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Comparison of the oral bioavailability of silymarin-loaded lipid nanoparticles with their artificial lipolysate counterparts: implications on the contribution of integral structure

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ABSTRACT

Both solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) were artificially broken down into lipolysates. Their oral bioavailability, with silymarin as a model drug, was compared in dogs to highlight the contribution of their integral structure. The lipid nanoparticles were prepared using a conventional hot homogenization method, whereas the lipolysates were obtained through lipolysis in phospholipid- and bile salt-enriched simulated intestinal fluid. More than 80% of vehicle-associated drugs could be transformed into the water-soluble form of mixed micelles. Pharmacokinetics analysis in dogs showed a decrease in bioavailability of 74.86% and 59.09% for lipolysates compared to integral NLCs and SLNs, respectively. It was indicated that lipolysates contributed to a majority of drug absorption. Integral nanoparticles were superior to their lipolysate counterparts, but only marginally; if the approximately 20% of the drug that precipitated during in vitro lipolysis was deducted from the overall absorption amount, the superiority of integral nanoparticles would be significantly compromised. In conclusion, lipolysis was the predominant in vivo absorption mechanism, and the contribution of intact lipid nanoparticles was limited.

1. Introduction

Lipid nanoparticles have drawn more and more attention for enhanced drug delivery in recent years (Harde et al., 2011). There are three types of lipid nanoparticles, i.e., solid lipid nanoparticles (SLNs), nanostructured liquid carriers (NLCs) and lipid-drug conjugates (LDCs) (Wissing et al., 2004). Due to the advantages of reduced particle size, biodegradability, biocompatibility, non-toxicity and absorption enhancement ability, lipid nanoparticles have been explored for various applications via both peroral and parenteral routes (Garg and Singh, 2014; Han et al., 2014; Jain et al., 2014; Jose et al., 2014; Liu et al., 2014; Rao et al., 2014). The effectiveness of lipid nanoparticles in oral bioavailability and enhancement of drug uptake is unique because it takes advantage of the physiology of lipid digestion and absorption (Fatouros et al., 2007; Porter et al., 2007; Qi et al., 2012). To date, a variety of drugs have been formulated into lipid nanoparticles with significantly enhanced oral bioavailability or obviously modified pharmacokinetics (Dwivedi et al., 2014; Funakoshi et al., 2013; Lin et al., 2014; Neves et al., 2013; Shangguan et al., 2014; Tian et al., 2013; Tran et al., 2014).

In spite of the large number of studies on lipid nanoparticles in this field, the in vivo fate and mechanisms of enhanced oral bioavailability remain unclear, mainly because of the complexity of digestive physiology (Qi et al., 2012; Roger et al., 2010). There is high demand for the elucidation of the exact mechanisms of enhanced absorption because it, in return, will greatly improve the design and development of lipid nanoparticle formulations. During the past several years, efforts have been made to elucidate and propose mechanisms, albeit ambiguous, from fragments of various investigations. Currently, there are two seemingly contradictory theories on the mechanisms of enhanced oral absorption by lipid nanoparticles. The first purported that lipid nanoparticles, either SLNs or NLCs, could be internalized by intestinal epithelia and suggested a mechanism of endocytosis, more specifically, clathrin- or caveolae-mediated endocytosis (Beloqui et al., 2013; Chen et al., 2013; Ravi et al., 2014; Zhang et al., 2010, 2012). However, these findings are significantly weakened by the fact that all of the conclusions are based on observations in Caco-2 cell lines. Moreover, the concept of intact uptake of lipid nanoparticles is elusive because fluorescent

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imaging, including that of free or released probes, may be mistaken for the imaging of lipid nanoparticles. Uptake as intact lipid nanoparticles by the intestinal epithelia and translocation to the lymph (approximately 70%) was proposed based on monitoring of fluorescent SLNs labelled by FITC-conjugated stearamine (Yuan et al., 2007). This conclusion can be compromised by the assumption that after SLN dissociation, FITC-stearamine can be released and absorbed via the lymph-oriented fatty acid absorption pathway (Mu and Høy, 2004). Therefore, the fluorescence observed in lymph after oral administration cannot be regarded as evidence of lymphatic translocation of SLNs. Most recent findings indicated that in mucus-coated cell lines, naked lipid nanoparticles have difficulty permeating through the mucus layer (Araújo et al., 2014; Chai et al., 2014; Yuan et al., 2013). It seems that the mechanism of internalization by intestinal epithelia as intact lipid particles, through either the M-cell or the enterocytic pathway, is non-persuasive due to the obvious neglect of the lipolysis mechanism that happens much earlier than when there is sufficient contact of the nanoparticles with the epithelia.

The currently well-accepted mechanisms governing enhancement of oral absorption by lipid-based delivery systems are directly related to the process of lipid digestion (Mu and Høy, 2004). Ingested lipids, mainly triglycerides from foods, will be first emulsified to form chymes in the stomach in response to gastric motion. Lipolysis occurs at the junction of oil/water surfaces resulting from the action of lipases and co-lipase. Triglycerides are regularly digested into diglycerides, then monoglycerides and then fatty acids. Monoglycerides and fatty acids form mixed micelles with endogenous phospholipids and bile salts that are secreted by the liver, which afterwards can be easily transported across the unstirred water layer to the vicinity of the epithelia. Monoglycerides and fatty acids are then absorbed into the systemic circulation through a passive diffusion pathway. The drugs embedded in lipid-based vehicles might be absorbed simultaneously (Porter et al., 2007).

