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Denatured globular protein and bile salt-coated nanoparticles for poorly water-soluble drugs: Penetration across the intestinal epithelial barrier into the circulation system and enhanced oral bioavailability

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ABSTRACT

Oral drug delivery is the most preferred route for patients; however, the low solubility of drugs and the resultant poor absorption compromise the benefits of oral administration. On the other hand, for years, the overwhelmingly accepted mechanism for enhanced oral absorption using lipid nanocarriers was based on the process of lipid digestion and drug solubilization in the small intestine. Few reports indicated that other bypass pathways are involved in drug absorption in the gastrointestinal tract (GIT) for oral delivery of nanocarriers. Herein, we report a new nanoemulsion system with a denatured globular protein with a diameter of 30 nm, soybean protein isolates (SPI), and bile salt as emulsifiers, aiming to enhance the absorption of insoluble drugs and explore other pathways for absorption. A BCS class II drug, fenofibrate (FB), was used as the model drug. The SPI and bile salt-coated Ns with a diameter of approximately 150 nm were prepared via a high-pressure homogenizing procedure. Interestingly, the present Ns could be converted to solid dosage form using fluid-bed coating technology, maintaining a nanoscale size. Most importantly, in a model of in situ rat intestinal perfusion, Ns could penetrate across the intestinal epithelial barrier into the systemic circulation and then obtain biodistribution into other tissues. In addition, Ns significantly improved FB oral absorption, exhibited as a greater than 2- and 2.5-fold increase in C_{max} and AUC_{0-t}, respectively, compared to the suspension formulation. Overall, the present Ns are promising nanocarriers for the oral delivery of insoluble drugs, and the penetration of intact Ns across the GIT barrier into systemic circulation may be a new strategy for improved drug absorption with the use of nanocarriers.

1. Introduction

Oral drug delivery is the most preferred route for patients because of its non-invasive nature and better compliance (Ensign et al., 2012; Pridgen et al., 2013). However, the low solubility of drugs and the resultant poor bioavailability, lack of dose proportionality and slow onset of action render the achievement of therapeutic levels through oral administration difficult (Chen et al., 2009; Rabinow, 2004). Moreover, insoluble drugs, including the drugs of BCS classification II and IV, would be problematic to develop because these drugs are always difficult to formulate via traditional approaches (Merisko-Liversidge and Liversidge, 2011). The use of nanoformulations, such as liposomes, nanoemulsions, polymeric micelles, and nanosuspensions, is one of the most promising ways to address the problem, due to their significant enhancement in the solubility, stability, and permeation of the drugs (Peer et al., 2007).

Among these nanocarriers, oil-in-water nanoemulsions (Ns) that the oil droplets with a diameter of 100–500 nm are dispersed

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in the aqueous phase and stabilized by the coating of emulsifiers, because of the high payload for insoluble drugs, have overwhelming benefits. Other advantages of Ns include controlled drug release, protection of the drug from environmental conditions, enhanced drug solubility and bioavailability, reduced patient variability and ease of fabrication (He et al., 2013b; Morais and Burgess, 2014). In the oral administration of Ns loaded with insoluble drugs, the enhanced oral bioavailability was obtained through a mechanism of lipid digestion and drug solubilization in the small intestine. The oil droplets that were translocated into the GIT would be broken and digested into monoglyceride and fatty acid; then, the lipid digestion products, endogenous biliary lipids such as bile salt, phospholipid and cholesterol from the gall bladder, and exogenous solubilizers from the nanoemulsion formulations form a series of colloidal structures, including multilamellar and unilamellar vesicles, mixed micelles and micelles, resulting in the increased solubilization capacity of the small intestine for drugs. Finally, the colloidal structures penetrate across the intestinal barrier into the systemic circulation via the lymphatic system (Porter et al., 2007; Trevaskis et al., 2008). However, such a model is established based on the hypothesis that all of the nanocarriers in the GIT are degraded (Hu et al., 2015). It is possible that some of the nanocarriers may not be destroyed within a short period of time (Hu et al., 2015; Ensign et al., 2012), thus raising a question as to whether these intact nanocarriers would be taken up by the epithelial cells and subsequently penetrate across the GIT barrier into systemic circulation, thereby contributing to enhanced absorption. Until now, the research results focused on this problem are disappointing because the nanocarriers taken up by epithelial cells would be broken by the endosomal–lysosomal system, leading to easy cellular uptake but no exocytosis (Zhao et al., 2012).

