Nanoparticle-based Platform Enables Increased Intracellular Antibiotic Delivery and Killing of *Brucella*

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Statement of Purpose: Infectious diseases caused by intracellular bacterial pathogens are extremely difficult to treat due to limited penetration of antibiotics into infected cells. Polyanhydride nanoparticles elicit unique cellular responses from immune cells that stimulate internalization, direct intracellular trafficking and slow particle degradation [1]. *Brucella abortus* survives and replicates within macrophages of infected animals requiring dual antibiotic therapy for several months for treatment. These studies were performed to examine the preventative and therapeutic efficacy of antibiotics encapsulated within polyanhydride nanoparticles.

Methods: Antibiotic-loaded polyanhydride nanoparticles based on copolymers of sebacic acid (SA), 1,8-bis(*p*-carboxyphenoxy)-3,6-dioxaoctane (CPTEG) and 1,6-bis(*p*-carboxyphenoxy) hexane (CPH) were fabricated (20:80 CPH:SA and 20:80 CPTEG:CPH) [1]. Intracellular viability was performed with RAW264.7 cells using a *Brucella:*monocyte multiplicity of infection (MOI) of 100:1 with IgG opsonized *Brucella* for 30 min [2]. Soluble or nanoparticle encapsulated antibiotic was administered at a final concentration of 10 µg/ml at 24 h post infection. Female BALB/c mice were infected intraperitoneally (i.p.) with 5 $\log_{10}$ CFU of *B. abortus* S19. Antibiotic therapy was administered i.p. as a single dose 3 days post infection. Animals were euthanized and bacteria were enumerated from the spleens and livers at 7 days post infection.

Results: Encapsulation of rifampicin increased the intracellular killing of *B. abortus* by 97% over equivalent amounts of soluble rifampicin that demonstrated no reduction in bacterial CFUs (Figure 1). Both 20:80 CPTEG:CPH and 20:80 CPH:SA nanoparticles exhibited equivalent antimicrobial efficacy.

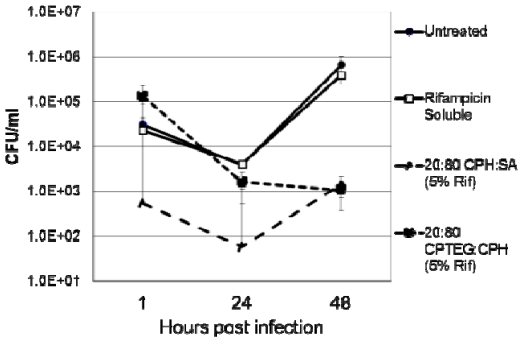

Figure 1. Viable intracellular *B. abortus* 2308 in murine macrophages treated with soluble, encapsulated or no antibiotics.

In vivo administration of a single dose of 0.5 mg doxycycline-loaded nanoparticles 3 days post infection with *B. abortus* S19 reduced bacterial burden in spleens and livers one week post infection compared to soluble antibiotic. Mice treated with 20:80 CPH:SA nanoparticles had greater reduction in CFUs in both the spleen and liver than 20:80 CPTEG:CPH, although both nanoparticle formulations performed better than soluble antibiotic at reducing bacterial burden.

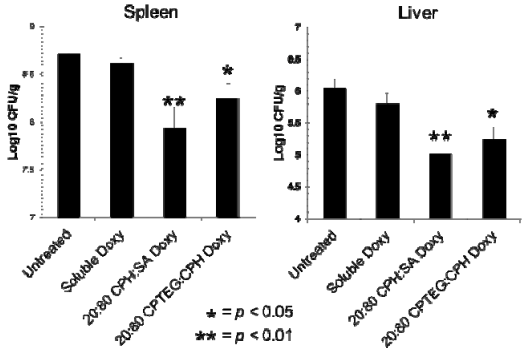

Figure 2. Antibiotic-loaded nanoparticles reduce *Brucella* colonization in spleen and liver.

Conclusions: Polyanhydride nanoparticles provide specific advantages for intracellular delivery of payload compared to other polymer or lipid based particle delivery systems. Their high hydrophobicity imparts a unique, native cellular affinity leading to a high degree of internalization. This is followed by extended intracellular persistence where the particles slowly erode and release the cargo molecule in a controlled manner. The nanoparticles are capable of persisting for extended periods of time in host tissues. Varying the chemistry of the particles by altering co-polymer composition impacts internalization, intracellular targeting, and ultimately the fate of the particle within cells. The results presented herein demonstrate the effectiveness of the nanoparticles for both prophylaxis and treatment regimens.

References: [1] Ulery *et al* Pharm Res 2009
[2] Ritchie *et al* Frontier Cell Microbiol 2012