International Journal of Pharmaceutics 515 (2016) 476–489

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Enhanced oral delivery of alendronate by sucrose fatty acids esters in rats and their absorption-enhancing mechanisms

Tammam Alama, Hiroko Katayama, Sachiko Hirai, Shizuka Ono, Ayumi Kajiyama, Kosuke Kusamori, Hidemasa Katsumi, Toshiyasu Sakane, Akira Yamamoto*

Department of Biopharmaceutics, Kyoto Pharmaceutical University, Misasagi, Yamashina-Ku, Kyoto 607-8414, Japan

ARTICLE INFO

Article history:
Received 23 June 2016
Received in revised form 17 October 2016
Accepted 21 October 2016
Available online 25 October 2016

Keywords:
Intestinal absorption
Absorption enhancer
Sucrose fatty acid ester
Poorly absorbed drug
Alendronate
Bisphosphonate

ABSTRACT

Oral delivery is the most fascinating route for interminable drug remedy. However, the intestinal absorption of alendronate (ALN), a bisphosphonate drug after oral administration is very poor. Absorption enhancers, which help to achieve the efficiency-safety balance, are considered one of the most promising agents for the improvement the intestinal absorption of drugs. In the current study, we focused on using sucrose fatty acid esters (SEs) as promising absorption enhancers to enhance the intestinal absorption of alendronate using an *in situ* closed-loop method in rats. The intestinal absorption of alendronate was significantly enhanced in the presence of SEs, especially L-1695. In addition, no considerable increase was observed in the activity of lactate dehydrogenase (LDH) or in protein release from the intestinal epithelium in the presence of sugar esters at concentrations equivalent to or lower than 1.0% (w/v), suggesting that these compounds are safe. Furthermore, mechanistic studies revealed increased membrane fluidity and loosening of the tight junctions (TJs) might be the underlying mechanism by which SEs improve the intestinal intake of alendronate, *via* transcellular and paracellular routes, respectively. These findings suggest that SEs are effective absorption enhancers for improving the intestinal absorption of alendronate, without causing serious damage to the enteric epithelium.

1. Introduction

Bisphosphonates, carbon-substituted pyrophosphate analogs, are useful in reducing the hazard of future fractures in osteoporosis patients who have already sustained a fracture due to the disease (Wells et al., 2008). Alendronate (4-amino-1-hydroxybutylidene-1, 1-bisphosphonate trihydrate) (Fig. 1A), which belongs to a nitrogen-containing bisphosphonates, is the most used bisphosphonate for the prevention and treatment of osteoporosis, particularly in women after menopause (Kanis et al., 1995; Karpf et al., 1997), corticosteroid-induced osteoporosis (Saag et al., 1998), and the treatment of Paget's disease (Reid et al., 1996). However, the oral absorption of alendronate in animals is limited under fasting conditions and negligible in the existence of food. Therefore, the oral bioavailability (BA) of alendronate is approximately 0.9–1.8% (Porras et al., 1999), because alendronate is highly polar and charged at physiological pH (*i.e.*, belongs to BCSIII). In addition, it was reported that alendronate caused severe membrane damage to the upper gastrointestinal tract after its oral administration. Consequently, if intestinal absorption of alendronate can be improved by some method, we can increase the efficacy of alendronate. Furthermore, we can reduce the side effect of alendronate in the gastrointestinal tract, because the dose of alendronate can be reduced by some absorption improving method (Porras et al., 1999).

On the other hand, absorption enhancers are considered a promising method in enhancing absorption, since they are easy to formulate with active pharmaceutical ingredients and lead to decreased cost of final products. Indeed, absorption enhancers have proved their efficacy in enhancing the absorption of drugs (Uchiyama et al., 1999; Yamamoto et al., 1996). Sucrose fatty acid esters are non-ionic surfactants that possess a sugar substituent as hydrophilic head and fatty acids as lipophilic groups and are called sugar esters (SEs). Fig. 1B shows the common structure of SEs. Since sucrose has eight hydroxyl groups, SEs ranging from sucrose mono- to octa- fatty acid esters can be synthesized. Stearic, palmitic, myristic and lauric acid can be used to obtain SEs. The type of fatty acid and the grade of esterification define the hydrophilic lipophilic balance (HLB) value and the melting point of these substances. SEs are tasteless, odorless and biodegradable components with HLB values from 1 to 16 and are usually used in pharmaceutical industries as solubilizing agents, lubricants and

* Corresponding author.
E-mail address: yamamoto@mb.kyoto-phu.ac.jp (A. Yamamoto).

http://dx.doi.org/10.1016/j.ijpharm.2016.10.046
0378-5173/

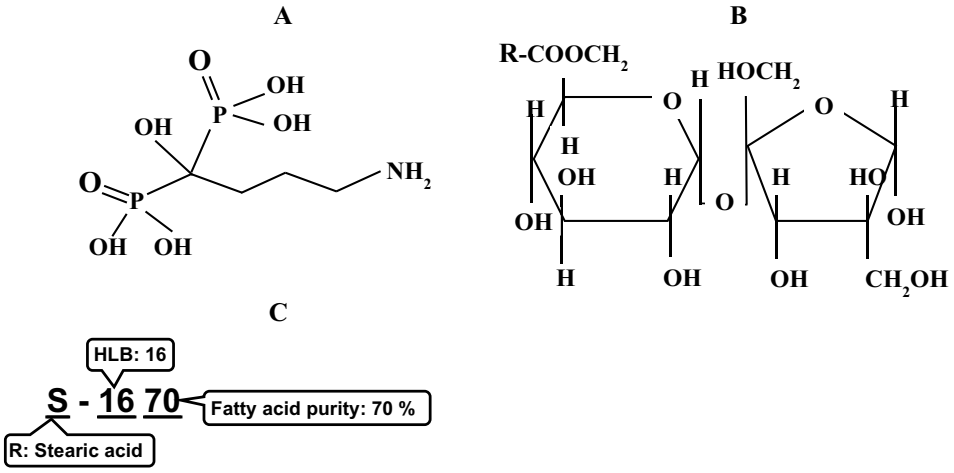

Fig. 1. (A) The chemical structure of alendronate. (B) The general chemical structure of sugar esters. The hydrophilic moiety of these agents is sucrose linked to the hydrophobic moiety, a fatty acid. (C) An example of sugar ester coding.

emulsifiers (Csóka et al., 2007). They are therefore expected to have applications in drug delivery systems as promising absorption enhancers with low toxicity to the intestinal membrane, biocompatibility, and biodegradability properties (Youan et al., 2003).