Herein, we report a new Ns system with soybean protein isolate (SPI) and bile salt as emulsifiers. SPI has a similar structure as the lipoprotein Apo A–E family with lower water solubility (Zhang et al., 2012). Interestingly, after being subjected to a heat-induced denaturation procedure, the hydrophobic residues that are buried inside the protein can be exposed on the protein surface, resulting in increased water solubility and enhanced adsorption on the hydrophobic surface (He et al., 2011). It thus becomes an amphiphilic globular protein with a diameter of approximately 30 nm (Fig. 1A). Importantly, nanoparticles that are coated by this type of protein, because of the presence of a hydrophobic area on their surface, would enter cells by bypassing the endosomal–lysosomal system, achieving cytosol delivery (He et al., 2015; Li et al., 2015). Bile salt, an endogenous surfactant that is present in the intestinal milieu, can form mixed micelles with phospholipids, resulting in the enhanced absorption of insoluble drugs via promoted permeability across the intestinal membrane and intestinal lymphatic transport (Chen et al., 2009; Zhang et al., 2013). Encouraged by these results, we hypothesized that SPI and bile salt-coated Ns could penetrate across the intestinal epithelium barrier into the systemic circulation and improve the oral absorption of insoluble drugs. Two hydrophobic compounds, fenofibrate (FB) and 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl indotricarbocyanine iodide (DiR), were used as model drugs. As expected, by using the present Ns, the oral bioavailability of FB was enhanced significantly, with a more than 2.5-fold increase in contrast to the suspension formulation. Most importantly, under cleansed intestinal conditions, we found that the Ns could translocate across the intestinal barrier into the systemic circulation and then obtain biodistribution into other tissues. Additionally, the present Ns could be converted into solid dosage form by a fluid-bed coater, indicating its potential clinical application. To the best of our knowledge, few reports have indicated that lipid-based nanocarriers have this ability. This finding provides a new strategy for the enhanced oral absorption of drugs with the use of nanocarriers.

2. Materials and methods

2.1. Materials

SPI was obtained from Hufeng Chemical Industry. Medium-chain triglycerides (Labrafil M 1944CS), which were used as the oil phase, were gifts from Gattefossé Co. (Saint Priest, Cedex, France). Bile salt was obtained from SCR Co., Ltd. (Shanghai, China). Fenofibrate (FB) was purchased from Enhua Pharma Co., Ltd. (Xuzhou, China). Polyvinylpyrrolidone (PVP) K30 was a gift from

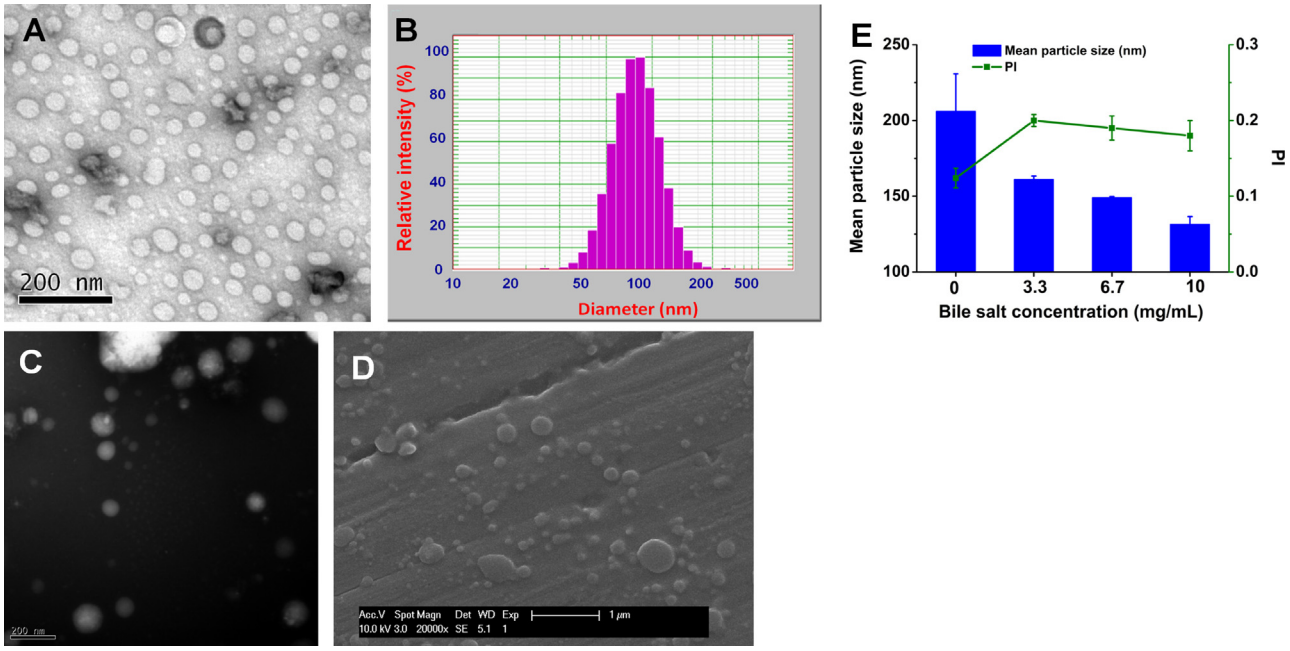

Fig. 1. (A) TEM image of denatured SPI and characterization of Ns, (B) particle size and size distribution, (C) TEM and (D) SEM images of Ns, (E) influence of bile salt on particle size of Ns.