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Effects of triglycerides on the hydrophobic drug loading capacity of saturated phosphatidylcholine-based liposomes

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ABSTRACT

A high drug-loading capacity is a critical factor for the clinical development of liposomal formulations. The accommodation of hydrophobic drugs within the liposomal membrane is often limited in saturated phosphatidylcholine (PC)-based liposomes owing to the rigidity of the lipid acyl chain. In the current study, we explored the possibility of improving the hydrophobic drug loading capacity of liposomes by incorporating triglyceride into liposomal membranes. Incorporation of Captex 300, a medium chain triglyceride, into liposomes composed of dimyristoylphosphatidylcholine and cholesterol greatly increased the fluidity and lamellarity of the resultant liposomes. Liposomal incorporation of medium or long chain, but not short chain, triglycerides greatly enhanced the concentration of loaded paclitaxel (PTX) in saturated PC-based liposomes. The enhancing effect of triglyceride saturated at a triglyceride content corresponding to the amount required to fluidize the liposome structure. In addition, the enhancing effect was not observed in unsaturated PC-based liposomes and was not associated with the solubility of PTX in each triglyceride. Triglycerides also enhanced the loading of docetaxel, another hydrophobic drug. Taken together, our results suggest that triglyceride incorporation in saturated PC-based liposomes provide an improved dosage form that enables increased hydrophobic drug loading by altering the fluidity and structure of liposomal membranes.

1. Introduction

Liposomes are spherical, enclosed vesicles composed of phospholipid bilayers that form spontaneously upon hydration in an aqueous media. The structure of liposomes allows the encapsulation of hydrophilic drugs in the inner aqueous compartment, and also accommodates hydrophobic drugs within the hydrophobic region of phospholipid layers. Extensive drug delivery studies have shown that loading drugs into liposomes is an effective way to increase their chemical stability and improve on their poor solubility, limited cellular entry and in vivo pharmacokinetics, thereby providing dosage forms with enhanced therapeutic efficacy and reduced side effects. These favorable properties, together with the biocompatibility of phospholipids, highlight the promise of liposomes as drug carriers (Allen and Cullis, 2013).

A high drug-loading capacity is a critical factor for the clinical development of liposomes as administrable and cost-effective dosage forms with optimum therapeutic efficacy. Drug loading in liposomes depends on the preparation method, the phospholipid composition/concentration, the initial input drug concentration, as well as the lamellarity and size of liposomes. For very hydrophobic drugs, multilamellar vesicle (MLV)-type liposomes are preferred to maximize drug loading in phospholipid layers. Studies have shown that the poor aqueous solubility of celecoxib, tamoxifen, dexamethasone, docetaxel, flavopiridol, triamcinolone acetonide, and paclitaxel (PTX) is improved by loading in MLVs (Bhardwaj and Burgess, 2010; Bhatia et al., 2004; Deniz et al., 2010; Goundalkar and Mezei, 1984; Immordino et al., 2003; Qu et al., 2013; Yang et al., 2009). In these studies, optimization of the membrane composition was required to improve the drug-loading capacity (van der Meel et al., 2014). Incorporation of Tween 80 or polyethylene glycol 400 into liposomal membranes was shown to greatly increase the amount of PTX and celecoxib loaded (Kang et al., 2013; Yang et al., 2007), an effect most likely attributable to the considerable solubility of these drugs in these surfactants. Incorporation of cationic lipids in liposomal membranes was shown to improve the loading and retention of PTX (Campbell et al., 2001), likely owing to the fluidizing effect of such lipids on liposomal membranes. Substitution of saturated PCs with unsaturated PCs significantly

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increases hydrophobic drug loading, also presumably due to the greater fluidity of PC membranes, enabling penetration of the drug into the hydrophobic domain of bilayers (Crosasso et al., 2000; Immordino et al., 2003; Zhang et al., 2005). In contrast, inclusion of cholesterol (CHOL) has been shown to decrease the encapsulation of hydrophobic drugs (Crosasso et al., 2000; Deniz et al., 2010; Zhang et al., 2005) because CHOL occupies the limited hydrophobic space in the membrane. These observations collectively suggest that hydrophobic drug loading is higher in MLVs whose membrane is composed of unsaturated PCs without CHOL and might be further modulated by incorporation of another component that affects membrane rigidity.

In addition to determining drug-loading capacity, membrane composition may affect the stability of liposomes (Sharma and Straubinger, 1994; Ulrich, 2002). For example, CHOL prevents the leakage of encapsulated drugs from liposomes and extends the circulation time of conventional liposomes in the blood (Deniz et al., 2010; Senior and Gregoriadis, 1982). Saturated PCs with longer fatty acid chain lengths tend to produce liposomes that retain loaded drugs longer than unsaturated PCs, owing to the absence of unsaturated bonds, which are prone to oxidation, and the higher phase-transition temperature of the liposomes (Lehtonen and Kinnunen, 1995; Mattjus and Slotte, 1996; Payton et al., 2013). This may explain why many commercialized liposomal drugs, including DaunoXome®, Doxil®, AmBisome® and Visudyne® (Chang and Yeh, 2012), employ saturated PCs together with CHOL in their liposome formulations. With this in mind, improving the hydrophobic drug-loading capacity of liposomes prepared with a mixture of saturated PCs and CHOL would help guarantee a formulation with higher stability, thereby accelerating their clinical development.