The mixed micelle-based theory has been used to interpret the enhancement of bioavailability of highly lipophilic components (Porter et al., 2007). However, discrepancies arose when this theory was used to interpret the fact that the oral bioavailability of some hydrophilic molecules, such as peptides or proteins, which could not be embedded in the lipophilic core of mixed micelles, could also be enhanced by SLNs or NLCs (Müller et al., 2006; Sarmento et al., 2007; Yang et al., 2011). However, enhanced absorption by lipid nanoparticles cannot be solely interpreted by lipolysis-based mechanisms. It is therefore important to evaluate the contribution of lipolysis and the intact uptake mechanism to overall absorption. To collect these data, we used an animal model.

Herein, we investigated the in vivo performance of SLNs and NLCs in beagle dogs by comparing their oral bioavailability with that of their lipolysate counterparts and fast-release formulations. The hypothesis was that the integrity of the lipid nanoparticles would be confirmed to be important if they showed superior performance. Otherwise, the contribution of the integral structure would be proved to be less important, and the conclusions based on intact uptake by intestinal epithelia should be reconsidered. Silymarin (SM), a poorly water-soluble drug, was used as the model drug (Shangguan et al., 2014).

2. Materials and methods

2.1. Materials

Precirol ATO 5 was a gift from Gattefossé Co. (Saint Priest, Cedex, France). Lecithin (Lipoid E100) was purchased from Lipoid GmbH Company (Ludwigshafen, Germany). Oleic acid was supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Silymarin (2.57% taxifolin, 16.58% silychristin, 4.02% silydianin, 8.35% isosilybin and 27.40% silybin by HPLC (Lu et al., 2007) was purchased from Panjin Huacheng Pharmaceuticals (Liaoning, China). Silybin was purchased from Shanghai Tauto Biotech Co., Ltd. (Shanghai, China). Non-pareil pellets (Suglets® sugar spheres PF101, 710–850 μm in diameter) were provided by NP Pharm (Bazainville, France). Silymarin capsules (Legalon®), used as a reference, were purchased from Madaus AG (Germany). HPLC-grade methanol was supplied by Tedia Company Inc. (USA). Deionized water was prepared by a Milli-Q purification system (Millipore, USA). All other reagents were of analytical grade and were used as received.

2.2. Preparation of silymarin-loaded NLCs and SLNs

SM-loaded NLCs were prepared by a hot high-pressure homogenization method following previously described procedures (Shangguan et al., 2014). Briefly, 2 g of ATO-5 and 0.9 g of oleic acid were melted at 80 °C by heating and mixed thoroughly by stirring. To the lipid melt, 400 mg of SM and 1.0 g of lecithin, which were first dissolved in 2 mL of ethanol and 0.6 mL of ammonium hydroxide solution, were added and mixed to form a uniform solution. Ethanol and ammonia were removed by evaporation under stirring. Next, the lipid mixture was dispersed into 30 mL of water containing 3.33% Tween-80 thermostatically maintained at 80 °C using a high-shear inner-cutting homogenizer (GF-1, Kylin-Bell Lab Instruments Co., Ltd., Jiangsu, China) at 8000 rpm for 1 min. Then, the primary emulsion was homogenized using a micro-jet homogenizer (Nano DeBEE, USA) using 20,000 psi for five cycles. The well-dispersed hot O/W nanoemulsions were cooled to room temperature with continuous stirring to obtain solidified NLCs. SLNs were prepared by following the same procedures but without the use of oleic acid; both blank NLCs and SLNs were prepared by following the same procedures but without addition of silymarin.

2.3. Preparation of lipolysates of NLCs and SLNs

The lipolysates of NLCs and SLNs were prepared following common in vitro lipolysis procedures simulating the fed-state intestinal conditions (Porter and Charman, 2001). Briefly, approximately 15 mL of either NLC or SLN suspensions (equivalent to 270 mg SM) were added to 40 mL of digestive medium, fed-state simulated small intestine fluid (FeSSIF), which was composed of a digestion buffer (50 mM Triz-maleate (pH 7.5), 150 mM NaCl, 5 mM $CaCl_2$), 5 mM cholates and 1.25 mM lecithin with a final pH of 7.50 ± 0.05. After cultivation at 37 °C for 10 min, lipolysis was started by addition of porcine pancreatic lipase solution to an activity of 6400 TBU/mL and continued for 2 h under stirring at 37 °C. The lipolysis product, which is primarily fatty acids, was titrated by 0.5 M NaOH continuously using a pH-stat titrator (TitraLab® 854, Radiometer Analytical) to maintain the pH at 7.5. Upon completion of the lipolysis, the lipase activity was immediately terminated by addition of 4-bromophenylboronic acid (4-BPBA) (Porter et al., 2004a).

2.4. Preparation of silymarin solid dispersion pellets and mixture with NLCs or SLNs

Silymarin/polyvinylpyrrolidone (1/4) solid dispersion pellets (SMSD) were prepared by our previously reported fluid-bed coating method (Sun et al., 2008). In brief, SM and PVP K30 were dissolved in ethanol to achieve a solid concentration of approximately 10% (w/v). The solution was then sprayed through a nozzle onto the fluidized non-pareil cores in a Mini-Glatt