COMMUNICATIONS

Polyketal Microparticles: A New Delivery Vehicle for Superoxide Dismutase

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There is currently great interest in developing microparticles that can enhance the delivery of proteins to macrophages. In this communication, we present a new acid-sensitive polymer for drug delivery, poly(cyclohexane-1,4-diyl acetone dimethylene ketal) (PCADK). PCADK is designed to hydrolyze, after phagocytosis by macrophages, in the acidic environment of the phagosome and enhance the intracellular delivery of phagocytosed therapeutics. Other key attributes of PCADK for drug delivery are its well-characterized degradation products and straightforward synthesis. PCADK hydrolyzes into 1,4-cyclohexanedimethanol, a compound used in food packaging, and acetone, a compound on the FDA GRAS list. PCADK was synthesized using the acetal exchange reaction between 1,4-cyclohexanedimethanol and 2,2-dimethoxypropane, and could be obtained on a multigram scale in one step. The hydrolysis kinetics of the ketal linkages in PCADK were measured by ^{1}H NMR and were determined to be pH-sensitive, having a half-life of 24.1 days at pH 4.5 and over 4 years at pH 7.4. The therapeutic enzyme superoxide dismutase (SOD), which scavenges reactive oxygen species, was encapsulated into PCADK-based microparticles using a double emulsion procedure. Cell culture experiments demonstrated that PCADK-based microparticles dramatically improved the ability of SOD to scavenge reactive oxygen species produced by macrophages. We anticipate numerous applications of PCADK in drug delivery, based on its acid sensitivity, well-characterized degradation products, and straightforward synthesis.

Reactive oxygen species (ROS) produced by macrophages play a central role in causing inflammatory diseases, such as acute liver failure, arthritis, and sepsis (*1*−*4*). Superoxide dismutase (SOD) is an enzyme that scavenges reactive oxygen species and has the potential to treat inflammatory diseases by suppressing ROS production by macrophages (*5*−*7*). Unfortunately, clinical trials with free SOD have been ineffective, due to its membrane impermeability, and SOD delivery vehicles are therefore being investigated. Liposome-based SOD delivery vehicles have shown promise for enhancing the delivery of SOD in animal models; however, their poor shelf life has prevented their progression into clinical trials (*8*, *9*). Polymeric microparticles, based on PLGA and polycaprolactone, are also being investigated for the delivery of SOD (*10*−*12*). Although polyester-based microparticles have an excellent shelf life and well-characterized degradation products, their application for the treatment of inflammatory diseases is potentially problematic because their acidic degradation products can cause inflammation (*13*).

Polymeric microparticles based on acid-degradable polymers also have the potential to enhance the delivery of SOD to macrophages. Acid-degradable polymers, such as polyorthoesters and polyacetals, hydrolyze in the acidic environment of lysosomes and phagosomes and have previously been used for intracellular drug delivery (*14*−*17*). The polyketals are a new family of acid-degradable polymers that have ketal linkages in their backbones (*18*, *19*). The polyketals have several properties that make them an attractive delivery vehicle for SOD. Polyketals degrade into neutral compounds and may avoid the inflammatory problems associated with the acidic degradation products of polyesters and polyorthoesters. Polyketals also have the potential to disrupt phagosomes, by degrading in the phagosome and osmotically destabilizing it. The ketal linkage has excellent hydrolysis kinetics for intracellular drug delivery, hydrolyzing approximately 1000 times faster at the phagosomal pH of 4.5 versus the pH 7.4 environment of the blood. Although the polyketals have potential for protein delivery to macrophages, poly(1,4-phenyleneacetone dimethylene ketal) (PPADK) is the only polyketal developed for drug delivery; however, it degrades into benzene dimethanol, an aromatic compound with potential toxicity, and new polyketals with better characterized toxicity profiles would be preferable.

