Antioxidant nanoparticles for inhibition of inflammation-mediated rheumatoid arthritis

David Cochran*, Lihua Yang**, Dr. Leslie Crofford**, Dr. Rebecca Bader***, Dr. Thomas Dziubla*
*Department of Chemical and Materials Engineering, University of Kentucky
**Department of Internal Medicine, Division of Rheumatology, University of Kentucky
***Syracuse Biomaterials Institute, Syracuse University

Statement of Purpose: Previously, we have reported on a novel antioxidant polymer, poly(trolox) (PTx), for controlled release applications. [1]. PTx has been shown to not only suppress oxidative stress, but prevent protein oxidation *in vitro*, a feature not seen in pure trolox[2]. It was hypothesized that nanoparticles containing PTx would provide the same protection in an *in vivo* model of inflammation-mediated rheumatoid arthritis.
Micelles comprised of poly(ethylene glycol)-poly(lactic acid) (PEG-PLA) and encapsulated PTx indeed have been shown to suppress markers of inflammation stemming from an antibody induced rheumatoid arthritis model *in vivo*. PEGylation of the antioxidant containing micelles permits extended circulation times and their size allows for accumulation in inflamed joint tissue. As the polymer degrades, it is hypothesized to release active trolox at the site of inflammation. These nanoparticles are potentially an attractive delivery system for the treatment of inflammation-mediated rheumatoid arthritis.

Methods: PEG-PLA (50K:5K MW) copolymer was synthesized in a single step reaction in the presence of stannous hexanote. A carbodiimide-based reaction scheme was used to synthesize poly(trolox) (1000 MW), as previously described[1]. PTx loaded nanoparticles were formulated utilizing nanoprecipitation and characterized by DLS and zeta potential. Drug content and loading were determined by UV-Vis spectroscopy and HPLC. For animal imaging Cy5.5, was incorporated into the nanoparticles.

Arthritis was induced in mice through the use of a collagen antibody-induced arthritis (CAIA) model[3]. Mice received 12.5 mg/mL of PTx loaded nanoparticles in 100 µl of PBS+BSA or 100 µl PBS+BSA via tail vein injections every day for 5 days then sacrificed. Trolox equivalent antioxidant content (TAEC) was measured from liver homogenates. Protein carbonyl content was measured from homogenized foot pad tissue and normalized against total protein content as determined via Bradford assay.

Results: Micelles of 163 nm were obtained utilizing a nanoprecipitation technique. Encapsulation efficiency was determined to be 50%, with a final drug loading content of 25%. Fluorescent imaging of Cy5.5 loaded particles indicate high accumulation in liver and kidneys of treated mice, whereas controls injected with equivalent mass of free dye resulted in undetectable systemic levels. Additionally, imaging analysis of inflamed paws revealed a 267% increase of fluorescence in treated mice over controls.
Animals treated with PTx nanoparticles show an elevated level of antioxidant content in the liver over untreated mice. In addition, mice treated with PTx nanoparticles also exhibit a significant reduction of protein carbonyl content in foot pad tissue, 0.25 nmol/mg protein in antioxidant treated mice vs. 5.3 nmol/mg protein in controls (Figure 2).

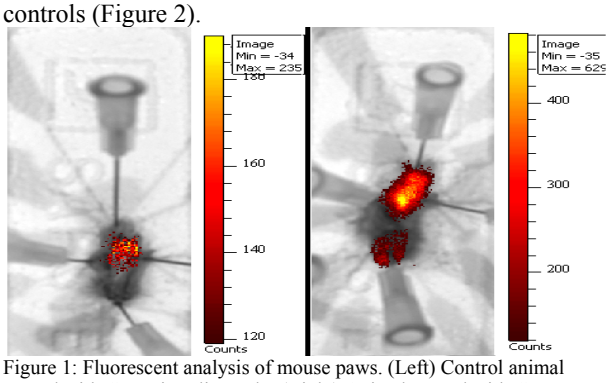

Figure 1: Fluorescent analysis of mouse paws. (Left) Control animal treated with Cy5.5 in saline only. (Right) Animal treated with Cy5.5 loaded PTx nanoparticles.

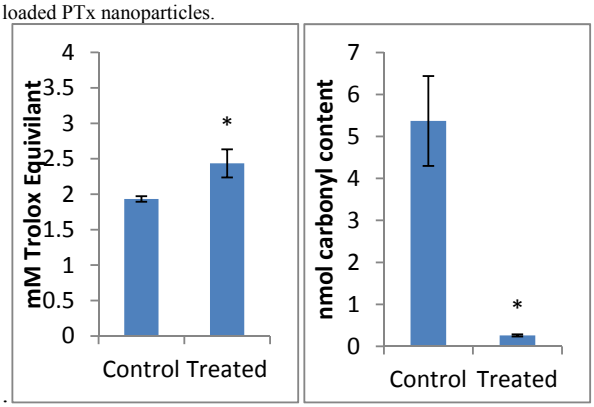

Figure 2: (Left) Trolox Equivalent Antioxidant Content (TAEC) of mice liver (p = 0.035). (Right) Protein carbonyl content of foot pad tissue (p=0.001)

Conclusions: PTx loaded PEG-PLA nanoparticles served to reduce a marker oxidative stress and replenish total antioxidant content in the organs of mice in an arthritic mouse model. Fluorescent imaging analysis of the organs indicates significant accumulation over a 5 day period as compared to control mice. Most importantly, imaging analysis suggests higher accumulation of nanoparticles in the inflamed joints possibly due in part to enhanced permeation and disruption of vasculature in the limbs. These nanoparticles also served to significantly reduce the levels of oxidized protein in the limbs, a marker of downstream damage due to inflammation. This preliminary data serves as a potential therapeutic delivery system for the treatment of rheumatoid arthritis and the accompanying inflammation-mediated damage caused.

References:

1. Wattamwar, P.P., et al. Advanced Functional Materials, 2009. **20**(1): p. 147-154.
2. Wattamwar, P.P., et al. Journal of Biomedical Materials Research Part A, 2011. **99**(2): p. 184-191.
3. Kojima, F., et al. The Journal of Immunology, 2008. **180**(12): p. 8361-8368.

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