Acid-responsive micelle-forming polymers as new anticancer theraperutics

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Statement of Purpose: Cancer is a major cause of mortality world-wide and is responsible for approximately 13% of all deaths, according to the World Health Organization. Cinnamaldehyde is a major component in cinnamon which is an important dietary factor and food additive. Interestingly, a number of studies have shown that cinnamaldeyhyde and its analogues inhibit growth of various human cancer cells and induce apoptotic cell death through ROS(reactive oxygen species) generation. Despite its potent anticancer activities, the use of cinnamadlehyde in clinical applications is limited by its poor stability and lack of specificity toward diseased tissues. One of strategy for controlled drug delivery involves polymeric prodrugs, in which therapeutic drugs are covalently incorporated into the backbone of biodegradable polymers. We present a polymeric prodrug of cinnamaldehyde, poly(cinnama-ldehyde β-amino ester)-co-poly(ethylene glycol)(PCAE), which causes ROS-mediated apoptotic cell death. PCAE incorporates covalently cinnamaldehyde in the hydrophobic backbone through an acetal linkage. It was designed to have two groups and acid-cleavable acetal linkages. PCAE are self-assembled in aqueous solutions to form stable micelles, which dissociate and release cinnamaldehyde at acidic pH. Here, we report the potential of dual pH-responsive micelle-forming PCAE as anticancer drugs and drug carriers.

Methods: PCAE was produced by two steps. First, we synthesized cinnamaldehyde containing diacrylate monomer and methoxy PEG monoacrylate. PCAE was synthesized from a Michael-type addition polymerization of cinnamaldehde containing diacrylate monomer, methoxy PEG monoacrylate and trimethylene dipiperidine in a 0.9:0.1:1.0 mixture. The chemical structure of PCAE block copolymers and micelle formation was confirmed by ^{1}H NMR. Its average molecular weight was determined using gel permeation chromatography. We therefore assessed the ability of PCAE micelles to induce the generation of ROS by confocal laser scanning microscopy (CLSM) using DCFH-DA. In order to investigate whether PCAE micelles induce apoptosis, flow cytometry was performed using fluorescein isothiocyanate (FITC)-labeled annexin V (annexin V-FITC) and propidium iodide (PI) as an apoptosis marker and a cell viability marker, respectively. We also performed the MTT assay to evaluate the cytotoxic effects of PCAE micelles on SW620 cells.

Results: The average molecular weight of PCAE was determined to be ~10,000*Da* with a polydispersity of ~1.3. PCAE was self-assembled to form thermodynamically stable micelles at a concentration higher than ~5μg/mL. The micelles were monodispersed spheres, with a mean hydrodynamic diameter of ~90 nm. PCAEG micelles showed a pH-dependent micellization/ demicellization behavior and cinnamadlehyde release kinetics due to the presence of amine groups and acid0labile acetal linkages.

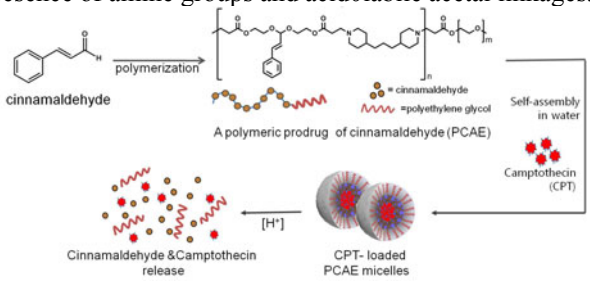
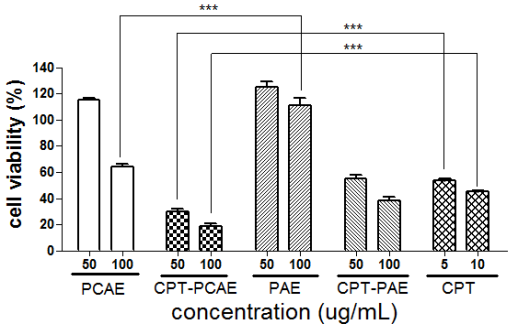


Figure 1. A diagram of dual pH-responsive PCAE micelles new anticancer therapeutics

Cinnamaldehyde and PCAE micelles induced the ROS generation in SW620 cells, evidenced by CLSM and flow cytomety. We also investigated the ability of PCAE micelles to deliver anticancer drugs, camptothecin (CPT) as a model drug, which triggers ROS generation to induce apoptotic cell death. CPT was loaded at a concentration of 10 wt% of micelles with ~90% encapsulation efficiency. CPT-loaded PCAE micelles induced significantly more ROS generation and apoptotic cell death than free CPT and PCAE micelles, suggesting that PCAE micelles have potential as drug carriers and are able to exert synergistic effects with CPT on ROS-mediated apoptotic cell death generation.



Frigure 2. Cell viability assay of SW620 cells.

Conclusions: we have developed, for the first time, polymeric prodrug micelles, which are able to serve as anticancer drugs and drug carriers. PCAE incorporates cinnamaldehyde in its pH-sensitive backbone via acid-cleavable acetal linkages and self-assembled to form stable micelles which encapsulate CPT. PCAE micelles induced apoptotic cell death through the generation of intracellular ROS and their apoptotic activities were significantly enhanced with a payload of CPT. We anticipate that dual pH-responsive PCAE micelles are able to serve as anticancer drugs as well as drug carriers and have enormous potential as novel anticancer therapeutics.

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