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| **Lipid nanocapsules for ceramide delivery induce apoptosis in breast cancer cells** |
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| **Background:** Ceramides have been known to be bioactive sphingolipids that play an important role in cell differentiation, senescence and induce pro-apoptotic effects in some cancer cells. However, the therapeutic application of ceramides is limited due to their poor water-solubility and susceptibility to enzymatic degradation if delivered in a free form. Therefore, encapsulating ceramides into nanoparticles may be required to increase intracellular levels and protect against degradation. Liposomes have been suggested for that purpose previously. In this project, we suggest that encapsulation in lipid nanocapsules (LNCs) could be equally beneficial. A short-chain ceramide, namely C6 ceramide, was used in this study since the cytotoxic activity depends on ceramide acyl chain length, with shorter chains showing stronger anticancer activity. Among these, C6 is considered the most potent ceramide and can inhibit proliferation and/or induce apoptosis in specific cancer cells, including breast, hepatocellular and pancreatic cancer. Here, we report the development of LNCs as potential nanocarriers for C6 ceramide delivery towards MDA-MB-231 breast cancer cells. |
| **Methods:** C6 ceramide was encapsulated into LNCs (LNC-C6) using a phase-inversion temperature method. As a control, LNCs without C6 ceramide (LNC-ghost) were prepared. Physicochemical properties, such as particle size, size distribution and surface charge, were evaluated using dynamic light scattering (DLS). *In vitro* cytotoxicity assay towards MDA-MB-231 cells after 72 hours of exposure to LNC-C6 were evaluated using cell-counting kit 8 (CCK8). In addition, to confirm that C6 ceramide delivery induces apoptosis in breast cancer cells, an annexin V-FITC/ PI staining assay was also performed following the treatment of MDA-MB-231 cells with LNC-C6 and then analyzed using flow cytometry. |
| **Results:** Two different sizes of LNC-C6 were successfully prepared by a phase-inversion temperature method and resulted in 44 ± 1 (LNC50-C6) and 100 ± 1 nm (LNC100-C6) in size, with narrow size distribution (PdI < 0.3) and slightly negative of zeta potential. The presence of C6 ceramide did not influence the physicochemical properties of LNCs. The 72 hours cytotoxicity results illustrated that the delivery of C6 ceramide in LNCs reduced cell viability when tested on MDA-MB-231, compared to the LNC-ghost. The IC50 decreased ca.1.5-fold and 2-fold for LNC50-C6 and LNC100-C6, respectively. Moreover, annexin V/ PI staining assay confirmed that C6 ceramide induced cancer cell death through an apoptosis mechanism. Both LNC-C6 formulations induced a greater response, compared to the LNC-ghost of similar size and composition. The percentages of total annexin-V positive cells were increased from 16.53% (LNC50-ghost) to 27.80% (LNC50-C6) and 8.43% (LNC100-ghost) to 16.30% (LNC100-C6) after 24 hours for MDA-MB-231 cells treated with the same nanoparticle concentration (1.25 mg/mL), corrected for ceramide content (12.5 μM) in LNC-C6. |
| **Conclusions:** Lipid nanocapsules showed great potential as nanocarriers for ceramide delivery that induced pro-apoptotic effect in breast cancer cells and offer a viable platform for future development of anticancer drug delivery systems. |