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Intestinal patches with an immobilized solid-in-oil formulation for oral protein delivery

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ABSTRACT

Oral administration of biomolecular drugs such as peptides, proteins, and DNA is an attractive delivery method because of the safety and convenience of delivery in contrast to injection administration. However, oral delivery of biomolecules has several potential barriers such as enzymatic degradation in the gastrointestinal tract and low permeability across an intestinal membrane. In this study, we proposed an intestinal patch system that included surfactant-coated insulin for oral delivery. The intestinal patches, which have mucoadhesive and drug-impermeable layers, induced sustained unidirectional insulin release toward intestinal mucosa and inhibition of insulin leakage from the patches. Moreover, the surfactant-coated insulin, which has high compatibility with cell membranes, enhanced insulin transport across the intestinal membrane. This study demonstrates that the intestinal patches might improve protein permeability in the intestinal mucosa, thereby offering an innovative therapeutic strategy.

1. Introduction

In pharmaceutical and biotechnology industries, peptides, proteins, and biopolymers are constantly being developed for the treatment of a variety of diseases [1,2]. The administration of these drugs is generally achieved by an injection that delivers them efficiently into the circulating bloodstream. However, the injection of these drugs has disadvantages such as low patient compliance, possibility of infection, and pain during repeated injections. In contrast, an oral delivery of the drugs is more natural and desirable to achieve safe and convenient insulin delivery that leads to high patient compliance [3,4]. However, oral delivery of these biomolecules has several barriers such as enzymatic degradation in the gastrointestinal (GI) tract and low permeability across the intestinal mucosa. To overcome these barriers, chemical modification of the biomolecules [5], and administration of protease inhibitors [6,7], absorption enhancers [8] and cell-permeable peptide [9] have been investigated. In contrast, drug carriers such as liposomes [10], emulsions [11], micro/nanoparticles [12–14], and hydrogels [15] have also been employed to remove enzymatic degradation and improve the permeability through the small intestinal membrane. Interestingly, it has been shown that the micro/nanoparticles formed from some polyacrylates or polysaccharides adhere to intestinal mucosa and therefore prolong their migration time and extend drug release [16–18]. Furthermore, these polymer particles are able to accelerate paracellular transport by opening the tight junction of the mucosal epithelium and inhibit the calcium-dependent hydrolytic enzyme because they interact strongly with calcium ion [19–21]. Although this approach improves the oral bioavailability of the biomolecular drugs, they have limitations as particle carriers, such as the disappearance of most drugs on release into the intestinal lumen and degradation of biomolecules exposed to GI fluid near the particle surface.

Recently, as a new concept, the GI patch system has been employed to improve the oral bioavailability of large molecules [22–28]. Patches are commonly used for the transdermal delivery of low molecule drugs [29,30]. In addition, buccal patches have also been used to administer drugs through the oral mucosa [31,32]. These patches are constructed from multiple layers that have adhesive, rate-controlling, and protective properties. GI patches mimic the aforementioned patches. Moreover, GI patches are formed from multiple functional layers, including mucoadhesive and drug-impermeable layers. The advantages of the GI patch system are (i) prolonged residence time in a single position in the small intestine, (ii) sustained unidirectional drug release towards the intestinal mucosa, and (iii) protection from enzymatic degradation by the impermeable layer.

In this work, we developed a new GI patch system, which can enhance transcellular transport of the biomolecular drugs. In a

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previous study, we developed a new type of orally administered protein formulation, solid-in-oil-in-water (S/O/W) emulsion, in which surfactant-coated proteins are dispersed into the oil phase [33,34]. Since the surfactant-coated proteins form a lipophilic nanoparticle, they should have high compatibility with the cell membrane. The S/O/W emulsion actually improved the absorption of the protein drug, insulin, in the GI tract and produced a hypoglycemic effect that lasted for half a day. Moreover, the solid-in-oil (S/O) formulation has been employed as oral delivery carriers for a human growth hormone and non-steroidal anti-inflammatory drug [35,36].

These formulations indicated the increase of drug absorption in the small intestine; however, they lacked the retention in the intestinal mucosa, similar to other carriers. In this study, we explored an intestinal-mucoadhesive patch including the S/O formulation for efficient protein delivery. The new patch consisted of two layers: a drug-impermeable layer and a mucoadhesive layer. The mucoadhesive layer is a porous matrix formed by a mucoadhesive polymer in which the S/O suspension was impregnated. Herein, the intestinal-mucoadhesive patches containing insulin as a model protein drug was prepared and the drug transport across the intestinal membrane was examined.

