Heat-Treated Emulsions with Cross-Linking Bovine Serum Albumin Interfacial Films and Different Dextran Surfaces: Effect of Paclitaxel Delivery

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ABSTRACT: In this study, a type of biocompatible and biodegradable oil-in-water emulsion for hydrophobic drug delivery was evaluated *in vitro* and *in vivo*. Bovine serum albumin (BSA)–dextran conjugates with different dextran molecular weights and different conjugation degrees were used as the emulsifier and stabilizer. Paclitaxel (PTX), a hydrophobic antitumor drug, was effectively loaded inside the oil droplets via high-pressure homogenization. The emulsions were heated at 90°C for 1 h to eliminate the anaphylaxis of BSA. By virtue of the cross-linked BSA films at the oil–water interfaces produced by the heat treatment and the hydrophilic dextran surfaces, the emulsions are stable in blood serum, as well as stable against long-term storage. *In vitro* cytotoxicity study verifies that the unloaded emulsions are biocompatible and the PTX-loaded emulsions have similar antitumor activity as PTX solution. *In vivo* investigation of murine ascites hepatoma H22-tumor-bearing mice demonstrates that the PTX-loaded emulsion with shorter and denser dextran surface has better tumor inhibition and survivability efficacy than the commercial PTX injection. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:1307–1317, 2013

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INTRODUCTION

About 40% of the new chemical entities discovered by the pharmaceutical industry are hydrophobic compounds, and parenteral delivery of the hydrophobic drugs is a challenging task.[1,2] For example, paclitaxel (PTX) is an effective antitumor drug and microtubule antiproliferative agent, but its efficacy is greatly limited by its extremely poor solubility in an aqueous solution[3] and poor absorption via the oral route.[4] To enhance its solubility and allow parenteral administration, PTX is currently formulated in a vehicle composed of polyethoxylated castor oil and alcohol.[5] Another marketed PTX formulation is PTX–human serum albumin (HSA) nanoparticles, produced by oil-in-water emulsion in which HSA acts as an emulsifier, then removing the solvents and redispersion in water.[6,7] PTX–HSA nanoparticles show a better antitumor activity and less toxicity.

Recently, emulsion has attracted much attention for hydrophobic drug delivery because of the improvement in efficacy of the therapeutic agents.[8–10] Besides HSA, many other proteins, such as bovine serum albumin (BSA), can also act as emulsifiers for the reason that they have a strong tendency to adsorb at oil–water interface to lower the surface tension.[2,11,12] BSA has been widely used in drug delivery studies because of its abundance, low cost, ease of purification, and unusual ligand-binding properties.[13] For example, Han et al.[2] used BSA as an emulsifier to produce emulsion. By dissolving drug and gelator into the oil phase, the oil droplets have a structure of BSA shell and drug-loaded gel core, which endows the droplets with a temperature-controlled release property. BSA–dextran conjugate, produced via a naturally occurring Maillard reaction between the ε-amino group in BSA and the reducing-end

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carbonyl group in dextran, can also be used as an emulsifier;[14,15] the oil droplets have a structure of BSA film at the oil–water interface and a dextran shell on the droplet surface. The dextran shell can change the surface charges and increase the thickness of the interfacial layers, enhancing the hydrophilicity and steric repulsion of the oil droplets. Furthermore, introducing dextran on the droplet surface can endow the droplets with a "stealth" property[16,17] that can avoid reticuloendothelial recognition and subsequent elimination of the droplets *in vivo*. These advantages make us think that BSA–dextran emulsion is a good system for the delivery of hydrophobic drugs. Recently, we found that BSA–dextran emulsion can effectively load PTX. Unexpectedly, we also found that this PTX-loaded BSA–dextran emulsion can cause acute death of murine ascites hepatoma H22-tumor-bearing mice after the administration via tail vein. The acute death may be caused by BSA–dextran conjugate, not by PTX, because no acute death was found for the commercial PTX injection. However, in our previous study, we have used BSA–dextran conjugate and doxorubicin hydrochloride to produce doxorubicin-loaded nanoparticles via a pH-adjusting and a heat-treatment process.[18] For the doxorubicin-loaded nanoparticles, no acute death happened during the treatments of H22-tumor-bearing mice. The different results between the emulsion and the heat-treated nanoparticle solution indicate that the acute death is caused by the BSA. As a foreign protein, BSA may cause anaphylaxis in mice, whereas the heat treatment can induce BSA denaturation[19] that may eliminate the anaphylaxis.

