Toxic or Not: Effect of Lipid, Surfactant and Lipid-Surfactant Mixture on Viability of Caco-2 Cells

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Purpose
The single epithelial layer lining the intestinal lumen constitutes the rate-limiting barrier to absorption of orally administered drugs. The human colon carcinoma cell line Caco-2 has been widely used to mimic the small intestine epithelium by experimentally differentiating into an epithelial monolayer with morphological and functional similarities. The Caco-2 monolayer model is, therefore, used extensively for in vitro prediction of intestinal drug permeation and to investigate the effects of various excipients and adjuvants as permeability enhancers. However, the toxicity of such agents to Caco-2 cells can cause a false positive increase in drug permeation by damaging the monolayer, and, therefore, it is of crucial importance to assess cytotoxicity of excipients of interests on Caco-2 cells prior to any permeability study. The objective of the present study is to investigate potential toxic effects on Caco-2 cells by several lipids, surfactants and lipid-surfactant mixtures that are commonly used in the development of lipid-based self-emulsifying drug delivery systems.

Methods
Caco-2 cells from different stages of maturation/differentiation were prepared, where 1-day and 5-day cells were cultured in regular tissue culture grade 96-well pates and 21-day cells were cultured in transwell plates. Different concentrations of medium-chain monoglyceryl lipids (Capmul MCM EP, Capmul PG8 NF and Capmul 708G) alone or in mixture with surfactant (Cremophor EL or Polysorbate 80), with and without a medium-chain triglycerol lipid (Captex 355), were prepared in Caco-2 growth medium. The preparations were then incubated with Caco-2 cells for 2 hours. Cell viability was evaluated using trypan blue exclusion test and MTT viability assay. Cell membrane integrity was assessed by CytoTox-ONE Homogeneous Membrane Integrity Assay and MultiTox-Fluor Cytotoxicity Assay.

Results
Cytotoxicity on Caco-2 cells was influenced by multiple factors, such as the chemical nature of lipids and surfactants, the composition of lipid-surfactant mixtures, and the differentiation status of Caco-2 cells. Neat lipids were more toxic compared to lipid-surfactant mixtures. All three monoglyceryl lipids caused 80% or more cell death in 1-day Caco-2 culture compared to the control (no lipid) at concentrations ≥ 0.1% (v/v). The preparation of emulsion or microemulsion (lipids + surfactant) significantly alleviated the toxicity of monoglyceride at 0.1% concentration, and such a capacity to reduce toxicity appeared to depend on the lipid-surfactant ratio. The inclusion of a triglycerol to the microemulsion system further reduced the toxic effect. A formulation composed of 30% surfactant, 35% of monoglyceryl lipid and 35% of triglycerol lipid showed the least cytotoxicity. Furthermore, the maturity of Caco-2 cell culture also influenced susceptibility to lipid toxicity. One-day culture was most sensitive to lipids, surfactants and lipid-surfactant mixtures, exhibiting 75% or more cell death at concentrations ≥ 0.2%. As cells became more mature and differentiated into monolayers, their resistance to lipid-surfactant mixtures increased markedly. After a 5-day culture period, Caco-2 cells were able to maintain a survival rate of 40 - 70% after exposed to lipid-surfactant mixtures at higher concentrations (0.2% and 0.5%). To most accurately mimic conditions used in permeability studies, Caco-2 cells were cultured in transwell plates for 21 days to fully differentiate into a monolayer prior to the cytotoxicity assessment. The 21-day Caco-2 cells were able to withstand the highest concentrations (0.5%) of some formulations (survival rate ≥ 70%), indicating a further enhanced tolerance to lipid toxicity upon monolayer formation. Remarkably, one formulation with a specific lipid-to-surfactant ratio (30% surfactant, 35% of monoglycerol lipid and 35% of triglyceryl lipid) reveals a consistent profile of reduced toxicity under all the conditions tested. Mechanistically, the observed cytotoxicity appeared to be due to lipid-induced rupture of cell membrane, as formulations that have less impact on cell membrane integrity render better cell survival.

Conclusion
The present study demonstrates that the cytotoxicity of lipid-based drug formulation to the Caco-2 monolayer model is influenced by the composition of formulation and the maturity of cells. Microemulsion or fine emulsion of lipid-surfactant mixtures has less cytotoxicity than lipid or surfactant alone. Based on these findings, well-tolerated lipid-based formulations will be selected for further assessment as potential permeability enhancer in Caco-2 monolayer models.