Polyketal Nanoparticles: A pH-Sensitive Biodegradable Drug Delivery Vehicle

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Introduction: In this presentation we describe the development of a pH-sensitive drug delivery vehicle, termed polyketal nanoparticles (PKNs), which are designed to target drugs to the lysosomes of phagocytic cells. The PKNs are fabricated from poly(1,4-phenylene-acetone dimethylene ketal) (PPADK), a new hydrophobic polymer containing acid-labile ketal linkages in the backbone (Figure 1). The PKNs hydrolyze into low molecular weight, water-soluble compounds and do not generate acidic degradation products as with polyesters. The pH-sensitivity of the PKNs allows for the accelerated release of drugs in the acidic lysosomes of phagocytic cells. Here we describe the synthesis and characterization of PKNs, as well as *in vivo* testing that demonstrates the potential of PKNs to treat macrophage-mediated conditions such as acute liver failure.

Methods:

Synthesis. PPADK was synthesized via a new polymerization strategy based on the acetal exchange reaction. 1,4-Benzenedimethanol (BDM) was polymerized with 2,2-dimethoxypropane in benzene at 100°C, by distilling off the methanol byproduct to drive the reaction forward (Figure 1). The recovered polymer was analyzed by GPC and ^{1}H NMR.

Hydrolysis Kinetics. The hydrolysis rates were measured by incubating finely ground PPADK in deuterated pH 1.0, 5.0, and 7.4 solutions. At 3, 24, 48, and 72 h, samples were pelleted and the supernatant was analyzed for hydrolysis products by ^{1}H NMR.

Nanoparticle Formation. Hydrophobic drugs and dyes were encapsulated in PKNs using a solvent evaporation method [1]. Fluorescein-loaded PKNs were made by dissolving 20 mg of PPADK and 1 mg of fluorescein in 500 µL of chloroform, and emulsifying in 5 mL of 0.2% w/v PVA (Aldrich) in 10 mM pH 9 sodium phosphate buffer. Particle sizes were measured by dynamic light scattering (DLS).

In Vivo Studies. To assess the ability of PKNs to deliver drugs to liver macrophage cells for the treatment of acute liver failure, fluorescein-loaded PKNs were injected into the tail vein of mice. The mice were sacrificed after 1 h, and frozen liver sections were fixed and stained with anti-fluorescein antibodies.

Results / Discussion:

PPADK was synthesized with M_w = 4000, and NMR peaks of 7.3 ppm (4 H), 4.5 ppm (4 H), and 1.5 ppm (6 H) confirm that the repeating unit contains a phenylene group and a ketal group. The hydrolysis half-life of PPADK is 102 h at pH 7.4 and 35 h at pH 5.0. These results demonstrate that a polyketal with pH-dependent degradation was successfully synthesized.

SEM images (Figure 2A) and DLS measurements indicate PKN sizes in the 200 to 600 nm range. The *in vivo* uptake of fluorescein-PKNs is shown in the frozen liver tissue slice in Figure 2B. The diffuse staining for fluorescein indicates that fluorescein is being released from PKNs after phagocytosis by liver macrophage cells.

Conclusions:

Polyketal nanoparticles (PKNs) are a new pH-sensitive biodegradable drug delivery vehicle, made from the polyketal PPADK. PKNs undergo pH-sensitive hydrolysis into water soluble compounds and do not produce acidic degradation products as with PLGA. We have demonstrated the rapid (< 1 hour post-injection) delivery of fluorescein dye to liver macrophage cells in mice. Future studies will include the delivery of functional enzymes to macrophages and the delivery of vaccine components to dendritic cells.

References:

1. Heffernan MJ. *Bioconjugate Chem.*, published on web 10/5/2005.

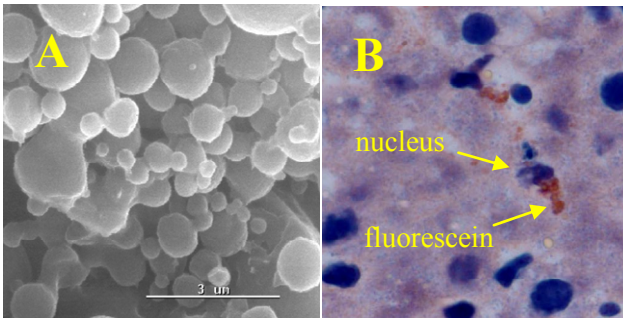

Figure 2. (A) SEM of PKNs produced by the solvent evaporation method. (B) In vivo release of fluorescein by PKNs in mouse liver cells.

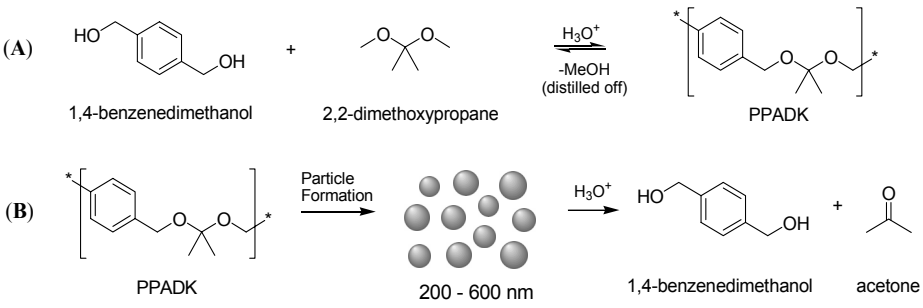

Figure 1. Synthesis of the polyketal PPADK. (A) Step-wise polymerization by the acetal exchange reaction. (B) Formation of polyketal nanoparticles (PKNs), and pH-sensitive degradation into water-soluble compounds.