A New Reactive Oxygen Species Sensitive Delivery Vehicle for Targeting Oxidative Stress

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Statement of Purpose: Oxidative stress, a cytopathic consequence of excessive production of reactive oxygen species (ROS) is implicated in the development and persistence of many inflammatory diseases, including inflammatory bowel disease, acute lung injury, and myocardial infarct.[1] In this presentation, we introduce a new ROS-sensitive drug delivery vehicle formulated from Poly(Thioketal) polymers (PTK) that has the ability to selectively release therapeutics inside cells that are under oxidative stress.

Methods: Poly(Thioketal) Polymerization. A schematic of the stepwise polymerization of cyclic and aliphatic dimercapto-monomers and 2,2-dimethyoxy propane (DMP) used to make PTKs is shown in Figure 1(A). A 3-neck flask was charged with equimolar amounts of the dimercapto-monomer and DMP dissolved in distilled benzene. This stirred solution was heated to 107°C before the addition of a catalytic about of *p*-toluenesulfonic acid. As the reaction proceeds at 107°C, methanol, benzene, and unreacted DMP are collected via distillation. In order to compensate for the removal of DMP and benzene from the reaction flask, DMP and benzene are added drop-wise throughout the duration of the reaction (24hrs).

Microparticle Formulation. Protein and small molecule-loaded thioketal microparticles (TKMP) were prepared by w/o/w (water/oil/water) double-emulsion and o/w single-emulsion methods. For the protein-loaded particles, a primary w/o emulsion is created by dispersing 100µL of FITC-labeled OVA albumin aqueous solution (20mg/mL) in an organic phase consisting of 100.0 mg PTK dissolved in 1.0 ml dichloromethane (DCM). This primary w/o phase is then added and dispersed by homogenization in a secondary aqueous solution containing 5% (w/v) surfactant to produce the final w/o/w emulsion. This w/o/w emulsion is then stirred under vacuum to remove the DCM. The particles are then isolated via centrifugation and lyophilized.

Polymer Degradation. PTKs were dissolved in DCM containing a phase transfer catalyst and potassium superoxide. After 8 hours the DCM was evaporated and the resulting polymer residue analyzed via gel permeation chromatography.

Intracellular Dye Release. RAW-264.7 macrophages were treated with empty TKMPs or TKMPs loaded with the hydrophobic dye CMFDA. The cells were incubated with the particles for 4 hrs before being washed 3 times with PBS. The particle-treated and untreated cells were then given fresh media or media containing 1.0 µg/ml of lipopolysaccharide (LPS). LPS is an endotoxin known to activate macrophages and lead to the overproduction of the ROS superoxide.[2] After 12 hours, the cells were then assayed for intracellular dye release by flow cytometry.

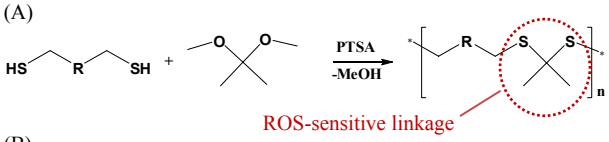
Results: PTKs synthesized according the schematic show in Figure 1(A) had number average molecular weights between 2,000 and 4,000 Da. Exposure of these polymers to potassium superoxide reduced the molecular weights to below 700 Da; however, polymers showed excellent stability to aqueous solutions with pH's of 1.0 and 10.0.

An SEM image of the OVA-loaded microparticles formulated from PTKs is shown in Figure 1(B). The florescent microscopy image of these particles verifies encapsulation of OVA in the particles.

Flow cytometry performed on cells receiving empty and CMFDA-TKMPs show an increase in the amount of dye released into cells that have been stimulated to overproduce superoxide by the addition of LPS. The red line in Figure 2 represents an increase in dye released in cells treated with CMFDA-TKMPs and LPS over the green line representing cells that only received CMFDA-TKMPs.

Conclusions: In this presentation, a new class of ROS sensitive polymers was synthesized from dimercapto-monomers and 2,2-dimethyoxy propane. PTK s were used to formulate small molecule and protein -loaded microparticles. Cell culture experiments demonstrate that dye-containing microparticles formulated from PTKs release their payload more rapidly in cells that overproduce superoxide. These results demonstrate the ability of TKMPs to target therapeutics to cells that overproduce the ROS super oxide.

(A)



(B)

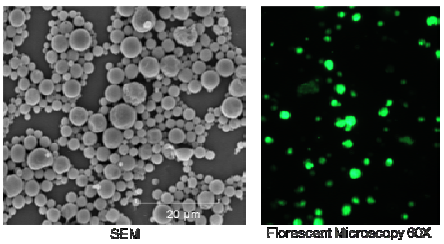


Figure 1. PTK, a new ROS-sensitive drug delivery vehicle: (A) PTKs are synthesized from dimercapto-monomers and DMP, (B) SEM and florescent microscopy images of TKMPs loaded with fluorescently-labeled OVA albumin.

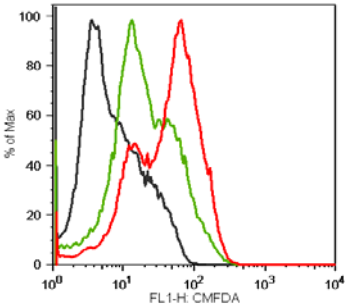


Figure 2. TKMPs target release in cells that overproduce ROS. Flow cytometry performed on RAW 264.7 macrophages treated with empty TKMPs and LPS (black), CMFDA-TKMPs (green), and CMFDA-TKMP and LPS (red) show that TKMPs have faster release kinetics in cells that have elevated levels of ROS (i.e. LPS treated cells).

References:

(1) Libby, P., *Nutrition reviews,* **2007**, 65, S140 – S146.
(2) Srisook, K., et al. *Biochem Pharmacol*. **2006**, 71, 307-18.