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Liposomes containing cholesterol analogues of botanical origin as drug delivery systems to enhance the oral absorption of insulin

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ABSTRACT

In fear of animal-associated diseases, there is a trend in searching for non-animal derived substitutes for existing excipients in the pharmaceutical industries. This paper aimed to screen cholesterol analogues as membrane stabilizers of liposomes from botanical sterols, including β-sitosterol, stigmasterol, ergosterol and lanosterol. Liposomes containing four kinds of sterols were prepared and evaluated *in vitro* and *in vivo* as oral delivery system of insulin. Liposomes containing β-sitosterol (Si-Lip), stigmasterol (St-Lip) and lanosterol (La-Lip) was found not to protect insulin against degradation. Only 10% of the initial insulin in liposomes was preserved after a 30 min exposure to simulated gastric fluids. However, the protective ability of liposomes containing ergosterol (Er-Lip) was similar to that of liposomes containing sodium glycocholate (Sgc-Lip) and superior to that of liposomes containing cholesterol (Ch-Lip). In addition, the blood glucose level can decrease to about 50% of initial level after oral Er-Lip which was significantly superior to the *in vivo* performance of Si-Lip and Ch-Lip and similar to Sgc-Lip. Er-Lips of ergosterol/phospholipids ratios of 1:4 or 1:6 exerts more pronounced protective ability of insulin in simulated gastrointestinal fluids and hypoglycemic effects in rats than other formulations. Furthermore, Er-Lips exerted low toxicity to Caco-2 cells through a cell viability study. Meahwhile, insulin permeability was significantly increased across Caco-2 monolayers by encapsulating in Er-Lip. It was concluded that ergosterol could be used as a substitute for cholesterol and bile salt derivatives in liposomes to enhance oral bioavailability of insulin.

1. Introduction

Liposomes have been used in various fields of drug delivery via different administrative routes, such as intravascular (Gabizon et al., 2004), oral (Niu et al., 2014), pulmonary (Chattopadhyay, 2013), ocular (Dai et al., 2013) and so on. Conventional liposomes are simple single- or multi-vesicular bilayer structures, which are solely composed of phospholipid and cholesterol, and are similar to living cells in our body. Due to favorable thermodynamics phospholipid and cholesterol in certain ratios produce strong and stable bilayer membranes. Although liposomes with partly substituted phospholipid or cholesterol (Schlegel et al., 2011) have been studied, phospholipid and cholesterol are indispensable components in most liposomes studied. Since conventional phospholipid/cholesterol liposomes are not stable in the gastrointestinal tract, the earlier stages of liposomes development did not target the oral administration. To improve the performance of liposomes via the oral route, bilosomes, also called as liposomes containing bile salts, were developed by incorporating bile salts into the lipid bilayers (Niu et al., 2011). The bilosomes showed enhanced gastrointestinal stability and oral absorption taking advantage of the permeation-enhancing and stabilizing effect of the bile salts. Our previous work indicated that bilosomes containing different kinds of bile salts could significantly enhance the integrity, stability and oral bioavailability of a model protein drug insulin (Hu et al., 2013; Niu et al., 2011, 2012; Niu et al., 2014).

However, in fear of contaminants derived from materials of animals and taking into account the demands of vegetarians and religious preferences, there is an emerging trend in the pharmaceutical industries to search for botanical substitutes to animal-derived excipients or materials, *e.g.* starch- or cellulose-based hollow capsules (Al-Tabakha, 2010; Vilivalam et al., 2000).

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As for the compositions of liposomes, phospholipids can be obtained from botanical sources such as soy beans, whereas cholesterol and bile salts mainly come from animal. Cholesterol is a major membrane component in animal cells, and is especially found in brain and nerve tissues. However, it is also found in substantial amounts in kidney, spleen, liver or bile juices. At the same time, bile salts primarily come from bile juices secreted by hepatocytes, including sodium or potassium glycocholate or taurocholate (Dawson and Karpen, 2014). Along with the increasing development of liposomal products, there is a demand to search for substitutes to both cholesterols and bile salts.

Some plant derived sterols that are structurally related to cholesterol have been utilized to decrease the blood plasma cholesterol level by inhibiting cholesterol absorption in the intestine (Katan et al., 2003; O'Neill et al., 2005), such as β-sitosterol or stigmasterol. In addition, two specific sterols, ergosterol and lanosterol, are derived from microorganisms that have also attracted some interest in comparative studies with cholesterol (Hildenbrand and Bayerl, 2005). The chemical structures of these four sterols are similar to that of cholesterol, but their tail moieties are different from each other (Fig. 1). The interaction between these sterols and phospholipids have been reported in literature (Cui et al., 2010; Nagadome et al., 2007; Schuler et al., 1990). For instance, β-sitosterol competitively inhibits the solubilization of cholesterol in lipid compositions (Matsuoka et al., 2012) and it influences the structure of egg yolk phosphatidylcholine bilayers similarly to cholesterol (Gallova et al., 2011). Many reports also demonstrated that ergosterol can form a stronger lipid domain with dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) than other sterols due to a stronger van der Walls interaction for its tail with the acyl chains of DPPC (Chen and Tripp, 2012; Gagos and Arczewska, 2012), such as cholesterol, lanosterol and 7-dehydrocholesterol. However, recent studies mainly focused on interaction with lipid bilayers or influence on structure of lipid membrane. There are no report about liposomes stabilized by botanical sterols as drug delivery systems.

