Comparison and Evaluation of Lopinavir, Atazanavir and Darunavir in pH-sensitive Lipid Nanoparticles containing Ritonavir

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**Purpose**
Current combination anti-HIV drug therapy is effective at clearing the virus from systemic circulation, but residual virus persists in lymphoid tissues. We have previously developed anti-HIV lipid nanoparticles (LNPs) with bound indinavir that demonstrate pH responsive release and drug accumulation in lymph nodes (Kinman et al 2003 & 2006). Our purpose was to evaluate three clinically-used HIV protease inhibitors, lopinavir (LPV), atazanavir (ATV) and darunavir (DRV), for pH sensitivity, stability and incorporation into LNPs intended to accumulate in lymphoid tissues.

**Methods**
The LNPs were prepared with disteroylphosphatidylcholine and methylpolyethyleneglycol disteroylphosphatidylethanolamine (DSPC:mPEG-DSPE 8:2 mole ratio) and LPV, ATV, or DRV plus RTV(115:10:5 lipid:LPV/ATV/DRV:RTV mole ratio) with thin film rehydration. The diameter of LNPs was measured with photon correlation spectroscopy (NicompTM 380ZLS). Drug encapsulation efficiency and pH sensitivity were determined with dialysis method followed by liquid chromatography tandem mass spectroscopy assay.

**Results**
Incorporation efficiency of LPV, ATV, and DRV were 98±0.3%, 98.5±3.4%, 97.2±6.4%, respectively, and 105.2±1.3% for RTV. The size of LNPs were 82.6±31.1 nm for LPV-RTV LNPs, 63.9±5.0 nm for ATV-RTV LNPs, and 23.7±1.8 nm for DRV-RTV LNPs. pH and osmolarity were evaluated to confirm physiological compatibility. Both LPV and ATV showed pH responsive release with half-maximum recorded at 5.5 at 37°C. LPV and ATV incorporated in RTV nanoparticles are stable in storage over 2 weeks, but DRV-RTV LNPs demonstrated less stability.

**Conclusion**
These data indicate that LPV, ATV, and DRV can incorporate completely in DSPC:mPEG-DSPE LNPs and exhibit pH-dependent dissociation from LNPs containing RTV. LPV and ATV appeared to associate stronger to LNPs containing RTV than DRV with the present composition. The size, pH, and osmolarity of all these anti-HIV LNPs, particularly those containing LPV and ATV, are suitable for clinical development.

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