Oral delivery of macromolecular heparin conjugate via functional transformation of bile acid transporter

Youngro Byun¹,² and Taslim Al-Hilal¹

¹Department of Molecular Medicine and Biopharmaceutical Science, Seoul National University, Seoul 153-741, Republic of Korea
²College of Pharmacy, Seoul National University, Seoul 151-742, Republic of Korea
E-mail address: yrbyun@snu.ac.kr

ABSTRACT SUMMARY
We describe the development of apical sodium-dependent bile acid transporter (ASBT)-targeted high-affinity oligomeric bile acid substrates that mediate the transmembrane transport of low molecular weight heparin (LMWH). The conjugated tetraDOCA interacted with several hydrophobic grooves in the substrate-binding pocket of ASBT. Orally absorbed LHe-tetraD successfully prevented thrombosis in a rat model of deep vein thrombosis.

INTRODUCTION
Apical sodium-dependent bile acid transporter (ASBT) is a membrane transporter, which moves endogenous small molecules like bile acids in either the conjugated or unconjugated form from the apical surface of enterocyte and cholangiocytes to the cytoplasm. The transport of bile acids involves the coordination of several transmembrane domains (TD) of ASBT, which creates a hydrophobic substrate-binding pocket. The binding of substrates in this extracellular pocket, in concert with sodium binding, drives the conformational change of ASBT, which in-turn, opens the cytoplasmic channel for ASBT to traverse the bile acids. Bilayer acids are then bound to ileal bile acid binding protein (IBABP), shuttled across the cytoplasm to the basolateral membrane and finally exported into the blood vessels by basolateral transporters, such as organic solute transporter (OST-α/β). This characteristic of ASBT is what makes the transport of bile acid conjugates larger than 800 Da most impedimental. Unlike the progress achieved in oral delivery of small molecules, the field of active delivery of macromolecules, particularly via bile acid transporters, largely remains a new frontier that needs to be explored. Here, we show the functional transformation of ASBT in response to high-affinity macromolecular substrates that pursue a new ‘receptor-like’ function and allow the transport of even high molecular weight bile acid conjugates.

EXPERIMENTAL METHODS
We designed new biological substrates of bile acid oligomers, preferably with deoxycholic acid (DOCA), and conjugated to a model macromolecule, low molecular weight heparin. The simultaneous binding of oligomeric structure of DOCA (oligoDOCA) to several TDs of ASBT was checked by computer simulation study. The binding affinity between human ASBT protein to oligoDOCA s and their macromolecular conjugates were analyzed by surface plasmon resonance (SPR). Caco-2, ASBT transfected MDCK (MDCK-ASBT), and SK-BR-3 cell lines were used for transport and mechanism study. Interactions among macromolecular substrate, ASBT, and IBABP were analyzed by Co-immunoprecipitation (Co-IP), proximity ligation assay (PLA), and confocal microscopy. ‘Receptor-like’ internalization of ASBT was observed by transmission electron microscope after immunolabeling with gold-labeled antibody against ASBT. Oral absorption of the macromolecular ASBT substrates was studied in preclinical models, such as in SD-rats in liquid formulations and in cynomolgus monkeys in capsule formulations.

RESULTS AND DISCUSSION
The affinity of tetrameric DOCA conjugated macromolecular substrate to that of ASBT was also very high, $K_D$: 0.072 μM. We observed that ASBT was spatially shifted from membrane to cytoplasmic fraction in both Caco-2 and MDCK-ASBT cells but not to the nuclear fraction when treated with macromolecular substrates. Using Co-IP and in situ PLA, we confirmed stable interactions both at membrane and cytoplasmic fractions. The interaction between ASBT and high-affinity macromolecular substrate leaves the membrane thermodynamically unstable. This induces the clustering of ASBT at the site of membrane bending and consequent vesicle mediated translocation of ASBT to the cytoplasm, as observed by the appearance of gold-marks to that of membrane curvatures. A distinct trimeric complex of ASBT/LHe-tetraD/IBABP was observed in the cytoplasm of SK-BR-3 cells. IBABP disrupts the complex to initiate membrane retro-translocation of ASBT and interacts with macromolecular substrates as well to initiate exocytosis. The association of high-affinity macromolecules with ASBT during this translocation process allows the transporter to adopt a ‘receptor-like’ drug uptake mechanism. It was also important to note that the conjugation of tetrameric DOCA, one of the oligoDOCA, tremendously enhanced the oral bioavailability of the conjugated macromolecule by 30.6 ± 4.1% in rats and 19.9 ± 2.5% in monkeys.

CONCLUSION
We believe that the ‘receptor-like’ functional transformation of ASBT can motivate the development of practical systems by synthesizing specific, high-affinity binding substrates, which enable transporter-based uptake of macromolecules. Thus, the functional transformation process of ASBT could lead to overcoming the size limitation in ASBT-mediated drug transport and can propose the new pathway for the oral macromolecular drug delivery.

REFERENCES