S-nitrosated poly(propylene sulfide) nanoparticles exhibit thiol-dependent transnitrosation and toxicity against adult female B. malayi filarial worms

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Statement of Purpose: B. malayi, filarial worms that infiltrate lymphatic vessels, infect an estimated 120M people primarily within the developing world [1] and cause elephantiasis, a painful and debilitating disease. Existing anti-filarial agents kill the microfilaria progeny that cause parasite spread, but there are no existing therapeutics capable of eliminating the source of microfilaria, adult female worms that reside within lymphatic vessels. Thus, infected individuals have no treatment recourse to prevent infection-induced disfigurement [2]. However, it is widely appreciated that nitric oxide (NO) functions as a cytotoxic effector molecule in the immune response against a variety of parasites [4]. To this end, we have developed a NO donating platform based on 30 nm diameter Pluronic-stabilized, poly(propylene sulfide) nanoparticles (NP) [3] that in vivo exhibit improved penetration into the lymphoid tissue, where these filarial worms reside. We demonstrate that S-nitrosated NP (SNO-NP) facilitate efficient NO donation via transnitrosation to physiological thiols to mediate B. malayi killing.

Methods: NP were synthesized as previously reported [3]. Free thiols remaining on the NP after synthesis were S-nitrosated using acidified sodium nitrite and subsequently dialyzed to physiological pH to form SNO-NP. S-nitrosothiol decomposition was assessed under physiological conditions in the presence of the thiol cysteine (CYS). Using size exclusion chromatography after SNO-NP/CYS co-incubation, the Saville assay was used to measure in eluted fractions separating SNO-NP from CYS the amount of S-nitrosocysteine (SNO-CYS) formation. Finally, the ability of SNO-NP to kill adult female B. malayi filarial worms under different CYS concentrations was assessed in vitro using a video-capture system and custom image analysis program assessing worm motility and time till death.

Results: SNO-NP incubated in endothelial bovine medium at 37ºC release NO with a half-life on the order of 24 hours. Co-incubation of SNO-NP with CYS at 1:0.1, 1:1, and 1:10 SNO-NP to CYS ratios (at a constant SNO-NP concentration) revealed that escalating CYS levels lead to accelerated NO release from the SNO-NP (Figure 1A). We found that this was the result of the increased formation of SNO-CYS with increasing ratios of CYS (Figure 1B). We explored whether we could exploit this ability of our SNO-NP to transnitrosate to CYS to create bioactive SNO-CYS in order to mediate killing of adult female B. malayi worms. We incubated worms in endothelial bovine medium at 37ºC with SNO-NP and different concentrations of CYS. We found that SNO-NP alone reduced the motility of the worms and eventually resulted in death, but this dampening of motility was significantly delayed relative to commercially available small molecule NO donor S-Nitroso-N-Acetyl-D,L-Penicillamine (SNAP). Given the capacity of CYS to accelerate the donation of NO as well as the transnitrosation to form SNO-CYS by SNO-NP, we hypothesized that addition of CYS to the culture medium would hasten SNO-NP-induced reduction in worm motility. Supporting this hypothesis, we found that at CYS concentrations of 1 mM or higher, B. malayi treatment with SNO-NP reduced motility significantly faster (Figure 1C) than low (0.1 mM) or no (0 mM) levels of CYS, as well as significantly shortened the time till death (data not shown).

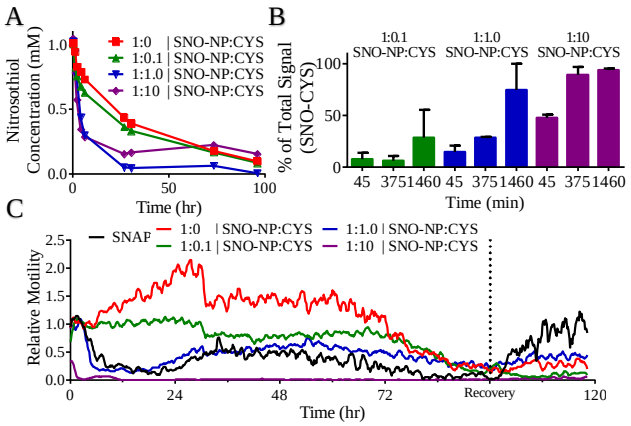

Figure 1: A) SNO-NP decomposition is accelerated in the presence of high CYS concentrations. B) Escalating concentrations of CYS result in higher levels of SNO-NP transnitrosation to CYS to form SNO-CYS. C) Adult female B. malayi worm motility is reduced by exposure to NO donors SNO-NP and SNAP. Reductions in motility caused by SNO-NP are accelerated in the presence of elevated CYS concentrations.

Conclusion: We have demonstrated that SNO-NP can donate a bioactive form of NO in the presence of CYS to facilitate killing of adult female B. malayi worms. These data suggest that the unique lymphatic targeting activity of SNO-NP could be exploited to eliminate filarial infections in vivo via targeted NO donation.

References:

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