2. Materials and methods

2.1. Materials

Chitosan, hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC), carboxymethyl cellulose sodium (CMC), ethyl cellulose (EC), soybean oil, and hexane were purchased from Wako Pure Chemicals Industries Ltd. (Japan). Carbopol was obtained from Mitsuya Kako Co. (Japan). Insulin (from bovine pancreas) was obtained from Sigma–Aldrich (Japan). Sucrose erucic ester (ER-290), sucrose oleic ester (O-170), sucrose lauric ester (L-195), decaglycerin erucic ester (ER-60D), and decaglycerin oleic ester (O-50D) were provided by Mitsubishi-Kagaku Foods Co. (Japan). Fluorescein-isothiocyanate (FITC) was obtained from Dojin Chemical Co. (Japan). All chemicals were of reagent grade.

2.2. Preparation of intestinal patches

S/O suspension was prepared following the same procedure as previously reported [33]. Briefly, an aqueous solution (5 ml) of FITC-labeled insulin (FITC-Ins, 1 mg ml^{-1}) and a hexane solution (10 ml) containing hydrophobic surfactant (ER-290, O-170, L-195, ER-60D, or ER-50D) were mixed with a homogenizer at 20,000 rpm for 2 min. The resultant water-in-oil (W/O) emulsions were lyophilized for 1 day. As the surfactant-coated FITC-Ins prepared was readily dispersed into soybean oil (10 ml) and the S/O suspension in which the coated FITC-Ins was dissolved entirely into the oil phase was prepared.

Mucoadhesive patches were prepared by a simple evaporation/lyophilization technique. The preparation flowchart is shown in Fig. 1. The drug-impermeable layer was prepared using an acetone solution with 5% w/w EC. The EC solution (40 μl per mold) was poured into circular molds (diameter: 6.0 mm; depth: 1.0 mm) of silicone and the solvent was evaporated at room temperature. Then, 3% w/w aqueous mucoadhesive polymer (chitosan, HEC, HPC, CMC, or carbopol) solution (100 μl per mold) was cast onto a thin EC layer and lyophilized for 1 day. Finally, the S/O suspension (20 μl per mold) was added into the mucoadhesive polymer layer.

2.3. Characterization of the patches

The surface and cross-sectional structure of the patches were investigated by scanning electron microscopy (SEM; JEOL JSM-6060, Japan). The impregnation property of the S/O suspension into the mucoadhesive layer of the patches was examined by dropping the suspension on the surface of the layer. The mucoadhesive property of the patches was investigated using the small intestine of rats (Male Wistar, 7 weeks). All animal procedures were performed using institutionally approved protocols of Oita University. Rats were killed and the small intestines were removed. The small intestines were rinsed with phosphate buffered saline (PBS) and cut into small pieces ($\sim 3 \times 3$ cm). Intestines were fixed in a beaker filled with PBS (pH 6.8), and five patches were placed on it. The PBS solution was stirred at 120 rpm and the time to detachment for each patch was investigated.

2.4. Membrane permeation experiment

The insulin permeation study was performed at 37 °C using horizontal diffusion cells. The small intestines of rat were removed as mentioned in Section 2.3. Small intestines were divided by tissue type, duodenum, jejunum, and ileum. The cells were filled with 5 ml PBS (pH 6.8) after a small intestine membrane was fixed between them. A patch was then placed on the surface of the intestine membrane. Samples in each cell were withdrawn at predetermined times. The fluorescence from FITC-Ins was detected using a fluorometer (excitation, 490 nm and emission, 520 nm). Leakage into the intestinal lumen and absorption into the body were estimated by measuring insulin release to the mucosal side and transport across the intestinal membrane, respectively.

3. Results and discussion

3.1. Formulation of intestinal mucoadhesive patch**es**

Surfactant-coated insulin was prepared by lyophilizing the W/O emulsion containing insulin. Since lyophilization permits drying under conditions that maintain the structure of the protein and position of surfactants, the lipophilic aggregations of insulin coated with the lipophilic surfactant was recovered easily. We developed an intestinal mucoadhesive patch containing surfactant-coated insulin for high compatibility with the cell membrane to improve insulin transport across the intestinal mucosa.

Intestinal mucoadhesive patches prepared in this study were disks of ~6 mm radius and 1 mm thickness that were multilayer constructions of porous mucoadhesive layers inside an impermeable layer that was fabricated into a dish-like structure. Fig. 2 shows the exterior surface of the mucoadhesive layer and a cross-sectional image of the mucoadhesive patch. Five polymer species (chitosan, HEC, HPC, CMC, and carbopol) were used as the mucoadhesive polymer. The mucoadhesive layer formed with chitosan had a porous structure, which was expected to include many substrates inside (Fig. 2b). Mucoadhesive layers made with other polymers also had structures similar to that of chitosan (data not shown). In contrast, the impermeable layer, formed with EC, was a smooth thin film without any cavities.

Next, water permanence, oil absorbency, and mucoadhesive properties were examined for patches formed with each of the mucoadhesive polymers (Table 1). The permanence of the patches in water is an important factor in maintaining their drug-carrying ability. Moreover, the mucoadhesive layer requires mucosal adhesive properties and immobilization of the S/O suspension to enhance the absorption of protein drugs by the epithelium (Fig. 2d).