Blended polyester nanoparticles to modulate retinoid signaling in a transgenic murine model of ALS

David X. Medina, Eugene P. Chung, Ricki Ceton, Robert P. Bowser, Rachael W. Sirianni
Barrow Brain Tumor Research Center, Phoenix AZ; Arizona State University, Tempe AZ

Statement of Purpose: Here, we sought to design drug delivery systems to study the neuroprotective role of retinoic acid (RA) signaling in amytrophic lateral sclerosis (ALS). RA is a derivative of vitamin A that has important roles in both neuronal development and neurodegeneration[1]. Recent evidence has shown that changes in the RA signaling pathway are correlated with ALS pathology[2]. Interestingly, our lab observed that the RA-receptor beta agonist adapalene is neuroprotective in cultured motor neurons. However, adapalene is poorly water soluble and cannot be delivered systemically. To address this delivery challenge, we engineered adapalene loaded NPs (Adap-NPs) composed of poly(lactic acid)-poly(ethylene glycol) (PLA-PEG) or PLA-PEG blended with other low molecular weight polymers for improved encapsulation and slow release of adapalene. We administered Adap-NPs to both healthy and transgenic mice bearing ALS pathology to study retinoid pathway activation in the CNS, and to test whether adapalene would be capable of slowing disease progression

Methods: NPs were fabricated by single emulsion. PLA-PEG polymer was blended at a 3:2 ratio with Poly-lactic acid (PLA; MW 2 kDa), Polycaprolactone (PCL, MW 1-5 kDa) or Poly (lactic-co-glycolic acid) (PLGA, MW 1-5 kDa). Retinoid signaling reporter mice (Stock #008477 Jackson) were used to assess induced retinoid signaling. $SOD1^{G93A}$ transgenic mice (Stock #004435, Jackson) were treated for 2 months with Adap-NPs. Motor function was assayed at 1 month after treatment by measuring total locomotion during 7 minutes in an open-field and by measuring the latency to fall off an accelerating rotarod. MRI was performed after 8 weeks of treatment on a 7T Bruker Biospec scanner. Regions of interest encompassing the quadriceps muscle were used to calculate muscle volume.

Results: Blending low molecular weight, hydrophobic polymers into a PLA-PEG base significantly improved adapalene loading while maintaining small nanoparticle size (Table 1). Administration of Adap-NPs directly to the brain, via convection enhanced delivery, or systemically, via tail vein injection, produced upregulation of expected biomarkers in healthy mice, including MAPK signaling in the brain and CRAPB activation in the spinal cord. When Adap-NPs were administered at the maximum deliverable dose (3x/week, 0.25 or 1.25 mg/kg adapalene) to $SOD1^{G93A}$ mice, treatment significantly slowed disease progression. We observed improved motor function as measured by increased locomotion in the open field test, and by increased latency to fall in the rotarod test. These behavioral data corresponded with decreased weight loss. In addition, immunohistochemistry of neurofilament and acetylcholine receptors demonstrated reduced loss of the motor neuron junctions, and MRI analysis revealed significant protection against muscle volume loss (Figure 1A). Early data suggests that intransal administration of Adap-NP in fact enables activation RARβ signaling in specific brain regions (e.g., Figure 1B), which opens the possibility for less invasive routes of administration in future work.

Polymer	Loading	Yield	Size
PLA-PEG	0.33%	74.63%	114.01
PLA 40%	1.37%	44.43%	85.22
PCL 40%	0.72%	74.33%	132.79
PLGA 40%	1.17%	78.14%	110.20

Table 1: Characterization of adapalene loaded NPs.

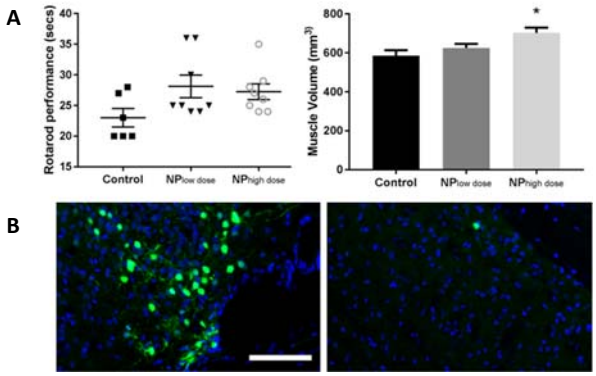

Figure 1: A) Adap-NP administration improved motor performance (left panel) and maintained muscle volume (right panel. (*=p<0.05, One-way ANOVA). B) Retinoid activity reporter mice demonstrate retinoid signaling 24 hours after Adap-NP administration (left panel) vs non-treated (right panel).

Conclusions: Blending PLA-PEG with low molecular weight, hydrophobic polyesters was an effective approach for improving encapsulation of adapalene while maintaining small nanoparticle size; our complete results suggest that loading is improved as a direct function of blended polymer fraction, and we anticipate that this approach will be useful to improve encapsulation of a wide variety of small, hydrophobic molecules. Adap-NPs were very well tolerated by mice, and we demonstrate that we are able to achieve activation of the retinoid pathway in brain and spinal cord following parenteral administration. Our current work is testing the hypothesis that targeted NPs will further enhance retinoid signaling within motor neurons of the spinal cord to synergistically improve treatment efficacy. Importantly, targeted modulation of retinoid signaling could be a significant therapeutic opportunity in ALS and other neurodegenerative disorders.

References: [1] Bailey SJ, Lane M. Progress in Neurobiol 2005; 75(4):275-293 [2] Kolarcik CL, Bowser R. Am J Neurodegen Disease 2012; 1(2) 130-145