Previously, we found that incorporation of 0.3 mol% tributyrin, a short-chain triglyceride (SCT), into 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC)-based liposomes increased the concentration of loaded celecoxib to an extent similar that of Tween 80 (Kang et al., 2013). The effect of tributyrin on the loaded celecoxib concentration did not appear to be associated with the solubilization of celecoxib in tributyrin. Considering that the ability of liposomal membranes to accommodate hydrophobic molecules is greatly affected by the ordering and mobility of lipid acyl chains (Campbell et al., 2001), it is conceivable that the small amount of incorporated tributyrin embeds itself between the hydrophobic chains of PCs, thereby decreasing the order and increasing the fluidity of DMPC membranes. If this were the case, the interaction between triglycerides and PCs would be dependent on the triglyceride's fatty acid chain length, which in turn would affect the membrane fluidity and hydrophobic drug-loading capacity of saturated PC-based MLVs. In the current study, we investigated this supposition by preparing triglyceride-containing liposomes incorporating SCTs (C2–C5 backbone), medium-chain triglycerides (MCTs; C6–C12 backbone), or vegetable oils, whose main components are long-chain triglycerides (LCTs; C16–C22 backbone).

2. Materials and methods

2.1. Materials

1,2-Dilauroyl-*sn*-glycero-3-phosphocholine (DLPC), DMPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) were purchased from Avanti Polar Lipid Inc., (Alabaster, AL, USA). CHOL, corn oil, glyceryl triacetate (triacetin) and glyceryl tributyrate (tributyrin) were bought from Sigma–Aldrich (St. Louis, MO, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Amresco (Solon, Ohio, USA). Docetaxel and PTX were purchased from LC laboratories (Boston, MA, USA). Taxol® was obtained from Bristol-Myers Squibb (New York, NY, USA). Captex 300 (C300) was obtained from Abitec (Columbus, Ohio, USA). Labrafac™ Lipophile WL 1349 (Labrafac) was bought from Gattefosse (Nanterre, France). Hexyl 4-hydroxybenzoate was obtained from Tokyo Chemical Industry (Toshima, Tokyo, Japan). 6-Carboxyfluorescein (6-CF) was purchased from Invitrogen (Eugene, Oregon, USA). Acetonitrile, methanol, *tert*-butyl methyl ether and distilled water were HPLC grade. All other materials were of reagent grade and used without further purification.

2.2. Cell lines and culture conditions

Human H460 lung cancer cell lines were obtained from the ATCC (Manassas, VA, USA) and maintained in RPMI medium (Welgene, Daegu, Korea). Human MCF-7/ADR breast cancer cell lines were kindly provided by Dr. K.H. Cowan (National Cancer Institute, Bethesda, MD) and maintained in DMEM/F-12 medium (Welgene, Daegu, Korea) (Kang et al., 2005). Each medium was supplemented with 10% heat inactivated fetal bovine serum (Gibco, NY, USA) and 100 units/ml penicillin/streptomycin. Cells were grown in incubators in a humid atmosphere of 95% air and 5% CO_2.

2.3. Preparation of liposomes

Liposomes were prepared by sonication method using cell disruptor-type sonicator as described in our earlier study (Hong and Lim, 2014). Briefly, total 40 μmol of lipid mixtures composed of 7:1 mol% of DMPC and CHOL were first dissolved in tertiary butyl alcohol. When the incorporation of triglyceirde or drug loading (PTX or docetaxel) were required, appropriate amount of them (0.1–4 mg triglyceride, 2.6 mg of drugs per 40 μmol of DMPC:CHOL mixture) were dissolved together in tertiary butyl alcohol. After rapid freezing at −80 °C, mixtures were subjected to freeze-drying by a freeze dryer (EYELA FDU-1200, Japan). Lipid cakes obtained after 24 h drying were hydrated with 1 ml of saline. Obtained liposome dispersions were subjected to bath sonication for 30 min at 37 °C in a ultrasonic cleaning bath (3510R-DTH, Bransonic, USA), followed by additional sonication for 7 min by using a cell disruptor (Bioruptor®, UCD-200T, Cosmo Bio Tokyo, Japan) set at 250 W.

To remove the unloaded/precipitated PTX or docetaxel from liposomes, the liposome dispersions were immediately filtered through 0.8 μm syringe membrane filter (Kang et al., 2011). The filtered PTX-loaded liposomes were stored at 4 °C until use.

When required, PTX-loaded liposome dispersions were diluted 1:1 with 5% sucrose solution (w/v, dissolved in distilled water) and freeze-dried for storage. The freeze-dried liposomes were reconstituted in distilled water, briefly vortexed and filtered through 0.8 μm membrane filter to remove the precipitated PTX. The PTX concentration retained in reconstituted liposomes was quantified by HPLC analysis as described below.

2.4. Physicochemical characterization of liposomes

The mean particle size and polydispersity index (PI) of liposome dispersions were determined by dynamic light-scattering method using fiber-optics particle analyzer (FPAR-1000, Otsuka Electronics, Japan) as described earlier (Lee et al., 2014). Prior to measurement, dispersions were diluted 100 times with filtered saline. Droplet size analysis data were evaluated using volume distribution to detect even a few large droplets. The PI was obtained as a measure of the uniformity of the droplet size distribution in colloidal dispersions.