Enhanced cytosolic drug delivery using fully biodegradable poly(amino oxalate) particles

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Statement of Purpose: Cationic biodegradable microparticles have attracted much attention because they can facilitate cell uptake and enhance the efficacy of their macromolecular payload by rapidly endosomal escape via the "proton sponge"effect. In this study, we report new protein delivery systems based on cationic biodegradable poly(amino oxalate) (PAOX) that are capable of disrupting endosomes and mediating cytosolic drug delivery.

Methods: PAOX was synthesized from a one-step reaction of oxalyl chloride, cyclohexanedimethanol and piperazinediethanol. The molecular weight was determined to be ~12,000 (polydispersity of 1.8) by a gel permeation chromatography (GPC) using polystyrene standards. The chemical structure of polymers was identified with a 400 MHz ^{1}H-NMR spectrometer (JNM-EX400 JEOL). The hydrolysis kinetics of PAOX was measured under physiological conditions. The PAOX particles were prepared by single emulsion or W/O/W double emulsion. The particles were characterized by scanning electron microscope (SEM) and particle analysis. The cytotoxicity of PAOX particles was evaluated by the MTT assay. The uptake of BSA-FITC-loaded PAOX particles was studied in living RAW264.7 macrophage cells using a confocal laser scanning microscope. A protein delivery efficiency of PAOX particles was evaluated using catalase as a model protein.

Results: We synthesized fully biodegradable cationic polyoxalate, PAOX from a reaction of oxalyl chloride with piperazinediethanol and cyclohexanedimethanol for enhanced cytosolic drug delivery of therapeutic proteins.

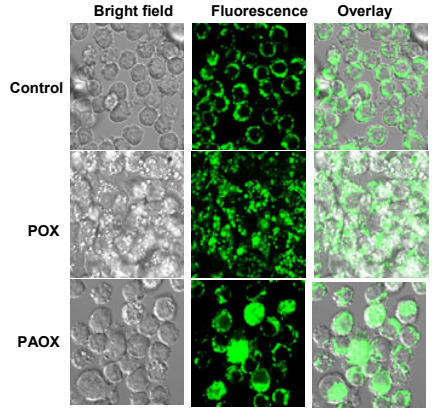

Figure 1. Confocal fluorescence micrographs of cells incubated with calcein. Cells of 7×10^5 were treated with 100 μL of POX or PAOX nanoparticles (100μg/mL).

The content of piperazinediethanol (15 mol %) in PAOX was chosen after careful consideration of the degradation kinetics and stability under aqueous conditions. The hydrolysis half-life of PAOX was ~ 36 h at pH 7.4 and 14 h at pH 5.5. This polymer is hydrophobic enough to be formulated into nanoparticles under aqueous conditions. PAOX nanoparticles were round spheres with an average diameter of ~ 450 nm with acceptable yields, > 70 %. A membrane impermeant fluorescent molecule calcein assay revealed that PAOX particles disrupted endosomes via "proton sponge" effect (Figure 1). Catalase-loaded PAOX microparticles significantly inhibited hydrogen peroxide generation in Phorbol-12-myristate-13-acetate (PMA)-stimulated macrophages, in a dose-dependent manner (Figure 2).

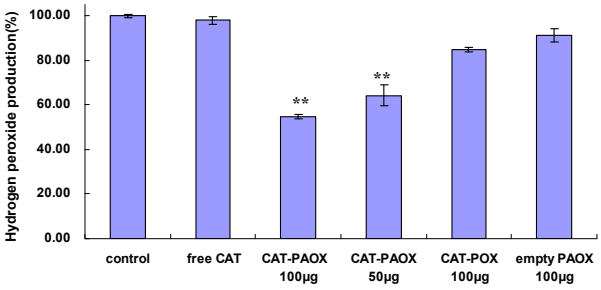

Figure 2. Enhanced delivery of catalase (CAT) to RAW 264.7 macrophage cells by PAOX microparticles. Macrophages were treated either free CAT, CAT-loadedPAOX, CAT-loaded POX or empty PAOX particles. For statistical analysis, three independent wells were measured for each sample. Significance of results was determined via the t-test with ** $p < 0.01$.

Conclusions: We developed poly(amino oxalate) as a protein delivery vehicle in order to enhance the cytosolic delivery of proteins. The incorporation of tertiary amine groups in the PAOX backbone induced hydrolytic nature and cationic nature, which results in fast drug release profile and the "proton sponge"effect. Based on excellent biocompatibility and physicochemical properties, we anticipate that PAOX is a promising cytosolic protein delivery system and useful for the treatment of acute inflammatory diseases.

References:

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