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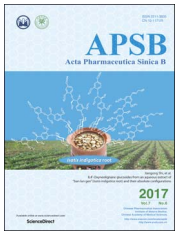

ORIGINAL ARTICLE

Biomimetic thiamine- and niacin-decorated liposomes for enhanced oral delivery of insulin

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Abstract Biomimetic nanocarriers are emerging as efficient vehicles to facilitate dietary absorption of biomacromolecules. In this study, two vitamins, thiamine and niacin, are employed to decorate liposomes loaded with insulin, thus facilitating oral absorption *via* vitamin ligand–receptor interactions. Both vitamins are conjugated with stearamine, which works to anchor the ligands to the surface of liposomes. Liposomes prepared under optimum conditions have a mean particle size of 125–150 nm and an insulin entrapment efficiency of approximately 30%–36%. Encapsulation into liposomes helps to stabilize insulin due to improved resistance against enzymatic disruption, with 60% and 80% of the insulin left after 4 h when incubated in simulated gastric and intestinal fluids, respectively, whereas non-encapsulated insulin is broken down completely at 0.5 h. Preservation of insulin bioactivity against preparative stresses is validated by intra-peritoneal injection of insulin after release from various liposomes using the surfactant Triton X-100. In a diabetic rat model chemically induced by streptozotocin, both thiamine- and niacin-decorated liposomes showed a comparable and sustained mild hypoglycemic effect. The superiority of decorated liposomes over conventional liposomes highlights the contribution of vitamin ligands. It is concluded that decoration of liposomes with thiamine or niacin facilitates interactions with gastrointestinal vitamin receptors and thereby facilitates oral absorption of insulin-loaded liposomes.

Abbreviations: AAC, area above the curve; CDI, *N*,*N*′-carbonyldiimidazole; CH, cholesterol; CH-Lip, conventional (cholesterol) liposomes; DMAP, dimethylaminopyridine; DMF, dimethylformamide; EDC, *N*-ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide; EE, entrapment efficiency; ESI-MS, electrospray ionization mass spectrometry; FAE, follicle-associated epithelia; GIT, gastrointestinal tract; ^{1}H NMR, ^{1}H nuclear magnetic resonance; HPLC/UV, high-performance liquid chromatography/ultraviolet; INS, insulin; NA, niacin; NA-Lip, niacin liposomes; SGF, simulated gastric fluid; SIF, simulated intestinal fluid; SPC, soybean phosphatidylcholine; TH, thiamine; TH-Lip, thiamine-decorated liposomes; USP, United States Pharmacopeia; VB1, vitamin B1

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1. Introduction

The oral delivery of labile macromolecules such as polypeptides and proteins is one of the top challenges in the field of drug delivery[1–3]. The fact that only a few modestly successful oral macromolecular products are marketed indicates the difficulty of overcoming the various physiological barriers that impede the oral absorption of labile macromolecular entities[4]. There are two main challenges: issues of stability, either enzymatic or acidic, and trans-epithelial absorption[5,6]. After oral ingestion, labile biomacromolecules first encounter the harsh gastric and intestinal environment, and are broken down by either gastric acid or diverse enzymes throughout the gastrointestinal tract (GIT)[7–10]. Beyond the detrimental gastrointestinal environment, the epithelial lining guards the entrance to the circulatory system, allowing only small molecules with amicable properties such as a balanced hydrophilicity and hydrophobicity. To date, there are still no efficient strategies devised to tackle these two issues simultaneously. As a result, oral delivery of biomacromolecules remains a top research interest in both academics and industry, and workable approaches or vehicles are yet to be explored.

In recent years, nanovehicles have found wide application in the field of oral delivery[11–13]. Nonetheless, while nanovehicles are efficient in protecting the payloads, they create new problems as well; for example, enlarged particle size that renders oral absorption extremely difficult. However, the epithelial lining of the GIT is not entirely inaccessible to particles: Some pathogens such as bacteria and viruses have developed mechanisms of invasion into the circulatory system[14,15]. On the other hand, the GIT has evolved specific mechanisms to break down mass nutrients such as fats into small particles before absorption[16,17]. These examples suggest that we can break through these physiological barriers by mimicking biological processes.