In this research, we inspected the impacts of SEs on the intestinal absorption of a poorly absorbed drug, alendronate. Additionally, intestinal membrane damage was estimated by measuring the activity of lactate dehydrogenase (LDH) released from intestinal membranes in rats, along with morphological observations. Furthermore, we performed mechanistic studies in order to identify the possible mechanisms underlying the enhancing effects of SEs on the intestinal absorption of alendronate. This is the first study that discusses the effects of SEs on the improvement of the intestinal absorption of alendronate.

2. Materials and methods

2.1. Materials

Male Wistar rats, weighing 250–300 g, were purchased from SLC, Inc. (Hamamatsu, Shizuoka, Japan). SEs (L-1695, M-1695, P-1670, S-1670, and O-1570) were kindly provided by Mitsubishi-Kagaku Foods Corporation (Tokyo, Japan). Alendronate was purchased from Teikoku Pharma USA, Inc. (San Jose, California, USA). LDH—Cytotoxicity Test Wako and tma-DPH (1-(4-(trimethylamino)phenyl)-6-phenylhexa-1,3,5-hexatriene-p-toluenesulfonate) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). DPH (1,6-diphenyl-1,3,5-hexatriene) and Hank's balanced salt solution (HBSS) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Dansyl chloride (DNS-Cl) was purchased from Santa Cruz Biotechnology, Inc. (Texas, USA). Anti-claudin-1 and anti-β-actin (rabbit monoclonal antibodies) and goat anti-rabbit IgG HRP-linked antibodies were purchased from Cell Signaling Technology® (Danvers, MA, USA). Anti-claudin-4 (mouse monoclonal antibodies) and rabbit anti-mouse IgG HRP-linked antibodies were purchased from Invitrogen™ (Carlsbad, CA, USA). Chemi-Lumi One Ultra kit, Dulbecco's modified Eagle medium (DMEM) with 4500 mg/L glucose, nonessential amino acids (MEM–NEAA), antibiotic-antimycotic mixture stock (10,000 U/mL penicillin, 10,000 μg/mL streptomycin, and 25 μg/mL amphotericin B in 0.85% sodium chloride), and 0.25% trypsin–1 mM EDTA solutions were purchased from Nacalai Tesuque (Kyoto, Japan). Human colon adenocarcinoma-derived Caco-2 cell line was purchased from Dainippon Sumitomo Pharma Co., Ltd. (Osaka, Japan). Fetal bovine serum (FBS) was purchased from Gibco® Life Technologies (Grand Island, USA). The bicinchoninic acid (BCA) protein assay kit was obtained from Pierce Biotechnology Inc. (Rockford, USA). All other reagents used in the experiments were of analytical grade.

2.2. Animal studies

Intestinal absorption of alendronate was examined by an *in situ* closed-loop method, as reported previously (Yamamoto et al., 1994). The experiments were carried out in accordance with the guidelines of the Animal Ethics Committee at Kyoto Pharmaceutical University. The rats were starved overnight for ∼16 h predosing, but water was freely available. After inducing anesthesia with sodium pentobarbital administered intraperitoneally at a dose of 32 mg/kg body weight, the rats were placed under a heating lamp to maintain body temperature at around 37 °C, and the intestines were exposed using a midline-abdominal incision. After the bile duct was ligated, the intestines were washed with phosphate buffered saline (PBS, pH 7.4) and the remaining buffer solution was expelled with air. Intestinal cannulation was performed at both ends using polyethylene tubing, and the distal parts of the small or large intestines were clipped by forceps. The dosing solutions (3 mL for the small intestine and 1 mL for the large intestine), with or without absorption enhancers, kept at 37 °C, were directly introduced into the lumen of the intestinal loop through a cannulated opening in the proximal part of the small or large intestinal loop, which was then closed by clipping with another forceps. The jugular vein was exposed and ∼0.3 mL of blood samples were collected *via* a direct puncture into heparinized syringes at predetermined time intervals up to 240 min. The samples were immediately centrifuged at 12,000 rpm (15,000 × *g*) for a period of 5 min to obtain the plasma fraction, which was stored on ice for further analysis. The concentrations of alendronate in these plasma samples were determined by reverse-phase high-performance liquid chromatography (RP-HPLC), as reported previously (Wong et al., 2004; Nakaya et al., 2016). The RP-HPLC system consisted of a (LC-10AS; Shimadzu, Kyoto, Japan) pump equipped with a fluorescence (RF-10AXL; Shimadzu, Kyoto, Japan) detector. The excitation and emission wavelength were 395 nm and 480 nm, respectively. The mobile phase was composed of an aqueous solution of 1 mM disodium EDTA and methanol at a ratio of 97:3 (v/v) and adjusted to pH 6.5. The mobile phase was injected in a separation column (4.6 mm × 150 mm inside diameter) packed with 5C18-PAQ (Nacalai Tesque, Kyoto, Japan) at a flow rate of 1 mL/