ABSTRACT SUMMARY

A series of β-lapachone (β-lap) prodrugs with diester structures were synthesized and encapsulated into polymeric micelles with high drug loading content and efficiency. In the presence of esterase, β-lap prodrugs can be efficiently converted to β-lap. In vitro study showed the prodrug micelles can selectively kill NQO1-overexpressing cancer cells and in vivo data showed lower toxicity and better antitumor efficacy in animal tumor models over HP-β-CD•β-lap complex (ARQ501).

INTRODUCTION

β-Lap is a novel therapeutic agent that kills cancer cells through a p53-, cell cycle-, and caspase-independent process. Prior studies showed that its mechanism of action is dependent on the expression of NAD(P)H: quinone oxidoreductase-1 (NQO1), a two-electron reductase enzyme that protects against quinone toxicity.\(^1\) NQO1 is highly elevated in a variety of tumors (up to 20-fold) over normal adjacent tissue, including those of the pancreas, lung, breast, and prostate.

The clinical use of β-lap is greatly hampered by the low water solubility (0.038mg/mL), the instability of the drug in gut and blood. Current delivery system for β-lap includes HP-β-CD•β-lap complex and polymeric micelles, which significantly enhanced the drug solubility or antitumor efficacy.\(^2\) However, HP-β-CD•β-lap complex shows narrow therapeutic window and acute toxicity. Here, we synthesized a series of β-lap prodrugs with diester structures. We anticipate that β-lap prodrugs with different chain lengths will be more compatible to PEG-PLA micelles to achieve high loading density. Our central hypothesis is that optimal β-lap prodrug micelles can achieve successful preclinical safety and antitumor efficacy outcomes in animal tumor models, which will provide critical data to initiate the clinical evaluation in cancer patients.

EXPERIMENTAL METHODS

β-Lap prodrugs and PEG-PLA block copolymer (Mn = 10,000 Da) were synthesized following a previously reported procedure. Solvent evaporation and film hydration methods were used for the prodrug micelles. Micelle size was determined by dynamic light scattering (DLS). H596 non-small cell lung carcinoma (NSCLC) cells were grown in DMEM with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/mL penicillin, and 100 mg/mL streptomycin at 37 °C in a 5% CO\(_2\)-95% air atmosphere. Cell survival was measured using a DNA assay as described.\(^2\) All animal procedures adhered to NIH guidelines, following approved protocols by the Institutional Animal Care and Use Committee at the University of Texas Southwestern Medical Center at Dallas. Antitumor efficacy of dC3 was performed in 6- to 8-week-old randomized tumor-bearing female nude scid mice (~15 g each). Log-phase A549 cells (1 × 10\(^6\)) were injected into mice via tail vein. Day 0 was designated as the first day of injection of tumor cells. Animals were treated with blank micelle, 25 mg/kg HP-β-CD•β-lap and 30, 50 and 65 mg/kg dC3 micelles administered i.v. via tail vein and repeated five times every other day over 9 days. Animals were monitored daily for survival.

RESULTS AND DISCUSSION

Diester prodrugs were synthesized with high yields (Fig. 1a). Both solvent evaporation and film hydration methods generated prodrug micelles with adequate size (<200 nm in diameter), high drug loading density and efficiency. From an initial 15% loading density, all prodrug loading density were more than 10% and loading efficiency more than 80% except dC2. All the prodrugs and micelle-delivered prodrugs can be converted to its parent drug β-lap in the
In vitro cytotoxicity showed that after a 2 h incubation, dC3 and dC6 micelles did not yield observable toxicity in either NQO1(+) or NQO1(−) cells (Fig. 2a). In the presence of 5 U PLE in the cell culture medium, the IC50 values of dC3 and dC6 micelles in NQO1(+) H596 nonsmall cell lung cancer (NSCLC) cells were 4.5 µM and 3.6 µM, respectively, and comparable to free β-lap. More important, their IC50 values in NQO1(−) H596 cells were >20 µM, indicating a larger therapeutic window over free β-lap.

In vivo studies in mice bearing A549 nonsmall cell lung tumors (five i.v. injections every other day of 65 mg/kg dC3 micelle or 100 mg/kg dC6 micelle) resulted in no deaths of healthy NOD/SCID mice. In comparison, the safe dose of HP-β-CD•β-lap complex was 25 mg/kg. The antitumor efficacy of β-lap prodrug micelles was then investigated in NOD/SCID mice bearing A549 NSCLC orthotropic tumors. Animal survival study shows that 50% of control animals died at day 112, while for 30 mg/kg dC3 micelle and 25 mg/kg HP-β-CD•β-lap complex, the 50% survival times were 138 and 139 days, respectively. In contrast, the medium survival times for animals treated with 50 and 65 mg/kg dC3 micelles were 162 and 200 days, respectively (Fig. 2b). Importantly, Kaplan-Meier curves indicate a statistically significant (p ≤ 0.0004) survival advantage with dC3 micelles over micelle carrier alone or HP-β-CD•β-lap complex.

CONCLUSIONS
A series of β-lap prodrugs were synthesized and successfully loaded into polymeric micelles with high loading density and efficiency. Micelle-delivered prodrugs were effectively converted into β-lap in the presence of esterase. In vitro cytotoxicity study showed that after 2 h incubation, prodrug micelles had a significantly increased toxicity in NQO1(+) cells over NQO1(−) cells. The safety and antitumor efficacy of β-lap prodrug micelles was dramatically increased as shown in an orthotropic nonsmall cell lung cancer model in mice.

REFERENCES

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