

Dual stimuli-responsive antioxidant nanoparticles based on polymeric prodrugs of vanillin

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Statement of Purpose: Excessive and unregulated production of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide and hydroxyl radicals is known to cause oxidative stress. Therefore, there has been great interest in the development of antioxidants as therapeutic agents for various ROS-associated inflammatory diseases. Vanillin is the major component of natural vanilla and has anti-inflammatory activity to suppress the expression of various pro-inflammatory cytokines. However, orally administrated vanillin is rapidly decomposed in the upper digestive tract and intravenously injected vanillin is rapidly cleared from blood circulation. Therefore, its successful clinical applications need a strategy to deliver vanillin to diseased cells or tissues and enhance its therapeutic efficacy. In this study, we developed a polymeric prodrug of vanillin, poly(vanillin oxalate)(PVO), in which vanillin is covalently incorporated in its backbone and can be released during its hydrolytic degradation. Herein, we report the synthesis and physicochemical properties of PVO and the potential of PVO nanoparticles as therapeutic agents for oxidative stress-associated diseases.

Methods: PVO was synthesized through two steps. First, 4-(5-hydroxymethyl)-5-methyl-1,3-dioxan-2-yl)-2-methoxyphenol were made using vanillin (4-hydroxy-3-methoxybenzaldehyde), 1,1,1-Tris (hydroxymethyl)ethane, dry tetrahydrofuran (THF) and p-TSA as a catalyst. Second, PVO was developed using 4-(5-hydroxymethyl)-5-methyl-1,3-dioxan-2-yl)-2-methoxyphenol, oxalyl chloride and pyridine in 1:1:2.5 mixture. The chemical structure of 4-(5-hydroxymethyl)-5-methyl-1,3-dioxan-2-yl)-2-methoxyphenol and PVO was confirmed by 1H NMR. Its average molecular weight was determined using gel permeation chromatography (GPC). PVO nanoparticles were prepared using an o/w single emulsion method. We investigated the release kinetics of vanillin from PVO nanoparticles. We assessed the H_2O_2 scavenging ability of PVO nanoparticles using Amplex Red assay and evaluated ability of PVO nanoparticles to reduce the generation of intracellular ROS through the confocal laser scanning microscopy (CLSM) and flow cytometry. Antioxidant and anti-inflammatory activities of PVO nanoparticles were assessed through detection of NO and RT-PCR. We also performed the MTT assay to evaluate the cytotoxic effects of PVO nanoparticles on RAW 264.7 cells.

Results: PVO contained both peroxalate ester bonds and acetal linkages in the backbone. Therefore PVO hydrolytically degrades into three components and its degradation is accelerated by low pH and H_2O_2 . Its molecular weight was determined to be $\sim 22,000$ Da with polydispersity index of ~ 1.6 by GPC. PVO had hydrophobic backbone and therefore could be formulated into nanoparticles of round spheres and a mean hydrodynamic diameter of ~ 230 nm in phosphate buffer (pH 7.4).

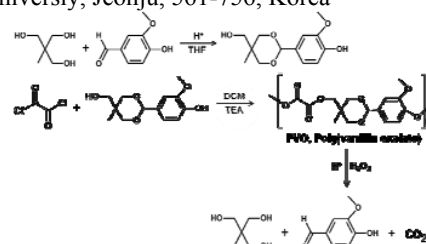


Figure 1. A synthetic route and degradation of PVO.

PVO nanoparticles showed higher H_2O_2 scavenging activity than free vanillin. This result can be explained by the combined effects of vanillin and H_2O_2 -reacting peroxalate ester bonds in the backbone of PVO.

PVO nanoparticles exhibited low cytotoxicity and excellent biocompatibility through MTT assay.

We investigated the ability of PVO nanoparticles to suppress LPS-mediated oxidative stress in RAW 264.7 cells, measured by CLSM and flow cytometry. As a result, the intracellular ROS generation was suppressed by PVO nanoparticles. PVO nanoparticles showed the stronger anti-inflammatory activity than vanillin may be due to their strong antioxidant activity. It can be explained by the PVO scavenges H_2O_2 generated during LPS-induced inflammatory responses and PVO releases vanillin during its degradation, which inhibits the further generation of other ROS.

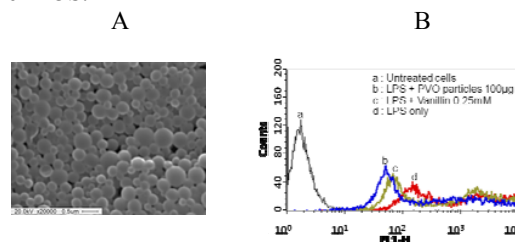


Figure 2. (a) Morphology of PVO nanoparticles (SEM). (b) Antioxidant activities of PVO nanoparticles in LPS-stimulated RAW 264.7 cells (Flow cytometry).

Conclusions: We developed antioxidant and anti-inflammatory nanoparticles based on dual stimuli-responsive polymeric prodrug of vanillin (PVO). PVO showed pH-dependent hydrolytic degradation kinetics due to its acetal linkage. Also, PVO nanoparticles contained H_2O_2 -responsive peroxalate ester bonds in their backbone and showed highly potent antioxidant activities by scavenging H_2O_2 and inhibiting the generation of oxidants in LPS-stimulated cells. In addition, PVO nanoparticles also exerted potent anti-inflammatory activity by inhibiting the expression of $TNF-\alpha$ and iNOS. We anticipate that PVO nanoparticles as a prodrug of vanillin have tremendous potential as therapeutics and drug delivery systems for oxidative stress-associated inflammatory diseases.