Journal of Controlled Release xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Safety and efficacy of self-assembling bubble carriers stabilized with sodium dodecyl sulfate for oral delivery of therapeutic proteins

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ARTICLE INFO

Article history:
Received 12 December 2016
Accepted 16 December 2016
Available online xxxx

Keywords:
Diabetes mellitus
Oral protein delivery
Endotoxin
Blood glucose-lowering effect
Permeability enhancer

ABSTRACT

Sodium dodecyl sulfate (SDS) is generally regarded as a potent permeability enhancer in oral formulations; however, one concern related to the use of any permeation enhancer is its possible absorption of unwanted toxins during the period of epithelial permeability enhancement. In this work, the safety and efficacy of an SDS-containing bubble carrier system that is developed from an orally administered enteric-coated capsule are evaluated. The bubble carriers comprise diethylene triamine pentaacetic acid (DTPA) dianhydride, sodium bicarbonate (SBC), SDS, and insulin. Upon exposure to the intestinal fluid, DTPA dianhydride hydrolyzes to yield acids, and SBC rapidly reacts with these acids to generate CO_2, producing bubble carriers, each containing a self-assembling water film. The hydrophilic insulin is entrapped in the self-assembled water film, which is stabilized by SDS. The SDS in the bubble carrier system can act as a dissolution enhancer in the dispersion of insulin molecules, as a surfactant that stabilizes the bubble carriers, as a protease inhibitor that protects the protein drug, and as a permeation enhancer that augments its oral bioavailability. Hence, a significant increase in the plasma insulin level and an excellent blood glucose-lowering response in diabetic rats are effectively achieved. Moreover, the enhancement of epithelial permeation by this SDS-containing formulation does not promote the absorption of intestinal endotoxins. The above facts indicate that the bubble carrier system that is stabilized by SDS can be used as a safe and potent carrier in the oral delivery of therapeutic proteins.

1. Introduction

The oral administration of therapeutic proteins such as insulin has been an elusive goal of the drug delivery community. However, increasing the oral bioavailability of protein drugs to a therapeutically effective level remains a major challenge, owing to their presystematic enzymatic degradation and poor intestinal absorption. To address these concerns, our group developed a bubble carrier system to treat diabetic rats by loading an acid initiator (diethylene triamine pentaacetic acid, DTPA, dianhydride), a foaming agent (sodium bicarbonate, SBC; $NaHCO_3$), a surfactant (sodium dodecyl sulfate, SDS), and a protein drug (insulin) into an enteric-coated gelatin capsule [1]. Following oral administration, the intestinal fluid that passed through the gelatin capsule saturated its loaded contents; concurrently, DTPA dianhydride formed an acidic environment, while SBC decomposed to generate CO_2 bubbles in the acid environment. The gas bubbles grew among the surfactant molecules (SDS) owing to the generation of CO_2. The walls of these bubbles comprised a self-assembled film of water, which contained insulin and was stabilized by two layers of SDS molecules. The bubble carriers bumped into the intestinal walls and burst, facilitating the transport of insulin molecules across the epithelium and enabling their eventual absorption into systemic circulation, where they had blood glucose-lowering effects (Fig. 1).

Surfactants are some of the most frequently used adjuvants in pharmaceutical formulations [2]. SDS, an anionic surfactant, is an FDA-approved excipient in oral formulations, primarily functioning as a dissolution agent and a stabilizer [3]; it may also serve as a protease inhibitor [4]. Furthermore, it can be utilized as a permeation enhancer for the absorption of protein drugs across the intestinal epithelium *via* both paracellular and transcellular routes, causing temporary and reversible

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http://dx.doi.org/10.1016/j.jconrel.2016.12.018
0168-3659/

