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Enhanced intestinal absorption of salmon calcitonin (sCT) from proliposomes containing bile salts

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Abstract

Purpose: The feasibility of using proliposomes containing salmon calcitonin (sCT) and absorption enhancing agents, as an oral delivery system, to improve the intestinal absorption of sCT was explored using rats and Caco-2 cell systems.
Methods: Seventeen surfactants were examined for their effects with reference to accelerating the permeability of sCT (300 μg/ml) across Caco-2 cell monolayers, and damage to the intestinal epithelial cells, as measured by the change in transepithelial electrical resistance (TEER) across the cell monolayer. Proliposomes containing sCT (0.75%, w/w) and sodium taurodeoxycholate (NaTDC, 2.5%, w/w) (TDC proliposomes) were prepared according to the standard method using sorbitol and phosphatidylcholine as core and wall-forming materials, respectively, administered intra-duodenally to rats, and plasma concentrations of sCT were subsequently determined by LC–MS.
Results: Among the surfactants examined, some bile salts including NaTDC appeared to be the most advantageous when estimated based on the balance between the permeation enhancement (e.g., a 10.8-fold increase in the permeability of sCT for 0.1% NaTDC) and damage to the cells (e.g., a 3.55-fold decrease in the TEER value for 0.1% NaTDC). The administration of TDC proliposomes resulted in a 7.1-fold increase in the bioavailability (i.e., 0.49%) of sCT, when administered duodenally to rats. The size of the reconstituted liposomes in water was significantly smaller (e.g., 23.2 nm, number weighted diameter), and the entrapment efficiency (EE) of sCT in the reconstituted liposomes was 2.8-fold larger (54.9%), for TDC proliposomes, compared to proliposomes prepared without NaTDC (sCT proliposomes).
Conclusion: A 7.1-fold increase in the bioavailability of sCT could be achieved from the TDC proliposomes. In addition to the intrinsic activity of the bile salt to fluidize the membrane, the simultaneous delivery of sCT and NaTDC to the site of absorption in the intestine via proliposomes and the subsequent formation of lipophilic ion-pair complexes between sCT and NaTDC at the site might have been contributing factors in this outstanding absorption enhancement.

Keywords: Salmon calcitonin; Caco-2 cell monolayer; Bile salts; Ion-pair complex; Proliposomes; Bioavailability

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1. Introduction

Non-parenteral delivery is by far the most convenient route to drug delivery, especially when repeated or routine administration is necessary [1]. Salmon calcitonin (sCT), which plays a crucial role in both calcium homeostasis and the treatment of bone disease such as osteoporosis [2,3], is such a drug for which appropriate oral dosage forms need to be developed. Like many other proteins or peptides, the oral bioavailability of sCT is very low due to enzymatic degradation in the gastrointestinal (GI) tract [4,5] and poor permeation across intestinal epithelial cells [6]. As a result, less than 0.1% of the bioavailability of sCT was obtained following intra-duodenal, -colonic and -ileac administration in rats and dogs [7,8].

In a previous study [9], we reported a 3.6-fold increase in the apparent permeability (P_{app}) of sCT across Caco-2 cell monolayers (i.e., 6.14×10^{-7} cm/s) when sCT was applied in the form of proliposomes, a free-flowing particulate dosage form that immediately forms a liposomal dispersion in water [9,10]. However, the P_{app} for the proliposomes is still quite low compared to the value that is generally known to be necessary for favorable intestinal absorption (i.e., 1×10^{-6} cm/s) [11]. In cases, the addition of appropriate absorption enhancers has frequently been attempted. However, in most cases, absorption enhancers also damage intestinal epithelial cells, limiting their use at high levels. Thus, the preferential delivery of absorption enhancers to the site of absorption rather than to the intestinal bulk fluid would be highly desirable [12]. Liposomes have often been considered to be potential candidates for achieving this objective [13]. In the present study, sCT and an appropriate absorption enhancer were formulated in the form of proliposomes, a precursor dosage form of liposomes, and examined for the feasibility as a dosage form that can increase intestinal absorption of sCT. Caco-2 cell monolayers were employed in the assessment of the permeability of sCT and the toxicity of absorption enhancers or dosage forms to the intestinal membrane, and the in vivo duodenal absorption of sCT in rats was measured, in an attempt to estimate bioavailability.

2. Materials and methods

2.1. Materials

Synthetic sCT (3431.9 Da, purity >99%) was purchased from A&PEP Inc. (Yeongi, Korea) and stored in a deep freezer (−70 °C) before use. L-α-Phosphatidylcholine (PC, powder type from frozen egg yolk, >99%), sorbitol (>98%), chloroform (anhydrous, >99%), trifluoroacetic acid (TFA), Dulbecco's modified Eagle's medium, non-essential amino acid solution, penicillin–streptomycin, Hank's balanced salts solution (HBSS) and *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), sodium glycocholate (NaGC), sodium taurocholate (NaTC), sodium dehydrocholate (NaDHC), sodium cholate (NaC), ursodeoxycholate (UDC), sodium tauroursodeoxycholate (NaTUDC), sodium taurochenodeoxycholate (NaTCDC), sodium deoxycholate (NaDC), sodium glycodeoxycholate (NaGDC), sodium taurodeoxycholate (NaTDC), sodium lauryl sulfate (SLS), lauroyl carnitine chloride (LCC), benzalkonium chloride (Bza), and benzetonium chloride (Bze) (all from Sigma Chemical Co., St. Louis, MO) were used as purchased. [^{14}C]Mannitol (50 mCi/mmol, New England Nuclear, Boston, MA), Triton X-100, Tween 80 (Shinyo Pure Chemicals Co., Osaka, Japan), Cremophor EL (BASF, Ludwigshafen, Germany), Fetal bovine serum (Hyclone Lab., Logan, UT) and Trypsin–EDTA (Life Technologies, Inc., Gaithersburg, MD) were used also as purchased. Methanol and acetonitrile from Fisher Scientific (Fair Lawn, NJ) were of HPLC grade. HPLC grade water was purified by the use of a Milli-Q (Millipore, Molsheim, France) system equipped with cellulose nitrate membrane filters (0.2 μm, Whatman, Maidstone, UK). All other reagents were of analytical grade or better.

2.2. Measurement of the transepithelial transport of sCT across Caco-2 cell monolayers

Caco-2 cells, a human colon adenocarcinoma cell line (American Type Culture Collection, Rockville, MD), were gown as monolayers in Dulbecco's Modified Eagle's medium containing 10% fetal bovine serum, 1% non-essential amino acid solution, 100 units/ml penicillin and 0.1 mg/ml streptomycin at 37 °C in an atmosphere of 5% CO_2 and 90% relative