Triptolide and Celastrol Loaded Silk Fibroin Nanoparticles: Formulation Development and Synergistic Effect Evaluation on Human Pancreatic Cells

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Purpose
Pancreatic cancer is one of the most deadly malignancies in the world. Though there are several available treatment options (surgery, chemotherapy, and radiation therapy), the prognosis remains extremely poor. Recently, the FDA has approved the combination therapy of gemcitabine with Abraxane® that can only increase the overall survival to 8.5 months. Therefore, novel therapy strategies are urgently needed to combat this deadly disease. The objective of our study was to formulate and study the anti-cancer effects of silk fibroin nanoparticles of triptolide (TPL) and celastrol (CL), two main anticancer agents in traditional Chinese medicine thunder god vine.

Methods
TPL and CL nanoparticles (TPL-NPs and CL-NPs) were prepared by the desolvation method. Both nanoparticles were characterized in terms of particle size, zeta potential, and morphology using dynamic laser spectroscopy (DLS) and transmission electron microscope (TEM). The entrapment efficiency, drug loading, and drug release profiles were determined using HPLC. The cytotoxicity and synergistic effect was investigated on MIA PaCa-2 and PANC-1 human pancreatic cell lines. Hemolysis test was conducted with fresh mouse blood.

Results
TPL-NPs and CL-NPs had a drug loading of 57.0 ± 4.7 and 63.5 ± 3.8 μg/mg, along with an encapsulation efficiency (EE) of 81.8 ± 2.8 and 87.0 ± 5.1%, respectively. Particle size of TPL-NPs and CL-NPs was 166.37 ± 4.57 and 170.44 ± 2.31 nm, with a mean zeta potential -27.2 ± 1.96 and -25.5 ± 2.57 mV, respectively. TEM images indicate that the nanoparticles were spherical in shape and monodisperse. The drugs were gradually released from the nanoparticles at pH 7.4 (plasma pH) and rapidly released at pH 4.5 (lysosomal pH). The cytotoxicity study results indicate that TPL-NPs (IC_{50} 3.8 and 4.8 nM) and CL-NPs (IC_{50} 0.37 and 0.63 μM) were 2-3 fold more potent against MIA PaCa-2 and PANC-1 cells than the free drugs TPL (IC_{50} 11.3 and 11.6 nM) and CL (IC_{50} 0.83 and 1.2 μM). The blank nanoparticles were non-toxic. Furthermore, co-treatment with TPL-NPs and CL-NPs increased the growth inhibition of cancer cells significantly in comparison with TPL-NPs or CL-NPs alone. Almost all combination index (CI) values, calculated using the Compusyn Software, were < 1, indicating that the growth inhibition effect of TPL-NPs in combination with CL-NPs was synergistic rather than additive. The hemolysis test demonstrated the nanoparticles were biocompatible with blood.

Conclusion
TPL and CL loaded silk fibroin nanoparticles were successfully formulated. These nanoparticles showed outstanding synergistic anticancer effect in pancreatic cancer cells with no sign of hemolytic effect, suggesting that this combination may offer a potential treatment for pancreatic cancer.