Oxidative stress amplifying polymeric micelles as novel anticancer therapeutics

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ABSTRACT SUMMARY

Modulating the level of oxidative stress provides a logical therapeutic strategy to kill cancer cells specifically. We synthesized acid-sensitive polymeric anticancer prodrug, amphiphilic PBCAE which induces ROS (reactive oxygen species)-mediated apoptosis. PBCAE self-assembles to form micelles which are able to encapsulate an antioxidant heme oxygenase-1 (HO-1) inhibitor, zinc protoporphyrin (ZnPP). ZnPP-loaded PBCAE micelles amplify oxidative stress in cancer cells to induce apoptotic cell death and hold great promise as novel anticancer therapeutics.

INTRODUCTION

ROS are a collective term of highly reactive chemical species produced as byproducts of normal oxygen metabolism, including hydrogen peroxide, superoxide anion and hydroxyl radical. Despite their essential and beneficial roles in cellular signaling and biological processes, overproduction of ROS causes oxidative stress and promotes carcinogenesis and tumor development by amplifying genomic instability. Cancer cells are therefore under oxidative stress and the elevated level of ROS is used to stimulate cell proliferation and maintain cancer phenotype. Ironically, many anticancer drugs are known to elevate ROS level to induce apoptotic cell death. However, cancer cells have adapted to oxidative stress by up-regulating cellular antioxidant systems such as glutathione, superoxide dismutase, catalase and HO-1 to counteract the damaging effects of ROS. In addition, the enhanced endogenous antioxidant systems make cancer cells resistant to anticancer therapies that depend on inducing ROS stress. Therefore, amplifying oxidative stress by elevating the ROS level along with depletion of antioxidant systems is an alternative therapeutic strategy to kill cancer cells through ROS-mediated apoptosis.

Benzoyloxyccinnamaldehyde (BCA) is an analogue of cinnamaldehyde, which is a major component of cinnamon and exerts anticancer activity through ROS-mediated apoptosis. BCA inhibits growth of various human cancer cells and shows stronger anticancer activity than cinnamaldehyde. There has been increasing interest in the use of cinnamaldehyde and its derivatives as an alternative cytotoxic agent due to its potential anticancer activity and excellent biocompatibility. However, its clinical applications are limited by their poor stability and lack of specificity toward diseased tissues. In order to overcome these drawbacks, we developed acid-sensitive anticancer polymeric prodrug of BCA (PBCAE), which possesses BCA covalently via acid-cleavable acetal linkages and protonable amine groups in its hydrophobic backbone. In addition, PBCAE was designed to self-assemble to form stable micelles under aqueous conditions and be activated by acidic tumor environments to release ROS-generating BCA. ZnPP is one of endogenous metalloporphyrins and is known to inhibit antioxidant HO-1, which is overexpressed in many human cancer cells and protects cancer cells from oxidative stress by generating potent antioxidant bilirubin. Inhibition of HO-1 is, therefore, a potential therapeutic strategy to kill cancer cells specifically and there is increasing interest in the use of ZnPP. In this regard, we developed oxidative stress amplifying PBCAE micelles, which encapsulate ZnPP in their hydrophobic core and dissociate at acid pH to release BCA and ZnPP. We reasoned that BCA generates ROS and ZnPP inhibits HO-1, which leads to amplified oxidative stress and enhanced apoptotic cell death. We demonstrated the synergistic anticancer effects of BCA and ZnPP using a cell culture model and a mouse xenograft model.
**EXPERIMENTAL METHODS**

We first synthesized BCA containing diacrylate and methoxy PEG monoacrylate. PBCAEE was synthesized from a Michael addition polymerization of BCA containing diacrylate, methoxy PEG monoacrylate and trimethylene dipiperidine in a 0.95:0.05:1.0 mixture. The ability of PBCAE micelles to induce the generation of ROS was studied by confocal laser scanning microscopy (CLSM) using DCFH-DA as a ROS probe. We performed western blot analysis to evaluate the effects of ZnPP-loaded PBCAE micelles on the apoptosis of SW620 and DU145 cells. The therapeutic efficacy of ZnPP-loaded PBCAE micelles was evaluated using SW620 cancer cells bearing mice.

**RESULTS AND DISCUSSION**

The average molecular weight of PBCAE micelles were determined to be ~10,000Da with a polydispersity of ~1.3. PBCAE was self-assembled to form thermodynamically stable micelles at a concentration higher than ~8µg/mL. Dynamic light scattering and TEM revealed that the micelles have an average diameter of ~115 nm. PBCAE micelles showed a pH-dependent micellization/demcillization behavior and BCA release kinetics.

![Figure 2. Cleavage of pro-apoptotic proteins in (A) SW620 cells and (B) DU145 cells treated with ZnPP-loaded PBCAE micelles, a) Untreated, b) ZnPP 5 µg/mL, c) BCA 20 µg/mL, d) BCA and ZnPP, e) PBCAE micelles 50 µg/mL, f) PBCAE micelles 100 µg/mL, g) ZnPP-loaded PBCAE micelles](image)

BCA and PBCAE micelles induced the ROS generation in SW620 cells in a dose dependent manner. ZnPP also showed moderate effects on ROS generation. However, ZnPP-loaded PBCAE micelles showed the remarkably enhanced ROS generation. ZnPP-loaded PBCAE micelles also showed significantly higher cytotoxicity than BCA and ZnPP. In order to further confirm apoptotic cell death of BCA and ZnPP, the expression of pro-apoptotic proteins such as caspase-3 and PARP-1 was investigated. ZnPP-loaded PBCAE micelles showed the stronger apoptosis inducing effects than free ZnPP and PBCAE micelles (Fig. 2). Next, we evaluated potential of ZnPP-loaded PBCAE micelles as novel anticancer therapeutics using a xenograft tumor mouse model. PBCAE micelles passively target the tumor site by EPR effects and then release BCA which induces apoptotic cell death. Treatment with ZnPP-loaded PBCAE micelles exhibited more reduction in tumor volume than free ZnPP and empty PBCAE micelles (Fig. 3), suggesting the synergistic effects of BCA with ZnPP.

![Figure 3. Anticancer effects of ZnPP-loaded PBCAE micelles in vivo. (A) Representative images of tumor bearing mice treated with various formulations, a) Untreated, b) ZnPP 5 µg, c) BCA 20 µg, d) BCA and ZnPP, e) PBCAE micelles 100 µg, f) ZnPP-loaded PBCAE micelles. (B) Change in tumor volumes of tumor bearing mice. (C) Biodistribution of ZnPP-loaded PBCAE micelles in tumor bearing mice.](image)

**CONCLUSIONS**

We have developed polymeric prodrug micelles, which are able to serve as anticancer drugs and drug carriers. PBCAE incorporates BCA in its pH-sensitive backbone via acid-cleavable acetal linkages. PBCAE is able to self-assemble to form stable micelles which encapsulate antioxidant HO-1 inhibitor, ZnPP. ZnPP-loaded PBCAE micelles induced apoptotic cell death through the generation of intracellular ROS and their apoptotic activities were significantly enhanced with a payload of ZnPP.

**REFERENCES**


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