β-Lapachone 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 and tumor-specific treatment modalities to combat the disease. NSCLC overexpresses 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 (HPβ-CD), limits 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 ^{1}H-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 lung cancer cells. Hemolysis of β-lap was examined *in vitro* following a 1 h incubation at 37°C in red blood cells. Pharmacokinetics of micelles was examined in subcutaneous tumor-bearing female nude mice (~25 g) mice at 2 and 24 h. *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 mice containing subcutaneous A549 lung tumors (100 mm^3) injected in both flanks.

Results: Resulting β-lap micelles (30 ± 6 nm) showed spherical morphology. Micelle core-shell formation and encapsulation of β-lap was demonstrated by ^{1}H-NMR, where micelle samples in deuterated chloroform ($CDCl_3$) showing prominent resonance peaks of β-lap, PLA, and PEG, while samples in deuterated water (D_2O) 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 cells over NQO1- cells, resembling free drug. *In vitro* hemolysis assays show that β-lap•HPβ-CD indeed caused hemolysis (52 ± 2% at a 1.5 mg/mL dose), but that hemolysis arised mainly from the HPβ-CD vehicle, which by itself caused a 94 ± 0.9% hemolysis at the aforementioned dose. By comparison, β-lap micelles did not lead to any perceivable hemolysis. Pharmacokinetic analysis shows that β-lap micelles were long-lived in the blood ($t_{1/2}$ ~ 28 h) and accumulated comparably well in tumors, showing long retention. *In vivo* data demonstrate that the micellar formulation of β-lap (30 mg/Kg) maintained tumor size at an average volume of 116 ± 75 mm^3 for 58 days after the initial injection. This contrasted remarkably with the vehicle (PEG-PLA micelles) and free β-lap (β-lap•HPβ-CD) controls, whose tumors grew to average volumes of 651 ± 160 mm^3 (p-value = 0.001) and 916 ± 313 mm^3 (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.

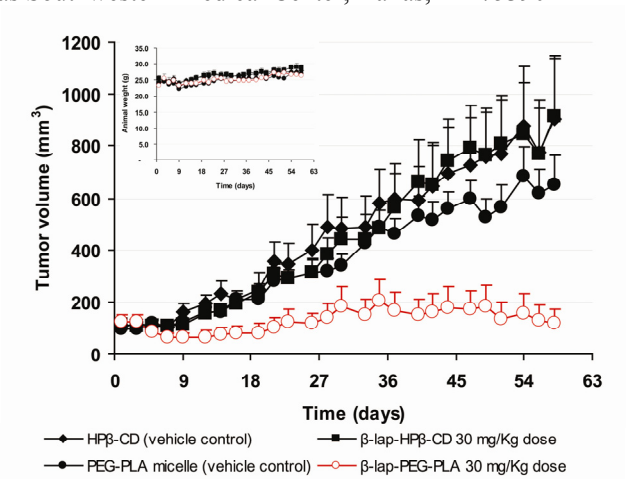

Figure 1. In vivo antitumor efficacy of β-lap micelles injected IV in nude mice bearing A549 subcutaneous tumors. The figure inset represents recorded weights of animals.

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. 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.

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).