

β -Lapachone Polymer Micelles as Novel Nanotherapeutics for Lung Cancer

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Statement of Purpose: Non-small-cell lung cancers (NSCLCs) account for >80% of lung cancer deaths, warranting more effective, tumor-specific treatment modalities to combat the disease. NSCLC has been found to overexpress the enzyme NAD(P)H:quinone oxidoreductase-1 (NQO1), making the enzyme an exploitable target for therapy.¹ β -Lapachone (β -lap) is a novel anticancer drug whose cytotoxic effect is significantly enhanced by the expression of NQO1. However, its poor aqueous solubility (0.04 mg/mL), as well as hemolysis resulting from a conventional formulation of β -lap complexed with hydroxypropyl- β -cyclodextrin (HP β -CD), limits its clinical translation. Our objective was to develop β -lap-containing polymer micelles to target lung cancer in a tumor- and site-specific manner using an NQO1 bioactivatable drug and highly efficacious delivery vehicle. We hypothesize that β -lap-containing micelles will provide for an effective nanotherapeutic platform for treatment of NQO1-overexpressing lung tumors.

Methods: Poly(ethylene glycol)-b-poly(D,L-lactic acid) (PEG5kD-PLA5kD) was synthesized using a ring-opening polymerization procedure, and β -lap-PEG-PLA micelles were fabricated using a film sonication method.² Micelle size was determined using dynamic light scattering (DLS) and verified via transmission electron microscopy (TEM). Core-shell architecture and encapsulation of β -lap inside of micelles was demonstrated via ¹H-NMR. *In vitro* release studies of β -lap PEG-PLA micelles were performed at 37°C in PBS at pH 7.4. β -lap micelle cytotoxicity following a 2 h treatment was examined *in vitro* in NQO1-expressing (NQO1+) and NQO1-null (NQO1-) H596 and A549 lung cancer cells. Hemolysis of β -lap in different formulations was examined *in vitro* following a 1 h incubation at 37°C in red blood cells. *In vivo* antitumor efficacy of a 30 mg/kg dose of a micellar formulation (given e.o.d. over the course of 8 d) was examined in female nude mice (~25 g) containing subcutaneous A549 lung tumors (100 mm³) injected in both flanks.

Results: Resulting β -lap micelles were of appropriate size (42 \pm 6 nm) and TEM shows spherical morphology and sample homogeneity. Micelle core-shell formation and encapsulation of β -lap was demonstrated by ¹H-NMR, where micelle samples in deuterated chloroform (CDCl₃) showing prominent resonance peaks of β -lap, PLA, and PEG, while samples in deuterated water (D₂O) showing only PEG peaks. Release kinetics of β -lap from micelles show a diffusion-based release profile, with a time for 50% of drug release ($t_{1/2}$) of 18 hours. *In vitro* cytotoxicity data showed that after a 2 h incubation with β -lap micelles, a marked increase in toxicity was shown in NQO1+ H596 and A549 cells over NQO1- cells, resembling free drug. *In vitro* hemolysis assays show that β -lap•HP β -CD indeed caused hemolysis (52 \pm 2% at a 1.5 mg/mL dose), but that hemolysis arised mainly from the

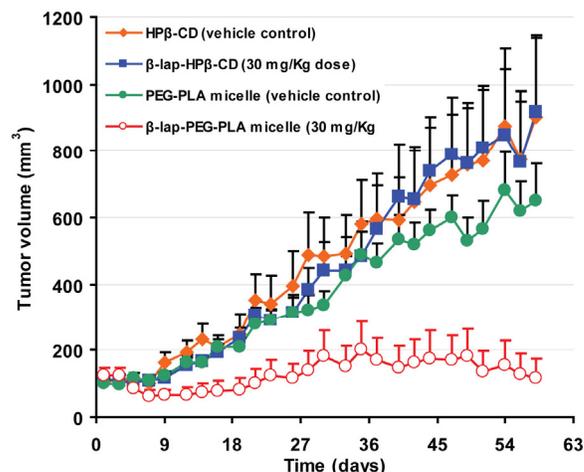


Figure 1. Tumor volume over time of A549 NQO1+ lung tumors after β -lap micelle administration.

HP β -CD vehicle, which by itself caused a 94 \pm 0.9% hemolysis at the aforementioned dose. By comparison, β -lap micelles did not lead to any perceivable hemolysis. *In vivo* data demonstrate that the micellar formulation of β -lap (30 mg/Kg) maintained tumor size at an average volume of 116 \pm 75 mm³ for 58 days after the initial injection. This contrasted remarkably with the vehicle (PEG-PLA micelles) and free β -lap (β -lap•HP β -CD) controls, whose tumors grew to average volumes of 651 \pm 160 mm³ (p-value = 0.001) and 916 \pm 313 mm³ (p-value = 0.005), respectively, after 58 days. It is important to note that minimal weight loss occurred in mice treated with micelles and that no statistical difference in weight loss was observed among groups.

Conclusions: Results from this study demonstrate the potential for a viable, novel nanotherapeutic platform of β -lap for the treatment of NQO1-overexpressing lung tumors. The micelles developed demonstrated favorable size ideal for preferential accumulation of micelles at tumor sites following IV injection through the enhanced permeability and retention (EPR) effect. Moreover, the outer corona of PEG proves highly effective at preventing micelle aggregation and protein adsorption, which can lead to non-specific uptake by the reticular endothelial system (RES) and shortened circulation times. Indeed, when tested *in vivo*, the micellar formulation proved effective at suppressing tumor growth for 58 days, far surpassing an alternate conventional form of the drug. Ongoing and future work consists of examination of efficacy in lung orthotopic models of mice, as well as pharmacokinetic studies of the micellar formulation.

References:

1. E. A. Bey *et al.*, *Proc Natl Acad Sci U S A* 2007 (**104**, 11832-11837).
2. E. Blanco *et al.*, *J Control Release* 2007 (**122**, 365-374).