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Enhanced Oral Delivery of Bisphosphonate by Novel Absorption Enhancers: Improvement of Intestinal Absorption of Alendronate by *N*-Acyl Amino Acids and *N*-Acyl Taurates and Their Absorption-Enhancing Mechanisms

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ABSTRACT

Bisphosphonates (BPs) are carbon-substituted pyrophosphate analogs that exhibit a high affinity to hydroxyapatite and specifically inhibit bone resorption. Alendronate sodium (sodium 4-amino-1-hydroxybutylidene-1,1-bisphosphonate trihydrate) is a typical BP compound in clinical use. BPs have very low bioavailability, typically <1% after their oral administration, and their intestinal absorption is further reduced by co-administered drugs or food. In this study, we examined the effects of *N*-acyl amino acids and *N*-acyl taurates on the small intestinal absorption of alendronate. All *N*-acyl amino acids and *N*-acyl taurates increased the small intestinal absorption of alendronate, especially 1% (wt/vol) sodium palmitoyl sarcosinate (PN), which elicited a 14-fold increase. In addition, the absorption-enhancing effects of these enhancers were reversible and they may not cause continuous and irreversible membrane toxicity in the rat small intestine. Furthermore, we examined the absorption-promoting mechanisms of PN and found that it increased the membrane fluidity of the lipid bilayers. In addition, it was found that PN may open the tight junctions by reducing the expression level of claudin-4, which is a major tight junction protein. These findings indicate that these enhancers are useful for promoting the intestinal absorption of alendronate.

Introduction

Osteoporosis is defined as a progressive, systematic skeletal disorder that is characterized by the loss of bone tissue, disruption of the bone architecture, and bone fragility that leads to an increased risk of fractures.[1] The removal and formation of bone occurs in a continuous remodeling cycle, which is a carefully regulated process involving many local and systemic factors.[2]

Bisphosphonates (BPs) are carbon-substituted pyrophosphate analogs that exhibit a high affinity to hydroxyapatite and specifically inhibit bone resorption.[3] Therefore, BPs are widely used as the first-choice drugs for the treatment of hypercalcemia and 3 forms of osteoporosis.[4,5] BPs are often administered via injection or oral administration in clinical application. Among various BPs, alendronate sodium (sodium 4-amino-1-hydroxybutylidene-1,1-bisphosphonate trihydrate) is a typical BP compound in clinical use.[6,7] However, the intestinal absorption of alendronate after its oral administration is very low. Alendronate has very low bioavailability, typically <1% after oral administration, and intestinal absorption of alendronate is further reduced by co-administered drugs or food.[8] In addition, the oral administration of alendronate has been associated with severe esophageal damage and stricture.[9-11] Therefore, it is recommended that alendronate is administered at least 30 min before taking drugs or food.

In general, oral administration is the most popular drug administration route and is used for the treatment of a variety of diseases. However, it is difficult to obtain sufficient intestinal absorption of hydrophilic or high-molecular-weight drugs after their oral administration. Therefore, absorption enhancers, including chelating agents,[12] bile acids,[13] surfactants,[14] and fatty acids,[15] have been used to improve the absorption of poorly absorbable drugs. Many kinds of surfactants have been used to increase the intestinal absorption of poorly absorbable drugs. However, these enhancers

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usually cause serious membrane toxicity to the intestinal epithelium. Accordingly, only sodium caprate has been clinically applied as an absorption-promoting agent. In practical application, safe and highly effective absorption enhancers are required to improve the intestinal absorption of poorly absorbable drugs.

In this study, we focused on various *N*-acyl amino acids and *N*-acyl taurates as novel types of absorption enhancers, and examined whether these compounds increased the intestinal absorption of alendronate. *N*-acyl amino acids and *N*-acyl taurates are anionic surfactants. *N*-acyl amino acids are composed of *N*-methyl glycine and fatty acids derived from edible fats. *N*-acyl taurates have similar structures to taurocholate. The chemical structures of these compounds are shown in Figures 1a and 1b. Few studies have examined the absorption-enhancing effects of *N*-acyl amino acids and *N*-acyl taurates, and the improvement in intestinal absorption of alendronate by these compounds. In this study, we examined the effects of *N*-acyl amino acids and *N*-acyl taurates on the intestinal absorption of alendronate, a typical BP, and elucidated the absorption-enhancing mechanisms of these enhancers.

