Novel Antioxidant Drug Delivery Vehicles based on Click Chemistry

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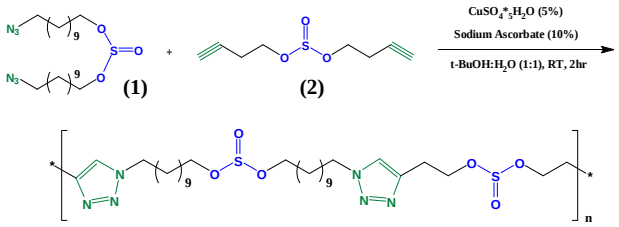
Statement of Purpose: The overproduction of hydrogen peroxide (HP) is central to the development and persistence of inflammatory diseases such as myocardial infarction, pulmonary fibrosis, and diabetic nephropathy. Antioxidants that diminish HP levels in inflamed tissue have been shown to reduce inflammation by preventing the oxidative activation of proinflammatory cytokines and the oxidative damage of cellular components[1]. In response to the wide-spread interest in the use of antioxidants to treat inflammatory diseases, we have developed an antioxidant microparticle delivery vehicle formulated from an HP-scavenging polymer. HP-scavenging polymers were synthesized from sulfite containing monomers using click chemistry (Figure 1.A). These antioxidant polysulfites were used to formulate polysulfite microparticles (PSMs) that can be loaded with anti-inflammatory therapeutics that work in concert with the antioxidant capabilities of the delivery vehicle to treat inflammatory diseases.

Methods: Click-polysulfites were synthesized according to the general procedure shown in Figure 1.

A. Polymer Synthesis



B. Antioxidant-Microparticle Formulation

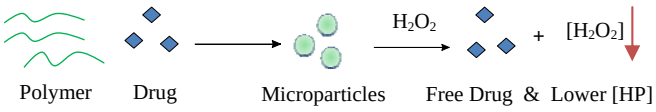


Figure 1. Synthesis of HP-scavenging click-polysulfites and the formulation of antioxidant drug carriers.

Monomer Synthesis. The two monomers necessary for the click-polymerization were synthesized by the addition of thionyl chloride to a stirred solution of tetrahydrofuran, 2 eq. pyridine, and either 2 eq. of 3-butyne-1-ol or 2 eq. of 1-azide –undecanol. The resulting diazide and dialkyne sulfite monomers **1,2** were purified via flash chromatography.

Polymer Synthesis. An equimolar ratio of monomers **1** and **2** were then stirred in a 1:1 mixture of water and t-butanol at room temperature in the presents of 0.05 eq. copper iodide and 0.1 eq. sodium ascorbate. After 2 hours, the polymer was collected via precipitation in methanol.

Microparticle Formulation. Dye-loaded and empty (PSMs) were prepared using a typical o/w single-emulsion method. An oil phase was generated by dissolving 100µg of click-polysulfite and 10 µg of Cell Tracker™ Red CMTPK into1.0 ml of chloroform. This oil phase was then added to 5 ml of a 5% PVA solution and homogenized to form an o/w emulsion that was then added to a stirred 50 ml 1% PVA solution. After stirring for 3 hours the particles were then isolated via centrifugation and lyophilized.

Hydrogen Peroxide Scavenging Assay. PSMs (0.2 mg/ml, 0.8 mg/ml) were added to a 25µM HP solution and incubated at 37ºC. After 4 and 8 hours, the PSMs were removed via centrifugation and the supernatant was assayed for hydrogen peroxide using a commercially available Amplex Red Hydrogen Peroxide/Peroxidase Assay.

Results: The synthetic strategy shown in Figure 1.B produced polysulfite with molecular weights between 4000-5000 Da. Exposing these polymers to a 1.0 mM hydrogen peroxide solution reduced the molecular weight of the polymers to less than 400 Da.

An SEM image of dye-loaded particles formulated from click-polysulfites is shown in Figure 2.A. Encapsulation efficiency studies showed that 89% of the dye used to formulate the particles is retained in the particles after formulation.

HP scavenging studies show that empty PSMs are capable of reducing HP levels *in vitro*. Figure 2.B shows that after 8 hr, at a concentration of 0.8 mg/ml, PSMs scavenge approximately 75% of the HP from a 25µM HP solution.

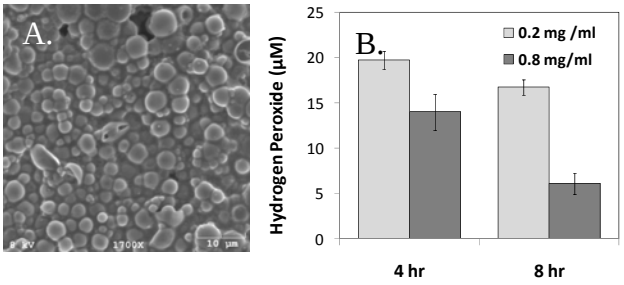


Figure 2. (A) SEM image of PSMs; (B) empty PSMs incubated with a 25µM HP solution significantly reduce HP.

Conclusions: Due to the deleterious roll of HP in inflammation and the prevalent interest in the development of antioxidant therapies for inflammation, we anticipate that antioxidant PSMs will find numerous applications in treating inflammation. Future research will focus on determining the ability of empty PSMs to reduce oxidative stress in phagocytes, as well as explore the therapeutic synergy between the antioxidant properties of the PSMs in conjunction with encapsulated anti-inflammatory therapeutics.

References:

(1) Cuzzocrea, S.; Riley, D.P.; Caputi, A.P.; Salvemini, D. *Pharmacol Rev*, **2001**, 53 (1), 125-59.