Synthesis and Characterization of Lipid Nanoparticle Formulation of a Poorly Soluble, Highly Potent, Novel Synthetic Analog of Curcumin for Cancer Therapy

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Introduction: Curcumin is used as an herbal supplement used in Ayurvedic medicine since a long time. It is a natural phenol isolated from the rhizome of Curcuma longa. It also shows anticancer properties and has undergone several clinical trials. However, till now it could not be used in clinics because of its various limitations such as poor solubility due to hydrophobicity and low bioavailability. Further, it gets metabolized very fast inside the body and is chemically unstable. The absence of any specific target inside the cells further limits its application and qualifies curcumin as Pan-assay interference compounds (PAINS). We have previously reported a novel curcumin derivative compound C1, which is ~10 times more potent than curcumin. It targets tubulin and depolymerizes microtubules. However, the compound C1 also has poor solubility and low bioavailability issues like curcumin and these limitations need to be addressed before its translation into a potential anticancer drug. To circumvent these problems, we have developed and characterized a lipid nanoparticle formulation of C1 for cancer therapy.

Methods: Lipid nanoparticles were synthesized using thin film hydration method using DPPC: Cholesterol: DSPE-PEG-2000. C1 (4-{5-(4-hydroxy-3-methoxy-phenyl)-2-[3-(4-hydroxy-3-methoxy-phenyl)-acryloyl]-3-oxo-penta-1, 4-dienyl}-piperidine-1-carboxylic acid tert-butyl ester) was loaded passively in liposomes. Liposome nanoformulation was characterized by TEM, SEM, and DLS. Zeta potential was measured to check the stability of the liposome. HeLa cell line was used for in-vitro characterization. SRB assay was used for determination of cell proliferation. Microtubule depolymerization was determined by indirect immunostaining and western blotting. Reactive oxygen species (ROS) level was determined by the level of DCFDA dye fluorescence. Apoptosis was confirmed by PARP cleavage.

Results: We have optimized a lipid nanoparticle formulation comprising unilamellar, nanosized (150±20 nm; Figure 1) liposomes. The polydispersity index of the liposomes was 0.09±0.02 and the zeta potential was about -19±1 mV. The half maximal inhibition of cell proliferation (IC50) value of the nanoformulation was found to be 3±1 µM in HeLa and 1.3±0.1 µM in Huh-7 cells. Moreover, in a multidrug resistant cell line EMT6/AR1, the IC50 was around 7±2 µM. The biocompatibility study was done using normal mouse fibroblast cell line (L929), and the particles were not toxic up to 270 µg/ml concentration. The nanoformulation depolymerized both mitotic as well as interphase microtubules (Figure 2), increased the level of reactive oxygen species and induced apoptosis via PARP cleavage.

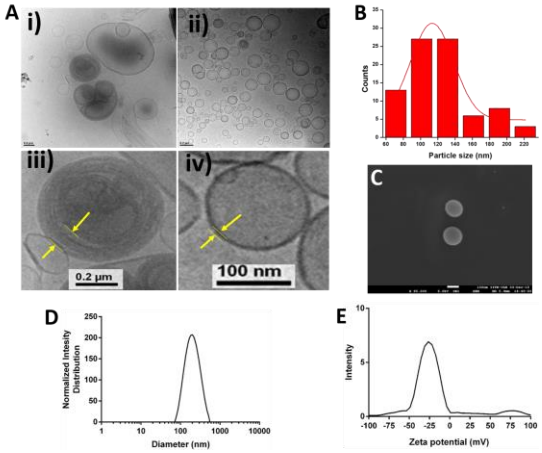

Figure 1: Characterization of lipid nanoparticles

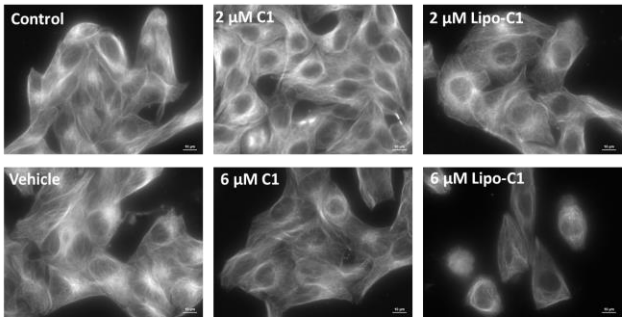

Figure 2: Nanoformulation induced microtubule depolymerization in HeLa cells

Conclusions: We have optimized lipid nanoparticle formulation which increases the solubility of the curcumin analog, C1 and has similar IC_{50} profile as the parent drug. These lipid nanoparticles are PEGylated and thus are expected to have higher half-life in blood circulation. In vivo efficacy and PK studies are currently underway.

References:

(Srivastava S. Biosci Rep. 2016;36:e00323–e00323) (Rengan AK. Nano Lett. 2015;15:842–848)