In this communication, we present a new polyketal for drug delivery, poly(cyclohexane-1,4-diyl acetone dimethylene ketal) (PCADK), which degrades into acetone, a compound on the FDA GRAS list, and 1,4-cyclohexanedimethanol, a compound with an excellent toxicity profile. For example, the LD_{50} of 1,4-cyclohexanedimethanol in rats is 3200 mg/kg for an oral dosage. 1,4-cyclohexanedimethanol also does not undergo significant enzymatic transformations in vivo; for example, a 400 mg/kg peritoneal gavage of 1,4-cyclohexanedimethanol in rats led to 97.5% of it being excreted intact (*20*). In addition, 1,4-cyclohexanedimethanol is a commonly used food packaging material and has approval for human consumption as an indirect food additive (Food Contact Notification (FCN) no. 000087).

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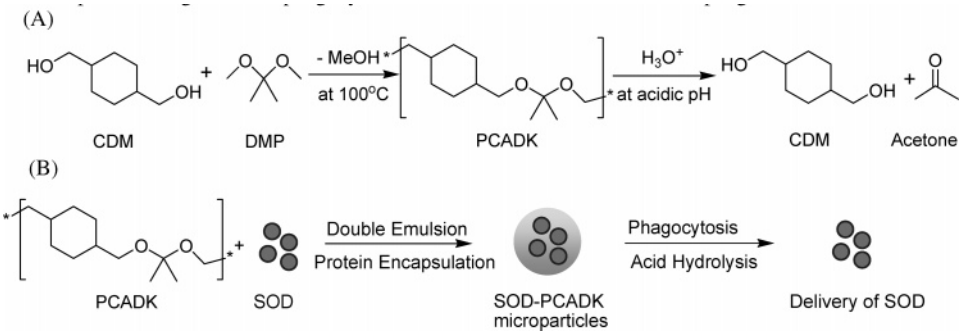
Scheme 1. PCADK, a New Polymer for Drug Delivery[a]



[a] (A) Synthesis of PCADK from 1,4-cyclohexanedimethanol (CDM) and 2,2-dimethoxypropane (DMP), and acid hydrolysis of PCADK into CDM and acetone. (B) Formulation of SOD-loaded microparticles by w/o/w double emulsion. Microparticles degrade after phagocytosis, in the acidic environment of the phagosome.

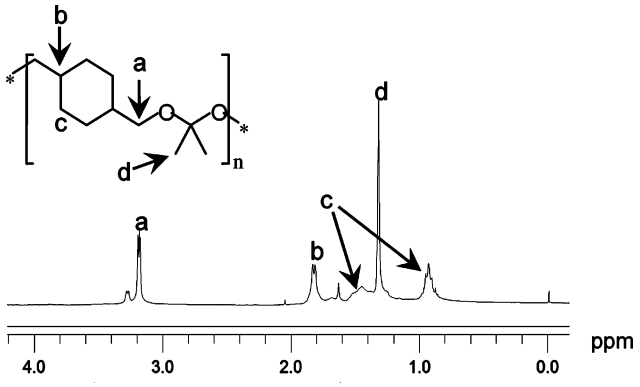


Figure 1. ^{1}H NMR of PCADK. ^{1}H NMR spectrum of PCADK in $CDCl_3$: per repeating unit, peaks at $\delta = 3.26-3.18$ (a, 4H), 1.83 (b, 2H), 1.60−0.93 (c, 8H), and 1.32 ppm (d, 6H).

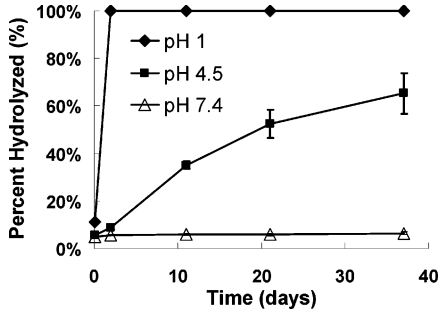


Figure 2. The hydrolysis of PCADK is pH-sensitive. Hydrolysis kinetics of PCADK (ground powder) was determined at pH 1.0, 4.5, and 7.4 at 37 °C by ^{1}H NMR. Half-lives of PCADK are 24.1 days (pH 4.5) and over 4 years (pH 7.4). PCADK was completely hydrolyzed in 2 days at pH 1.0.

