Nanoparticles for drug delivery to respiratory tract for prophylaxis of free radical-induced damage

Vertegel, A.,[1] Reukov, V.,[1] Maximov, V.,[1] Mulligan, R.,[2] Atkinson, C.,[2] Schlosser, R.[2]
[1]Clemson University, Clemson, SC, USA; [2]Medical University of South Carolina, Charleston, SC, USA

Statement of Purpose: Free radical-induced damage is one of the most important and well understood aspects of tobacco smoking. Airborne smoke particles are known to produce high levels of reactive oxygen species (ROS), such as superoxide, peroxide, and OH-radical.[1] If this free radical-induced damage could be eliminated, one can expect reduced incidence and/or milder manifestations of pro-inflammatory conditions associated with the inhalation of tobacco smoke. Superoxide dismutase (SOD) has recently been proposed as a potentially powerful therapeutic agent for the resolution of inflammation.[2] It can thus be expected that SOD will be effective in the treatment of free radical-induced damage caused by the inhalation of tobacco smoke. However, one problem associated with therapeutic use of SOD for respiratory conditions is ensuring that topically administered therapeutics actually reach the target tissues, i.e. respiratory mucosa, and remain there long enough to be effective. Normal mucociliary clearance typically results in rapid removal of topical medications within 15-20 minutes when it would be more desirable to maintain mucosal contact for hours to days.

Here, we report targeted delivery to and retention of SOD on respiratory epithelium using biodegradable polylactic acid (PLA) nanoparticles as delivery vehicles. Specifically, we simultaneously coated nanoparticles with SOD and a targeting antibody, which binds to an epitope on the surface of epithelial cells. The strategic goal of this study was to develop a topical spray, which could be used by those exposed to second hand tobacco smoke as a therapeutic measure against its pro-inflammatory action on respiratory epithelium.

Methods: In a pilot study, SOD and antibody to epithelial membrane antigen (MUC1), a cell surface marker specific for airway epithelium, were covalently attached to ~100 nm polylactic acid (PLA) nanoparticles. The conjugates were then fluorescently labeled by a green fluorescent dye (Alexa Fluor® 488). Human turbinate explants were then incubated with fluorescently-labeled SOD- and antibody-coated nanoparticles (MUC1-SOD-NPs) for 20 minutes. Nanoparticles lacking the targeting antibody (SOD-NPs) served as the negative control. Imaging was repeated every 24 hours post-treatment for 4 days to determine whether or not nanoparticles were cleared by the cells. Cell viability was evaluated by measuring ciliary beat frequency. To evaluate in-vivo efficacy in acute model of cigarette smoke exposure, mice were exposed to 3 days of cigarette smoke at a dose of 4 cigarettes/mouse/exposure with mice receiving two exposures per day. Mice were either treated with the solution of phosphate buffer (PBS control) or MUC1-SOD-NP daily 30 minutes prior to the first daily exposure. Lungs were harvested 12 h following the final exposure, and the degree of alveolar inflammation, ROS production and lung histology analyzed.

Results: Fluorescent imaging of turbinate explants 4 days after treatment with Alexa Fluor® 488 labeled nanoparticles demonstrated lack of binding in NPs without targeting antibody, while MUC1-SOD-NPs demonstrate significant binding to respiratory epithelial cells even after 4 days. Measurement of ciliary beat frequency (CBF) over 72 hour period demonstrated cell viability and no impairment of ciliary function due to prolonged binding of MUC1-SOD-NPs even at higher concentrations.

In vivo biodistribution studies showed that MUC1-SOD-NP Alexa 488 treated mice had NP localized to the alveolar walls at 12 h post instillation. At the same time, animals treated by SOD NPs lacking the antibody showed no obvious deposition of NP in the alveolar or ciliary epithelium.

In vivo studies using acute model of cigarette smoke exposure were indicative of the efficacy of MUC1-SOD-NPs. Histological studies demonstrated that animals treated with PBS showed evidence of neutrophil accumulation within the parenchymal wall and surrounding vascular structures, and evidence of epithelial cell vacuolization and cytoplasmic blebbing, features associated with cell death and damage (Fig.1B). In contrast animals treated with MUC1-SOD-NPs showed few parenchymal inflammatory infiltrates and no obvious evidence of epithelial hyperplasia (Fig. 1C). We also found significantly lower levels of reactive oxygen species in mice treated by MUC1-SOD NPs versus PBS treated controls.

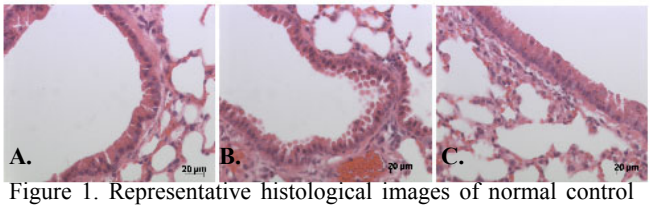

Figure 1. Representative histological images of normal control (A), PBS treated (B) and MUC1-SOD-NP (C) treated lungs following three days of cigarette smoke exposure. The epithelial changes seen in PBS treated animal (B) demonstrate signs of cytoplasmic blebbing and vacuolation, which is absent in control (A) and anti-MUC1-SOD-NP (C) (n=4-6).

Conclusions: We prepared PLA NPs simultaneously coated by SOD and targeting antibody (MUC1) and demonstrated targeting to nasal epithelial cells and lack of toxicity *in vitro*. In vivo biodistribution studies showed retention of MUC1-SOD NPs on respiratory epithelium for at least 12 h, while nanoparticles lacking the targeting antibody were removed by mucociliary clearance. Finally, *in vivo* study using acute mouse model of cigarette smoke exposure demonstrated therapeutic efficacy of MUC1-SOD nanoparticles.

[1] J. Hobson, et. al, *Am. J. Path.*, 139(3) (1991), 573-580.
[2] K. Yasui, et. al, 55(9) (2006), 359-363.