In order to demonstrate that the heat treatment is an effective method to eliminate BSA anaphylaxis and BSA–dextran emulsion is a universal vehicle for hydrophobic drugs, in this study, we fabricated thermostable PTX-loaded oil-in-water emulsions, in which BSA–dextran conjugates with different dextran molecular weights and different conjugation degrees were used as the emulsifier and stabilizer. The antitumor effects of the heated emulsions were evaluated *in vitro* and *in vivo*. The results confirm our supposition.

MATERIALS AND METHODS

Materials

BSA (fraction V, 99%) and dextran (molecular weight 10 kDa) were supplied by Sangon Biotech (Shanghai) Co. Ltd. (Shanghai, China). Dextran (molecular weight 62 kDa) was from Amershan Biosciences AB (Uppsala, Sweden). Soybean oil for injection was from Jiangxi Golden Crabapple Medicinal Oil Company Ltd. (Yushan, China). PTX was from Jiangsu Yew Pharmaceutical Company Ltd. (Wuxi, China). Fluorescein isothiocyanate (FITC) was from Tokyo Chemical Industry Company Ltd. (TCI, Shanghai, China). Human oral squamous carcinoma KB cell line was from American Type Culture Collection (ATCC, Manassas, Virginia). Dulbecco's modified Eagle's medium (DMEM) cell culture medium and fetal bovine serum were from GIBCO BRL Life Technologies Inc. (Shanghai, China). MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] was from Promega Company (Beijing, China). Commercial PTX injection prepared from polyethoxylated castor oil and alcohol was from Harbin Pharmaceutical Group Company Ltd. (5 mL, 30 mg PTX; Harbin, China). Male ICR mice were from Sino-British Sippr/BK Lab. Animal Ltd. (Shanghai, China). All other reagents were of analytical grade. All solutions were prepared by deionized water, which was filtered by 0.45 μm film to remove dust and bacteria.

Preparation of BSA–Dextran Conjugates

BSA–dextran conjugates were prepared by Maillard reaction as reported previously.[20,21] In this study, 10 and 62 kDa dextran molecules were used to prepare BSA–dextran conjugates. Briefly, BSA and dextran were dissolved together in water to reach molar ratio (MR) of BSA to dextran 1:1–1:7. The solution was adjusted to pH 8.0 with 0.5 mol/L NaOH and then lyophilized. The lyophilized power reacted at 60°C and 79% relative humidity for 48 h. The resultant BSA–dextran conjugates were kept at −20°C before use.

Conjugation Degree Analysis of BSA–Dextran Conjugates

For the conjugates prepared from 10 kDa dextran, the free dextran in the conjugate solution was separated by high-flow ultrafiltration membrane (cutoff molecular weight = 100 kDa; Microcon YM-100; Millipore Corp. Billerica, MA) and was collected in ultrafiltrate. The dextran concentration in the ultrafiltrate was analyzed by gel permeation chromatography (1515 GPC; Waters Corp., Milford, MA) to calculate the conjugation degree. Considering the noncovalent interactions between BSA and dextran as well as the membrane adsorption, a mixture of BSA and dextran with the same MR, the same concentration, and the same ultrafiltration process was used as a standard to calibrate the dextran concentration in the ultrafiltrate.

Preparation of PTX-Loaded Emulsions

BSA–dextran conjugates were used to produce emulsions without purification. For the purpose of simplifying the description, in this paper, only BSA concentrations were presented to denote the conjugate concentrations.