Assessment of Cytotoxicity and Permeation Enhancement of Lipid-Based Self-Emulsifying Drug Delivery Systems with Caco-2 Cell Model: Polysorbate 80 as the Surfactant

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Purpose
Oral administration of therapeutic agents has the most advantages as compared to other routes. However, the majority of existing or newly discovered drug candidates are poorly water-soluble, which necessitates the use of suitable drug delivery systems to improve their solubility, dissolution rate and ultimately oral bioavailability. In addition, the epithelial layer lining the intestinal lumen constitutes the rate-limiting barrier for absorption of orally administered drugs, where it is necessary to improve the permeability of certain drugs by using appropriate excipients. The lipid-based self-emulsifying drug delivery system is an attractive approach, which not only increases the solubility of drugs but also promotes their absorption in the gastrointestinal tract and overall bioavailability. A widely used in vitro model that mimics the intestinal epithelium is the Caco-2 cells, which has been commonly employed to predict intestinal permeation of oral dosage forms. However, toxicity of lipid-based drug delivery systems can damage cell monolayers and cause artificial increase in permeation studies. The present study has been undertaken to assess safety of commonly used lipid-based excipients and their formulations on the Caco-2 cells and to evaluate their effects on permeation enhancement using the Caco-2 monolayer model under nontoxic conditions.

Methods
Various concentrations of medium-chain monoglyceride (Capmul MCM EP and Capmul 708G) or propylene glycol monoester (Capmul PG8 NF) of medium-chain fatty acids in mixtures with the surfactant polysorbate 80 (Tween 80), with and without a medium-chain triglyceride (Captex 355), were prepared and the particle size of each solution was measured. Caco-2 cells from a series of culture stages were prepared, where 1-day and 5-day cells were cultured in regular tissue culture plates and the 3-week (21-23 days) monolayers were cultured in transwell plates. Caco-2 cells with different maturation statuses were incubated with lipid-based formulations at various concentrations for 2 hours. Cell viability and membrane integrity were assessed using MTT assay and MultiTox-Fluor Cytotoxicity assay, respectively. The formulations that were found to be nontoxic were further evaluated for their permeation enhancement effects using Caco-2 monolayers.

Results
The toxicity of a lipid-based formulation is determined by several factors, such as the concentration and composition of the lipid-surfactant mixtures, the physicochemical properties of lipids and surfactant within, and the droplet sizes of the mixtures. Furthermore, the maturity or differentiation status of Caco-2 cells markedly influenced the cytotoxicity outcome. One-day Caco-2 culture was most sensitive to both the surfactant alone and their lipid-surfactant mixtures. However, the inclusion of a triglyceride (Captex 355) to the lipid-surfactant mixture containing monoglyceride Capmul 708G significantly alleviated the toxicity at 0.1% (v/v) concentration. After Caco-2 cells were cultured for 5 days, their tolerance to higher concentrations of lipid-surfactant mixtures increased markedly. Mixtures containing a triglyceride consistently showed reduced toxicity, particularly at the highest concentration (0.5%, v/v). Remarkably, upon forming a monolayer after a 3-week culture, the Caco-2 cells showed most robust resistance towards any adverse effects of lipid-surfactant mixtures. These monolayer cultures not only withstood effects of the treatment of surfactant alone at all three concentrations tested, they also tolerated most of the lipid-surfactant mixtures at low (0.1%) and medium (0.2%) concentrations. A formulation containing 35% of triglyceride in combination with 30% of surfactant (Tween 80) and 35% of monoglyceride or propylene glycol monoester formed microemulsions in aqueous media that exhibited the greatest toxicity-reducing capacity in the 3-week monolayer cultures at the highest concentration (0.5%). Even treated with such a high concentration of the formulations, the monolayers were able to maintain survival rates of 60-85%. Furthermore, the extent of cell membrane rupture caused by lipid-surfactant mixtures positively correlated with levels of cytotoxicity, suggesting a potential underlying mechanism. Formulations that were well tolerated were selected for subsequent assessment of permeation enhancement effect using the Caco-2 monolayer model. Formulations containing 35% of triglyceride in combination with 30% of surfactant and 35% of monoglyceride or propylene glycol monoester not only were best tolerated but also exhibited significantly permeation enhancement effects in the Caco-2 monolayer model.

Conclusion
Toxicity assessment of excipients-containing oral formulations is a prerequisite for good tolerance and permeation enhancement at the site of absorption. Lipid-based drug delivery systems that self-emulsify into microemulsions have significantly less toxicity than coarse emulsions. Additionally, a microemulsion with the combination of triglyceride and monoglyceride has significantly less toxicity than the one containing monoglyceride alone. Fully differentiated Caco-2 monolayer is more relevant to in vivo situations and is shown to be able to withstand lipid-surfactant mixtures, representing a more accurate in vitro model.