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Curcuminoid in Nanoemulsion Formulations Cause Enhanced Cell Death in the HeLa Cancer Cell Culture Model

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Purpose

To formulate and characterize curcuminoid (insoluble chemoprevention agent) loaded nanoemulsions and evaluate their cytotoxicity in HeLa cells.

Methods

The solubility of curcuminoid in oil (glyceryl monooleate, Peceol™) was determined using RP-HPLC using a C18 column (5 µm silica particles, 150 X 4.6 mm), isocratic mobile phase [40% acetonitrile and 60% glacial acetic acid in water (1%)] at the flow rate of 1.5 ml/min with UV-Vis detection at 425 nm. The nanoemulsion formulations comprised of oil (Peceol), surfactant (Labrasol, Pluronic P85™, Pluronic L61) and co-surfactant (Poloxamer 188, Tween 80, Lauroglycol™ 90) were prepared using high shear followed by high-pressure homogenization process. Curcuminoid was pre-dissolved in the oil phase to prepare the drug-loaded formulations. The oil, surfactant, co-surfactant and the aqueous phase were separately heated to 55°C ± 5°C. The warm aqueous phase was slowly added to the warm oily phase under continuous mechanical agitation. The resulting crude emulsion was homogenized with a laboratory scale T18 digital Ultra-Turrax mixer (IKA, Staufen, Germany) at 3500 rpm for 5 min for size reduction which leads to the formation of the primary emulsion in the micron size range. A microfluidization system (M-110L, Microfluidics, Massachusetts, USA) was used to further reduce the particle size to obtain nanoemulsions of curcuminoid by passing the primary emulsion through the high impact force capillary chamber for 20 consecutive cycles at a constant pressure of 18000 psi. The nanoemulsions were characterized for droplet size, surface charge, polydispersity index and encapsulation efficiency of curcuminoid. Droplet (particle) size was determined by photon correlation spectroscopy using a NICOMP 380ZLS particle size analyzer at a scattering angle of 170° at 25°C after dilution with deionized water to avoid multiple scattering effects. The physical stability of blank nanoemulsions was studied at 4, 25 and 40°C for one month to identify formulations suitable for drug loading. The physical and chemical stability of blank and curcuminoid loaded nanoemulsions stored in glass vials was studied at 4, 25 and 40°C over a period of three months. The MTT cell survival assay was conducted to assess cell viability with free curcuminoid, blank nanoemulsion, and curcuminoid loaded nanoemulsion against media controls using HeLa cells.

Results

Blank nanoemulsions containing various surfactants (Pluronic P85, Pluronic F68, Tween 80, Pluronic L61, Labrasol, Lauroglycol™ 90) at concentrations ranging from 0.1% v/v to 5% v/v were prepared with oil concentrations ranging between 5-10%v/v. Based on physical stability data, the nanoemulsion formulation selected for further studies consisted of 10%v/v oil (Peceol), 0.5%v/v surfactant (Pluronic P85) and 0.1 %v/v co-surfactant (Tween 80). The average droplet size and polydispersity index (PDI) of these blank nanoemulsions were in the range of 110-300nm and 0.05-0.52, respectively. The average solubility of curcuminoid in Peceol was 2.4 mg/mL. Three replicates of curcuminoid loaded nanoemulsions were prepared for stability studies. Following drug loading, no significant change in average droplet size of curcuminoid loaded nanoemulsion was observed in comparison to blank nanoemulsions. The average droplet size, zeta potential & polydispersity index of the curcuminoid loaded nanoemulsions were in the range of 120-290nm, -0.60 to -5.30 mV and 0.05-0.13, respectively. No significant change in droplet size and PDI for blank nanoemulsion and curcuminoid loaded nanoemulsion were found during the stability studies. The curcuminoid loaded nanoemulsions were also found to be chemically stable over the study period. Encapsulation efficiency of curcuminoid in nanoemulsion was calculated to be greater than 95%. MTT assay indicated that the curcuminoid loaded nanoemulsion led to significantly higher cell death in HeLa cells compared to free curcuminoid, blank emulsion and media control.

Conclusion

Physicochemically stable curcuminoid loaded nanoemulsions with low concentrations of surfactant and co-surfactant were successfully formulated using a high shear and high-pressure homogenization process. Cell survival studies indicated that the curcuminoid loaded nanoemulsions led to significantly higher cell death in HeLa cells compared to free curcuminoid, blank emulsion and media control. This novel nanoemulsion formulation of curcuminoid could be further developed for used in cancer chemoprevention.