Herein, we attempt to substitute cholesterol in liposomes with other sterols, including β-sitosterol, stigmasterol, lanosterol and ergosterol. In this study, liposomes containing various sterols were utilized as oral delivery systems for insulin. As indicated by stability studies in simulated gastrointestinal fluids, the liposomes containing ergosterol was more stable than others. Moreover, animal studies also suggested that the liposomes containing ergosterol improved oral bioavailability of insulin more significantly in comparison with others. Hence, we further studied the influence of liposomal formulations containing ergosterol on the *in vitro* stability and *in vivo* bioavailability of insulin. The cytotoxicity and transport mechanisms of the sterol-containing liposomes were evaluated in the Caco-2 cell model. The primary goal was to confirm the possibility of substituting cholesterol with sterols as membrane stabilizers of liposomes.

2. Materials and methods

2.1. Materials

Recombinant human insulin (rhINS, 27 IU/mg) was kindly gifted by Novo Nordisk (Maalov, Denmark). β-Sitosterol (Si) was obtained from Acros organics (New Jersey, USA). Ergosterol (Er), lanosterol (La) and stigmasterol (St) were purchased from Tokyo Chemical industry (Tokyo, Japan). Soybean phosphatidylcholine (SPC), Dipalmitoylphosphatidylglycerol (DPPG) and cholesterol (Ch) were supplied by Lipoid (Ludwigshafen, Germany). Sodium glycocholate (Sgc) was purchased from Amresco (Solon, OH, USA). Fluorescein isothiocyanate (FITC) was obtained from Sigma–aldrich (Shanghai, China). Sephadex G-50 was purchased from Pharmacia (Peapack, USA). HPLC-grade acetonitrile and methanol were purchased from Tedia Company (USA). The other solvents and chemicals used in this article were of analytical grade.

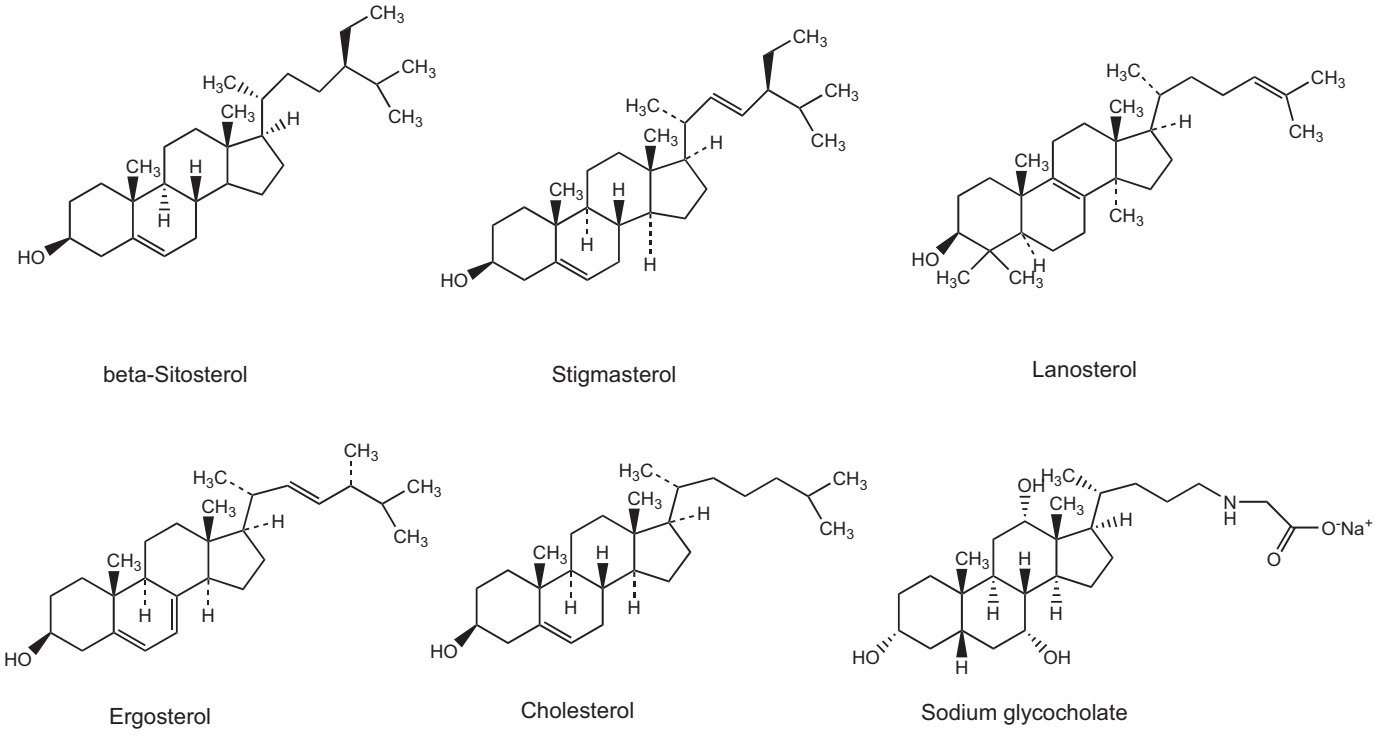

Fig. 1. The chemical structures of β-sitosterol, stigmasterol, lanosterol, ergosterol, cholesterol and sodium glycocholate.