Materials and Methods

Materials

Alendronate sodium trihydrate was obtained from Tokyo Chemical Industry (Tokyo, Japan). *N*-acyl amino acids and *N*-acyl taurates were obtained from Nikko Chemical Co. Ltd. (Osaka, Japan). An LDH (lactate dehydrogenase) cytotoxicity test was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and 5(6)-carboxyfluorescein (CF) was obtained from Eastman Kodak Company (Rochester, NY). For cell culture, Dulbecco's modified Eagle's medium, fetal bovine serum, and modified Eagle's medium non-essential amino acid solution were purchased from Life Technologies Corporation (Carlsbad, CA). Trypsin-EDTA (ethylenediaminetetraacetic acid), 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES), and antibiotic-antimycotic mixed stock solution (10,000 U/mL penicillin, 10 mg/mL streptomycin, 25 mg/mL amphotericin B, 0.85% saline) were prepared by Dojindo Laboratories (Kumamoto, Japan). Hank's balanced salt (HBS) and 1,6-diphenyl-1,3,5-hexatriene (DPH) were obtained from Sigma-Aldrich Chemical Co. Ltd (St. Louis, MO). 1-(4-Trimethyl-ammonium phenyl)-6-phenyl-1,3,5-hexatriene-ρ-toluenesulfonate (tma-DPH) was supplied by Santa Cruz Biotechnology, Inc. (Dallas, TX).

Preparation of the Drug Solution

For the *in situ* closed loop absorption studies, alendronate sodium was dissolved in phosphate-buffered saline (PBS, pH 7.4). Sodium palmitoyl sarcosinate (PN), sodium myristoyl sarcosinate (MN), sodium cocoyl sarcosinate, sodium lauroyl sarcosinate, or sodium oleoyl sarcosinate was added to the dosing solutions at a concentration of 1.0% (wt/vol). Additionally, sodium methyl palmitoyl taurate, sodium methyl myristoyl taurate, sodium methyl cocoyl taurate (CMT), sodium methyl lauroyl taurate, or sodium methyl stearoyl taurate was added to the dosing solutions at a concentration of 1.0% (wt/vol). For the *in vitro* studies, Hank's balanced salt solution (HBSS, pH 6.0) was prepared to examine the absorption-enhancing mechanisms of these surfactants. PN was added to the dosing solutions at concentrations of 0.01%-0.1% (wt/vol).

Intestinal Absorption of Drugs Using an In Situ *Closed Loop Method*

The intestinal absorption of alendronate sodium was performed by an *in situ* closed loop method in rats, as reported previously.[16] The experiments were carried out in accordance with the guidelines of the Animal Ethics Committee at Kyoto Pharmaceutical University. The rats were fasted for 16 h but water was freely available. Prior to the experiment, the animals were anesthetized with sodium pentobarbital (32 mg/kg of body weight, intraperitoneal administration). The experiment was performed on rats lying under a heating lamp to maintain constant body temperature. The intestine was exposed via a midline abdominal incision. After ligating the bile duct, intestinal contents were removed by slow infusion of PBS (pH 7.4) and air. Alendronate solution was administrated at a dose of 10 mg/kg. The small intestine was cannulated at both end, and the distal part of the small intestine was clipped by a forcep. The drug solutions, with or without the absorption enhancers, that were kept at 37°C were introduced. Blood samples (0.35 mL) were taken at 8 time points (0, 15, 30, 60, 90, 120, 180, and 240 min). The samples were immediately centrifuged at 12,000 rpm (9660 × *g*) for 5 min to obtain the plasma fraction, which was then frozen until further testing. The concentrations of alendronate sodium in these plasma samples were measured by HPLC.

In the pretreatment experiment, PN (1.0% [wt/vol]) or CMT (1.0% [wt/vol]) was administered into the small intestinal loop. After the pretreatment for 60 min, the PN or CMT solution was removed by washing the small intestine with PBS (pH 7.4). After washing, the alendronate sodium solution was administered into the small intestinal loop and then the plasma samples were collected to determine the concentrations of alendronate sodium.

The plasma peak concentrations (C_{max}) and the time to reach the plasma peak concentrations (T_{max}) were determined from the plasma concentration-time profiles. The area under the curve (AUC) was calculated using the trapezoidal method from time zero to the final sampling time. The absorption enhancement ratios

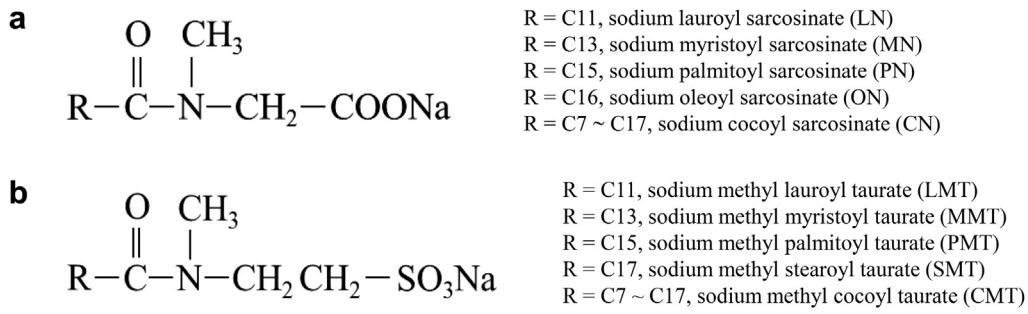

Figure 1. The chemical structure of *N*-acyl amino acids (a) and *N*-acyl taurates (b).