**Thioketal nanoparticles target orally delivered siRNA to inflamed intestinal tissues and suppress intestinal inflammation**

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**Statement of Purpose:** Here, we present a new oral delivery vehicle for siRNA, called the thioketal nanoparticles (TKNs), which can target orally delivered siRNA to inflamed intestinal tissues, and thus manipulate gene expression at sites of disease development. TKNs are formulated from a new polymer, poly-(1,4-phenyleneacetone dimethylene thioketal) (PPADT), that degrades selectively in response to reactive oxygen species (ROS). Therefore, when delivered orally, TKNs target the release of encapsulated agents to the elevated levels of ROS specific to sites of intestinal inflammation. Using a murine model of ulcerative colitis (UC), we demonstrate that orally administered TKNs loaded with TNFα-siRNA diminished TNFα messenger RNA (mRNA) levels in the colon and protected mice from intestinal inflammation[1].

**Methods: Poly(Thioketal) Polymerization.** PPADT was synthesized from 1,4-benzenedimethanethiol **1** and 2,2-dimethoxypropane **2** via a step-growth polymerization that produces polymers with ROS-sensitive thioketal linkages in their backbones **3**. To investigate the specificity of PPADT for ROS, we incubated PPADT with either a superoxide solution, 0.5 N HCl solution, or a 0.5 N NaOH solution and then analyzed the resulting product's molecular weight.

**Targeting Orally Delivered siRNA to Inflamed Tissues.** Experimental UC was induced in female C57BL/6 mice by replacing their drinking water on day zero with a 3% solution of dextran sodium sulfate (DSS). DSS supplementation induces a robust inflammatory response that is confined to the colon[2]. Starting on day zero, mice receiving either DSS or normal drinking water were given a daily oral gavage of TKNs loaded with a Cy3-tagged scrambled siRNA (Cy3siRNA). On day 7, the biodistribution of siRNA was measured by fluorescence.

**Treating DSS-induced UC with orally delivered TNFα-loaded TKNs.** Due to the essential role played by TNFα in the onset and persistence of intestinal inflammation, we chose to treat mice suffering from DSS-UC with a known TNFα-siRNA sequence. Mice receiving DSS were given TNFα-siRNA or scrambled siRNA encapsulated in TKNs (TNFα –TKNs, Sc-TKNs) via oral gavage once daily for five days. Mice receiving DSS were also treated with TNFα-siRNA complexed with the cationic lipid DOTAP (TNFα-DOTAP) or encapsulated in PLGA nanoparticles (TNFα-PLGA). After 7 days, the colonic TNFα-mRNA levels were determined via RT-PCR. We also monitored the weight loss of the animals during the experiment and performed histological analysis on the colons to determine if TNFα-TKNs could prevent the clinical manifestations of DSS-induced UC (DSS-UC).

**Results:** PTKs synthesized according the schematic show in Figure 1a had number average molecular weights of approximately 9,000 Da. Exposure of these polymers to potassium superoxide reduced the molecular weights to below 900 Da; however, polymers showed excellent stability to aqueous solutions with pH's of 1.0 and 14.0. Our results also demonstrate that the TKNs can localize orally delivered siRNA to sites of intestinal inflammation. For example, Figure 1b shows a greater than 3-fold increase in the amount of Cy3siRNA delivered to the colons of mice suffering from DSS-UC and receiving Cy3siRNA-TKNs as compared to mice receiving normal water and treated with Cy3siRNA-TKNs (* $p \leq 0.05$). As shown in Figure 1c, mice receiving DSS and treated with TNFα-TKNs experienced a dramatic ten-fold decrease in colonic TNFα mRNA (** $p \leq 0.001$, * $p \leq 0.05$) as compared to all other treatment groups. These results suggest that the ability of the TKNs to target siRNA to inflamed intestinal tissues is an important factor in their improved efficacy. Furthermore, mice receiving TNFα-TKNs experience less intestinal tissue damage associated with DSS-UC and lost significantly less weight loss as compared to mice treated with the other treatments.

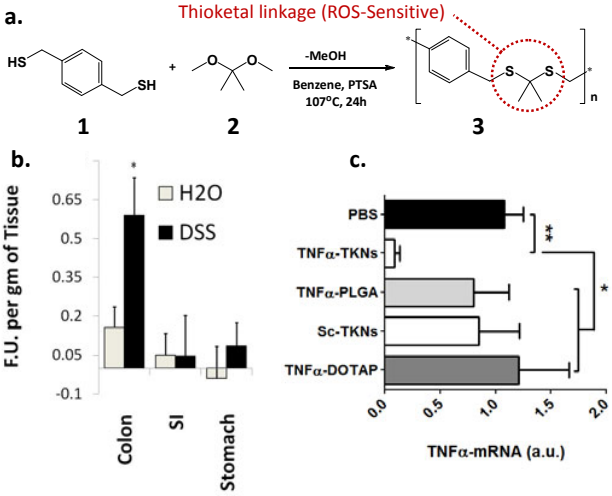


**Figure 1.** (a) PPADT is a new polymer composed of ROS-sensitive thioketal linkages (circled in red). (b) Biodistribution of Cy3-tagged siRNA in the organs of mice treated with a daily gavage of Cy3siRNA-TKNs. Fluorescent units (FU) per gram of tissues depicted as the mean ± s.d. for n = 10 mice per group. (c) Relative colonic TNFα mRNA levels in mice receiving DSS and treated with either scrambled- or TNFα-siRNA.

**Conclusion:** Oral administration represents the most patient friendly way to deliver siRNA to diseased intestinal tissues. Here we show that TKNs have the chemical and physical properties needed to overcome the harsh environment of the intestinal tract and deliver siRNA to inflamed intestinal tissues. Based on our results, we expect that TKNs will make a significant contribution to the treatment of numerous diseases linked to intestinal inflammation.

**References:**

1. Wilson, D.S., et al. *Nat Mater.* **9**, 923-8 (2010).
2. Yan, Y., et al. *PLoS One* **4**, e6073 (2009).