In this report, SOD was encapsulated into PCADK-based microparticles via a double emulsion procedure. Cell culture experiments with macrophages demonstrated that these microparticles dramatically improved the ability of SOD to scavenge superoxide produced by macrophages. On the basis of these observations, we anticipate numerous uses for PCADK-based microparticles in drug delivery.

PCADK was synthesized, on a multigram scale, using the acetal exchange reaction between 1,4-cyclohexanedimethanol and 2,2-dimethoxypropane, as shown in Scheme 1, generating polymers with an M_w of approximately 6000 (Supporting Information Figure S1). A ^{1}H NMR spectrum of PCADK is shown in Figure 1, confirming its chemical structure. Although the acetal exchange reaction has been extensively used for the protection of alcohols, it has been rarely used for the synthesis of polyketals. In 2005, Heffernan and Murthy reported the synthesis of PPADK through the reaction of 2,2-dimethoxypropane and 1,4-benzenedimethanol (*18*). While PPADK has many of the characteristics needed for drug delivery, its degradation products have potential toxicity. PCADK, in contrast, degrades into 1,4-cyclohexanedimethanol and acetone, both of which have excellent toxicity profiles.

PCADK has ketal linkages in its backbone and should therefore degrade after phagocytosis, in the acidic environment of the phagosome. The hydrolysis kinetics of PCADK, in the form of ground powder, was measured at the pH values of 4.5 and 7.4, to estimate the behavior of PCADK-based delivery vehicles after phagocytosis. Figure 2 demonstrates that the hydrolysis of the ketal linkages in PCADK is indeed pH-sensitive, having a half-life of 24.1 days at pH 4.5 and an estimated half-life of over 4 years at pH 7.4. The hydrolysis of the ketal linkage in PCADK is considerably slower than that of a water-soluble ketal, which has a half-life of approximately 1.6 min at pH 5.0 (*21*). This observation leads us to speculate that the rate-limiting step in the hydrolysis of PCADK is the diffusion of hydronium ions into the polymer matrix, rather than the cleavage of ketal bonds. This hypothesis is further supported by the faster hydrolysis rate of the more hydrophilic polyketal, PPADK, which has a half-life of 35 h at pH 5.0.[1] This trend suggests that the hydrolysis of polyketal microparticles can be tailored by manipulating their hydrophobicity.

SOD was encapsulated into PCADK microparticles using a w/o/w double emulsion procedure. An experimental protocol was developed on the basis of procedures used to formulate SOD into PLGA-based microparticles (*10*). Briefly, a 100 μL aqueous solution of SOD (40 mg/mL) was dispersed by homogenization (21 500 rpm, 30 s) into 1.0 mL of methylene chloride, containing 125 mg of PCADK, generating a water in oil (w/o) emulsion. This w/o emulsion was then dripped into 5 mL of an 8% (w/v) aqueous polyvinyl alcohol (PVA) solution and was stirred with a homogenizer at 6000 rpm for 5 min. The resulting w/o/w emulsion was then poured into 25 mL of pH 7.4 buffer and was stirred for several hours, evaporating the methylene chloride. The resulting particles were isolated by centrifugation and freeze-dried, generating a white solid powder. The protein encapsulation efficiency of the SOD−PCADK microparticles was 36.7%, as determined by UV absorbance at 280 nm. An SEM image of the SOD−PCADK microparticles, shown in Figure 3, demonstrates that they are 3 to 15 μm in diameter, which is suitable for both intracellular and extracellular delivery. This may be beneficial in the case of SOD delivery, because superoxide causes both intracellular toxicity and extracellular tissue damage during inflammation.

