Anti-inflammatory and anti-oxidant activity of novel biodegradable copolyoxalate nanoparticles

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Statement of Purpose: 4-hydroxybenzyl alcohol (HBA), one of the well-known phenolic compounds in diverse plants, is known to play a protective role against oxidative damage-related diseases, such as coronary heart disease, stroke and cancer and exerts anti-inflammatory effects. In this study, we introduce a novel polymer, termed copolyoxalate, in which HBA is incorporated into a polymer backbone and is released upon degradation by water hydrolysis. We report the physicochemical properties of copolyoxalate and the potential of copolyoxalate nanoparticles in pharmaceutical applications.

Methods: Polymerization. 1,4-cyclohexanedimethanol (21.96 mmol) and 4-hydroxybenzyl alcohol (5.49 mmol) were dissolved in 20mL of dry tetrahydrofuran (THF), under nitrogen, to which triethylamine (60 mmol) was added dropwise at 4°C. Oxalyl chloride (27.45 mmol) in 25 mL of dry THF was added to the mixture dropwise at 4°C. The reaction was kept under nitrogen atmosphere at room temperature for 6 h and polymers were obtained through the extraction using dichloromethane and isolation by precipitating in cold hexane.

Particle preparation. Fifty milligrams of polymers dissolved in 500 μL of DCM was added to 5mL of 10 (w/v)% poly(vinyl alcohol) solution. The mixture was sonicated using a sonicator (Fisher Scientific, Sonic Dismembrator 500) for 30 seconds and homogenized (PRO Scientific, PRO 200) for 1 minute to form a fine oil/water emulsion. The emulsion was added into 20 mL PVA (1w/w%) solution and homogenized for 1 min. The remaining solvent was removed using a rotary evaporator. Nanoparticles were obtained by centrifuging at 11,000*g* for 5 min at 4°C, washing with deionized water twice and lyophilizing the recovered pellet.

Physicochemical characterization. Hydrolysis kinetics of copolyoxalate was studied by grinding the polymers into fine powders and measuring their molecular weight by a gel permeation chromatography. The morphology and size of polyoxalate nanoparticles were observed by a scanning electron microscopy. The cytotoxicity of polyoxalate nanoparticles was investigated using RAW 264.7 cells by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay.

Nitrite analysis. The anti-inflammatory effect of copolyoxalate nanoparticles was evaluated by measuring accumulated nitric oxide (NO) in the media obtained from the cell culture. The concentration of NO was determined using a colorimetric assay based on the Griess reaction.

Results: Copolyoxalate synthesized from a one step reaction of oxalic chloride with cyclohexanedimethanol and HBA had a molecule weight of ~ 25,000Da with a polydispersity of 2.0. The chemical structure of copolyoxalate was confirmed by NMR analysis. This polymer readily degrades by water hydrolysis and generates HBA, which exerts anti-inflammatory and anti-oxidant effects. The half-life of copolyoxalate was dependent on the content of HBA on the polymer. The hydrolysis rate increased with increasing the HBA content. Copolyoxalate with 20% of HBA had a half-life of hydrolysis ~ 12h and was used for the further study. Copolyoxalate nanoparticles prepared by an emulsion/solvent displacement method were round spheres with smooth surface, with an average diameter of ~ 600 nm. The cytotoxicity of copolyoxalate nanoparticles was evaluated by an MTT method and the cell viability was compared with PLGA nanoparticles. Both nanoparticles exhibited cytotoxicity in a dose-dependent manner. However, copolyoxalate nanoparticles exhibited a significantly higher cell viability than PLGA. Nitrite analysis revealed that copolyoxalate nanoparticles possessed an inhibitory activity on NO production in LPS stimulated RAW 264.7 cells.

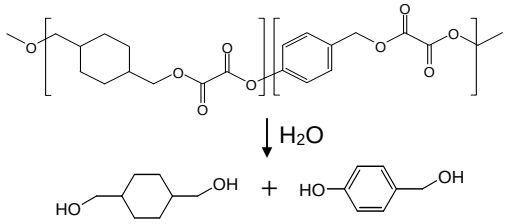

Figure 1. Chemical structure of copolyoxalate which generate hydroxybenzyl alcohol upon degradation.

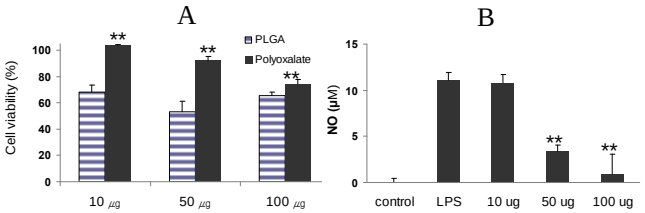

Figure 2. Cell viability of copolyoxalate nanoparticles (A) and suppression of NO in LPS-stimulated RAW 264.7cells. ** $P < 0.05$.

Conclusions. We synthesized a new biodegradable copolyoxalate that could be formulated into nanoparticles and release therapeutic hydroxybenzyl alcohol upon degradation. Copolyoxalate nanoparticles exhibited a minimal cytotoxicity and possessed an inhibitory activity on NO production because of incorporated HBA. Given therapeutic activity attributed to HBA and the ease of synthesis, we anticipate that copolyoxalate nanoparticles have great potential as drug carriers for the treatment of inflammatory diseases and cancer.

References:

1. E. Lim, *J. Pharma. Pharmacol*, 2007, 59, 1235-1240.
2. H. Kim, *Brain Res*. 2007, 1181, 130-141.