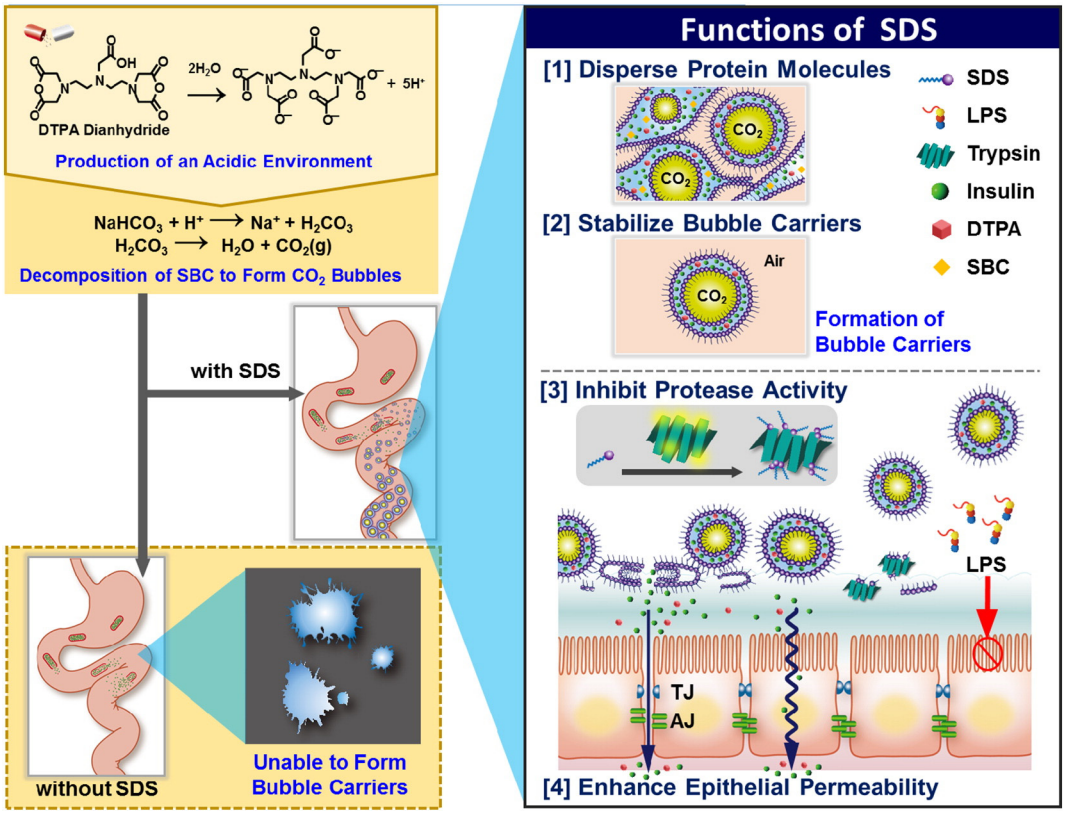

Fig. 1. Mechanism of formation of SDS-containing bubble carriers that are developed from an orally ingested test capsule, and enhancement of oral bioavailability of insulin by SDS.

alteration of the properties of the mucosal/epithelial barrier [5]. To enhance the intestinal permeability *in vivo*, the protein drug and a sufficient concentration of the permeation enhancer must be simultaneously delivered to the absorption site [6]. However, an overdose of a permeation enhancer may cause irreversible damage to local intestinal walls, resulting in the absorption of toxic substances, and especially endogenous endotoxins [7].

This work optimizes the dose of SDS that is used in the bubble carrier system to facilitate the crossing of its encapsulated insulin molecules through the epithelial barrier, while eliminating the possibility of damage to the intestinal walls and absorption of endogenous endotoxins.

2. Materials and methods

2.1. Materials

SDS, DTPA dianhydride, SBC, bovine insulin (Catalogue No. I1882), and fluorescein isothiocyanate (FITC)-lipopolysaccharide (LPS) were purchased from Sigma-Aldrich (St. Louis, MO, USA), while cyanine 5 (Cy5) and FITC-insulin (bovine) were obtained from Lumiprobe Corp. (Broward, FL, USA) and Invitrogen Corp. (Carlsbad, CA, USA), respectively. All used chemicals and reagents were of analytical grade.

2.2. Preparation of enteric-coated test capsules

To prepare enteric-coated capsules for use in the animal studies, hard gelatin capsules (size 9; Torpac Inc., Fairfield, NJ, USA) were manually filled with a powder mixture of DTPA dianhydride (8.0 mg), SBC (8.0 mg), insulin (0.4 mg), and a pre-determined dose of SDS [0 mg (w/o SDS), 1.6 mg (low dose; LD SDS), 3.2 mg (medium dose; MD SDS), or 4.8 mg (high dose; HD SDS)] as per the manufacturer's instructions. The FDA Inactive Ingredients List allows up to 95 mg SDS to be used in oral capsules for humans [2]; this dose is equivalent to a dose of 3.4 mg for a rat model of 300 g, based on the animal's surface area [8]. The as-prepared capsules herein were dipped into a methanol solution that contained Eudragit® L100-55 (15% w/v, Evonik Industries, Parsippany, NJ, USA) and subsequently dried using an air-blower; this process was performed three times.

2.3. Characterization of bubble carriers

FITC-insulin was used in this investigation to observe the formation and development of bubble carriers. The contents of each test capsule [w/o SDS, LD SDS, MD SDS, or HD SDS] were firstly exposed to deionized (DI) water. The formation of the bubble carriers and changes in their sizes and number in DI water were then monitored using a fluorescence microscope (Axio Observer; Carl Zeiss, Jena, Germany) and analyzed using the "Analyzing Particles" tool in ImageJ. The loading efficiency of the bubble carriers was calculated by subtracting the fluorescence intensity of free FITC-insulin from the total fluorescence intensity [9]. The fluorescence intensity of FITC-insulin was determined using a microplate spectrophotometer (SpectraMax M5, Molecular Devices, CA, USA).

The formed bubble carriers were also characterized by small-angle X-ray scattering (SAXS) using the BL23A1 beamline at the National Synchrotron Radiation Research Center (NSRRC), Hsinchu, Taiwan. The procedure and conditions that were used in the SAXS experiment have been described in our previous study [1]. The SAXS experiment yielded the scattering intensity profile as a plot of the scattering intensity $I(q)$ as a function of scattering vector, $q = (4\pi/\lambda)\sin(\theta/2)$ with a scattering angle of θ.

2.4. In vitro enzymatic degradation

Insulin is very sensitive to trypsin, which is a proteolytic enzyme in the small intestine [10]. The ability of SDS to protect insulin against proteolytic attack by trypsin was investigated *in vitro*. Briefly, free-form insulin and SDS at various doses were diluted in Tris buffer that contained