[1] PCADK was determined to be more hydrophobic than PPADK on the basis of the log *P* values of their respective monomers, 0.359 for 1,4-cyclohexanedimethanol versus −0.149 for 1,4-benzenedimethanol.

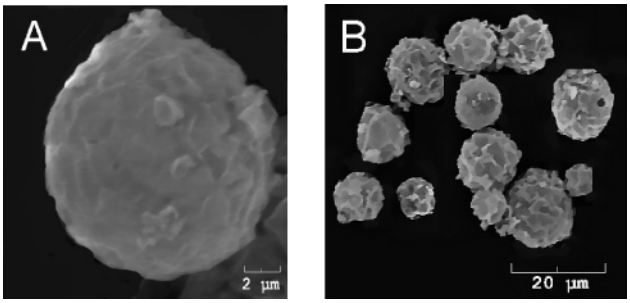


Figure 3. SEM images of SOD−PCADK microparticles: (A) 6000× magnification, (B) 1000× magnification. SEM images were taken on a Hitachi S-800.

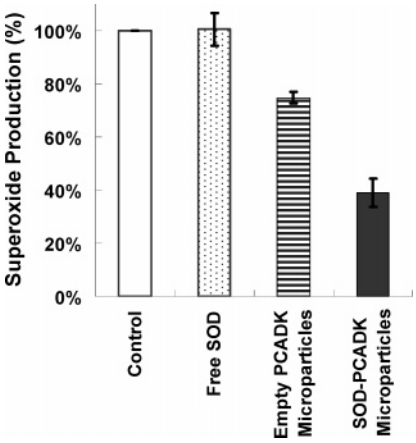


Figure 4. PCADK microparticles enhance the delivery of SOD to macrophages. Macrophages were incubated with either SOD−PCADK microparticles (black bar), empty PCADK microparticles (horizontal stripe), free SOD (dotted bar), and media only (white bar). For statistical analysis, three independent wells were measured for each sample. Significance of results was determined via the *t*-test with $p < 0.05$.

The ability of SOD−PCADK microparticles to scavenge superoxide from macrophages was investigated in cell culture. TIB-186 macrophages (1×10^5 cells/well, 96-well plate) were incubated with 0.1 mg/mL SOD−PCADK microparticles (1.14 μg SOD in 0.1 mg microparticles), free SOD (1.14 μg/mL SOD), or empty PCADK microparticles for 2 h. The cells were washed 3 times and then stimulated with 0.2 μg/mL phorbol myristate acetate (PMA) for 30 min. The superoxide production from these macrophages was then measured using a cytochrome c based assay, following the procedure of Voter et al. (*22*). Figure 4 demonstrates that free SOD by itself caused very little inhibition of superoxide production, whereas SOD−PCADK microparticles caused a 60% reduction in superoxide production. Empty microparticles also reduced superoxide production by macrophages by approximately 20%. This is most likely due to the toxicity of the particles, which caused approximately 20% toxicity under these experimental conditions (0.1 mg/mL for 2 h) as determined by an MTT assay (Supporting Information Figure S3).

In conclusion, we have developed a new acid-sensitive polyketal for drug delivery, PCADK, which is designed to hydrolyze in the acidic environment of the phagosome and enhance the intracellular delivery of phagocytosed therapeutics. Key attributes of PCADK are its well-characterized degradation products and straightforward synthesis. PCADK was synthesized on a multigram scale in one step and hydrolyzes into acetone and 1,4-cyclohexanedimethanol. The hydrolysis of PCADK is pH-sensitive, having a half-life of 24.1 days at pH 4.5 and over 4 years at pH 7.4. The therapeutic protein SOD was encapsulated into PCADK-based microparticles, 3−15 μm in size, using a double emulsion procedure. In cell culture experiments, PCADK microparticles significantly improved the ability of SOD to scavenge superoxide generated by macrophages. We anticipate widespread interest in PCADK for drug delivery, based on its acid sensitivity, well-characterized degradation products, and ease of synthesis.

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Supporting Information Available: Additional experimental details as mentioned in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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