DOCTORAL THESIS

Development of halofuginone, artesunate liposomes and crocetin γ-cyclodextrin inclusion complex

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ABSTRACT

The water solubility of drug molecules plays an important role in consideration of formulation development to treat a wide range of diseases. In this project, two kinds of drug delivery systems, cyclodextrins and liposomes, were developed for insoluble drug delivery to treat Alzheimer's disease (AD) and colorectal cancer (CRC), respectively.

AD is an irreversible neurodegenerative disorder associated with the accumulation of amyloid-beta (Aβ) fibrils. Approximately 10% of people aged 65 and above have AD. Crocetin (CRT) is an active compound isolated from the fruits of gardenia (*Gardenia jasminoides* Ellis) and the stigmas of saffron (*Crocus sativus* L.). It has been reported to show various neuroprotective activities. However, poor water solubility and bioavailability are the major obstacles in developing pharmaceutical formulations of CRT. To address the issues, CRT liposomal formulations and CRT-cyclodextrin inclusion complexes were developed and evaluated. CRT-cyclodextrin inclusion complexes significantly increased the water solubility of CRT from the range μg/mL to mg/mL. The CRT-γ-cyclodextrin inclusion complex (1:3 molar ratio of CRT/γ-cyclodextrin) was chosen for further studies as it showed the highest encapsulation efficiency (94.73 ± 0.86%). The formulation had no toxicity to neuronal cells nor AD model cells within the experimental concentration range (0.625 to 100 μM of CRT). It could downregulate the expression of C-terminus fragments and decrease both intracellular and extracellular levels of Aβ, which are hallmarks of AD. It also showed dose-dependent neuroprotective and antioxidant effects against H_2O_2-induced cell death. Pharmacokinetics and biodistribution studies showed that this CRT-γ-cyclodextrin inclusion complex was suitable for intravenous administration. The

formulation significantly increased the bioavailability of CRT and facilitated CRT crossing the blood-brain barrier to enter the brain.

Similar to AD, CRC is increasingly prevalent with aging populations. Approximately 60% of CRC patients are aged 70 and above. Halofuginone (HF) is an active pharmaceutical ingredient (API) originated from Chinese quinine (*Dichroa febrifuga* Lour.) and artesunate (ART) is a semi-synthetic derivative of artemisinin (ATS) extracted from annual wormwood (*Artemisia annua* L.). Both APIs show anticancer activities by inhibiting the growth of CRC. However, low aqueous stability limits their applications. Liposome formulation with surface functionalization by CPP2 cell-penetrating peptide was developed to deliver HF and ART for targeted CRC therapy. CPP2 is a peptide that can selectively penetrate colon cancer cells. The liposomal drug formulations had uniform particle size (about 100 nm), high encapsulation efficiency (over 80%) and good stability upon 14 days of storage. In cellular uptake study, CPP2-modified liposome showed stronger permeability and selectivity to colon cancer lines without inducing lysosomal degradation. CPP2 surface-modified liposomal drugs demonstrated greater anticancer activities than free form of drugs or conventional liposomal drugs. Combinations of HF and ART formulations notably decreased cancer cell viability as compared to single formulation alone, which indicated that HF and ART formulations exhibited synergistic anticancer effects at specific ratios.

To conclude, the drug delivery systems, cyclodextrins and peptide-modified liposomes, which were developed for AD and CRC treatment, successfully improved the aqueous solubility of insoluble APIs extracted from Chinese medicinal plants.

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