Paclitaxel and carboplatin co-loaded nanovesicles prepared with novel multifunctional amphiphilic phosphonated calix[4]arene

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ABSTRACT SUMMARY
A novel modularly clickable phosphonated calix[4]arene was synthesized for the purpose of developing a vesicle formulation of paclitaxel (PTX) and carboplatin (CPT) to obtain synergy for the treatment of ovarian cancer. PTX and CPT-co-loaded vesicles of the phosphonated calixarene measuring 118 ± 6 nm were prepared at (calixarene (compound 1, fig. 1)+CPT):PTX ratios of 50:1 w/w. High PTX and CPT loading and pH-responsive drug release can be achieved.

INTRODUCTION
Paclitaxel is a highly effective drug for the treatment of a wide variety of tumors, including ovarian, breast, head and neck and lung cancers.¹ One of its biggest shortcomings, however, is a low aqueous solubility, and much effort has been expended to develop novel formulations to ameliorate the Cremophor EL-induced toxicity of the Taxol™ product. Carboplatin combined with paclitaxel is an accepted standard treatment for patients with advanced ovarian cancer, but there is no formulation to date that incorporates both drugs into one dosage form. Calixarenes are bowl-shaped macrocyclic molecules with emerging potential for biomedical applications. The central scaffold of the calixarene molecules is readily synthesized from the condensation of commercially available phenols and aldehydes, and it can be elaborated at the lower and upper rims to impart desirable physicochemical properties.

In this paper, we seek to fabricate via click chemistry a multifunctional supramolecular vesicle with a novel amphiphilic phosphonated cali[4]xarene molecule (Figure 1). In the development of targeted delivery platforms, ligand attachment to the carrier is best accomplished under mild reaction conditions that preserve the integrity of all associated molecules. This requirement is easily fulfilled by click chemistry.

Figure 1: Scheme showing the attachment of multifunctional groups to the amphiphilic O-octyl p-Phosphonated calix[4]arenes by click chemistry

Functional groups attached to the upper rim of the phosphonated calixarene carrier (Figure 1) provide a targeted theragnostic system (Figure 2) to co-deliver paclitaxel and carboplatin for the treatment of ovarian tumors.

Figure 2. Diagram depicting the multifunctional vesicles

EXPERIMENTAL METHODS
5,11,17,23-Tetra-diethylphosphonomethyl-25,26,27,28-tetra-octyloxy calix[4]arene (P4C8) and modularly clickable phosphonated
calix[4]arene were synthesized and characterized using methods established in our laboratory. P4C8 and CPT separately dissolved in dilute NaOH (pH 10) were mixed at equimolar concentration (10 mM) by variable angle rotating tube processing (VARTP, Figure 3) (45°, 5000 rpm, 10 min) to yield host-guest complexes (HGC).

Figure 3 Schematic diagram of VARTP

Vesicles containing paclitaxel in combination with HGC were prepared hydration of a lipid film, followed by extrusion through polycarbonate filters with 100 nm pore size. Triplicate batches of vesicles were analyzed by particle size (DLS, Malvern Zetasizer Nano ZS, Malvern, UK), morphology and drugs loading. Simultaneous analysis of carboplatin and paclitaxel were developed by reverse phase HPLC on C-18 column with a mobile phase comprising of water–acetonitrile run on gradient mode at a flow rate of 1 ml/min at 227 nm. The drugs loads are calculated as the weight of the drug encapsulated in the vesicles divided by the total weight of the vesicles. The unit of drug load is μg drug per mg vesicles. For in vitro drugs release study, the multi-functional vesicles were dispersed in buffers with different pH values (pH=7.4 or pH=5) containing 0.1% v/v Tween-80 and 5 ml of solution was placed in a dialysis bag. The incubation buffer was collected and replaced by fresh incubation buffer at every designated time points. The released drugs in collected buffer were measured by HPLC following the same procedure mentioned above.

RESULTS AND DISCUSSION

P4C8 was obtained as a white fluffy powder at a final yield of approximately 65%. The molecular weight and chemical structure of P4C8 and HGC were confirmed using high resolution mass spectra, and 1H NMR and 13C NMR spectra. Blank P4C8 vesicles (104 ± 5 nm) were successfully prepared when the P4C8 thin film was rehydrated with Milli Q water. Once the vesicles were assembled, they could be stably reconstituted in distilled water. Vesicle preparation in the presence of paclitaxel was successful when the HGC:paclitaxel weight ratio was ≥ 50:1. High drugs loadings (the paclitaxel load is 20 μg/mg and the carboplatin load is 52 μg/mg) were obtained for HGC:paclitaxel weight ratios of 50:1, the corresponding vesicles having particle sizes of 118 ± 6 nm. The in vitro drugs release profiles of multi-functional vesicles at different release buffers in 72 h are shown in Fig. 4.

Fig. 4. Cumulative release profiles of paclitaxel and carboplatin-loaded multi-functional vesicles at pH=7.4 and pH=5 buffers.

CONCLUSION

The novel multifunctional amphiphilic phosphonated calix[4]arene, P4C8, is capable of self-assembly into nano-vesicles in aqueous media for the efficient co-encapsulation of paclitaxel and carboplatin, which show a pH-sensitive release of paclitaxel and carboplatin.

REFERENCES

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