By mimicking the invasion of pathogens, nanovehicles targeting M cells are able to be internalized and transported very quickly to follicle-associated epithelia (FAE) in the Peyer's patches[18–23]. Whatever the fate of the particles might be, trapped there or transported to other locations, new opportunities of oral delivery are created. However, due to the limited population of M cells, less than 1% of the total enterocyte population, attention has been drawn to targeting the enterocytes that are responsible for absorption of nutrients[24,25]. By targeting the receptors residing in the membranes of enterocytes, nanocarriers bearing payloads can be taken up *via* endocytosis, thus increasing the chance of delivering the nanovehicles to the circulatory system[26–28]. The decoration of the nanovehicles with ligands such as vitamins, amino acids and polypeptides endows them with biomimetic capabilities to work as Trojan horses to protect and transport the payloads into the body simultaneously[29–31]. Taking advantage of neonatal Fc receptors, insulin, encapsulated into poly(lactic acid-*co*-glycolic acid) (PLGA) nanoparticles, has been successfully delivered into the body and elicits therapeutic effects[32]. Vitamins such as folates, VB12 and biotins are among the most popular ligands for active targeting of the intestinal epithelia due to their versatility and availability[33–37]. In this study, we tested the feasibility of using two vitamins, thiamine (VB1) and niacin, as ligands for oral delivery. It has long been established that there is wide distribution of receptors for thiamine and niacin in the intestinal epithelia[38,39]. Therefore, it makes sense to decorate nanovehicles with thiamine and niacin for enhanced oral delivery. In this proof-of-concept study we employed liposomes as model vehicles and insulin as the model drug, both of which have been used extensively in our previous studies or by other researchers[36,40–47].

2. Materials and methods

2.1. Materials

Recombinant human insulin (INS) was provided by Jiangshu Wanbang Biopharmaceuticals Co., Ltd (Xuzhou, China). Cholesterol (CH) and soybean phosphatidylcholine (SPC, Lipoid S100) were supplied by Lipoid (Ludwigshafen, Germany). Sephadex G-50 was obtained from Pharmacia (Shanghai, China). Thiamine (TH) and niacin (NA) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Stearamine was provided by Aladdin Industrial Corporation (Shanghai, China). *N*,*N*′-Carbonyldiimidazole (CDI), dimethylaminopyridine (DMAP), *N*-Ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide (EDC), phosphoric acid and triethylamine were supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Streptozotocin was obtained from Sigma–Aldrich (Shanghai, China). Deionized water was prepared by a Milli-Q purification system (Millipore, Molsheim, France).

2.2. Synthesis of thiamine–stearamide and niacin–stearamide

TH–stearamide was synthesized utilizing CDI as a coupling reagent by the methods previously reported with modifications (Scheme 1)[48–50]. Briefly, 3 mmol TH and 3.6 mmol CDI were added to anhydrous dimethylformamide (DMF) to react for 24 h at room temperature. The activated TH (TH–CDI) was obtained by washing the reactants with cold anhydrous ether three times to remove DMF, residual CDI and other by products. Subsequently, 2.4 mmol TH–CDI and 2 mmol stearamine were allowed to react in anhydrous chloroform at 80 °C for 12 h. All reactions were conducted under the protection of nitrogen. After evaporation of chloroform, the product was purified by silica gel column using chloroform/methanol (20/1, *v*/*v*) and preparative high performance liquid chromatography with a mobile phase of acetonitrile/water successively.

NA–stearamide was synthesized by the common amidation reaction utilizing DMAP and EDC as catalysts by referring to our previous work[36]. Briefly, 1.0 mmol NA was dissolved in anhydrous dichloromethane, followed by addition of 1.5 mmol DMAP and 1.5 mmol EDC. After stirring for 30 min, 1.0 mmol stearamine was subsequently added and allowed to react at room temperature overnight under the protection of nitrogen under mild stirring. Upon evaporation of dichloromethane, water was added and extracted with ethyl acetate. After washing with 1 mol/L HCl, the organic phase was then dried with anhydrous sodium sulfate and concentrated in vacuum. The crude compound was finally purified by silica gel column using methanol/dichloromethane (1/20, *v*/*v*).

The chemical structures of TH–stearamide and NA–stearamide were confirmed by mass spectrometry (MS) and ^{1}H nuclear magnetic resonance (NMR), respectively.

2.3. Preparation of thiamine- and niacin-decorated liposomes

INS-loaded plain and decorated liposomes were prepared by a reversed-phase evaporation method following previous procedures with modifications[37,45]. The general procedures for preparation of

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