0.1 mmol/0.8 g of HSA sol) were added to HSA solution and stirred vigorously. In the case of the DOX containing HSA–TAD, the DOX was added to HSA solution before adding TAD.

3.3. Measurement of bonding strength

Bonding strength of HSA–TAD with/without DOX was measured by using collagen casing which adhered on test pieces (10×10 mm) at the one end of a PET film (10×40 mm). Seventy microliters of HSA–TAD with/without DOX was first applied to collagen casing. The other test piece was then placed on the first layer. The bonding area was set at 10×10 mm. After 10 min at 37 °C, the bonding strength, as shearing bonding strength, was measured by tensile testing machine (TA-XT2i, Eko Instruments Co., Ltd., Tokyo, Japan). Three samples were tested to measure the same bonding strength ($n = 3$).

3.4. Determination of DOX

Determination of DOX was carried out by RF-HPLC. The HPLC system consisting of HPLC pump (PU-2080 plus), intelligent autosampler (AS-2055 plus), fluorescence detector (FP-2020 plus), column ovens (CO-2060) and degasser (DG-2080-53) was purchased from Jasco Inc. (Tokyo, Japan). The separation was carried out using reverse-phase