Effects of Polyketal Delivered Superoxide Dismutase on Bleomycin-Induced Lung Fibrosis

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Background: Lung fibrosis is an untreatable and deadly disease accounting for over 250,000 deaths in the U.S. annually. Alarmingly, the incidence of lung fibrosis has increased 150% over the past three years. While the exact mechanisms initiating and sustaining pulmonary fibrosis remain under investigation, it is clear that the chronic recruitment of alveolar macrophages (AM) and other pro-inflammatory and non-professional phagocytic cells into the alveolar space aggravates the underlying pathology. As a part of their normal physiology, these cells exhibit raised levels of reactive oxygen species (ROS), which when released, further contribute to the disease progression (1). It has long been postulated that delivering antioxidants or ROS modifying enzymes such as superoxide dismutase (SOD) may, at a minimum, prevent the further progression of the disease. However, therapeutic delivery remains a critical hurdle. Microparticles constructed from polyketals represent a novel delivery vehicle for therapeutic lung delivery. This newly formed class of polymers exhibit acid catalyzed hydrolysis, short half-lives, and degradation into neutral byproducts, making them ideal for lung delivery (2).

Methods: Particle synthesis was conducted according to the procedures of Lee et al (3). Briefly, SOD (Sigma, St. Louis, MO) was encapsulated in polyketal polymer (copolymer of 1,4-cyclohexanedimethanol and 1,5-pentanediol; PK3) via a w/o/w double emersion procedure. Two separate studies were done to examine both the biocompatibility of PK3 microparticles in the lung and the therapeutic efficacy of SOD loaded PK3 particles in bleomycin-induced fibrosis. For biocompatibility, C57 mice were intratracheally injected with 1.5 mg (50 µL PBS) of empty PK3 or empty PLGA particles for comparison. Mice were sacrificed at 1, 4, and 7 days for temporal analysis of inflammation due to particle instillation. For therapeutic testing, intratracheal co-injections were performed with 50 µL of bleomycin (2.5 U/mL) and 100 µg of particles of either PK3/SOD, empty PK3, empty PLGA, or free SOD in PBS. Mice were sacrificed at 14 days for analysis of whole tissue sections via hydroxyproline (collagen) assay and immunohistochemistry, and bronchoalveolar lavage (BAL) analysis of cell-type recruitment.

Results: In contrast to PLGA, PK3 injected particles inflamed lung tissue to a lesser degree. At day one, macrophage numbers remained similar to control values; at day 4 macrophage influx was markedly less than that of PLGA groups. Macrophage infiltration subsided by day 7 closer to basal level, whereas PLGA particles continued to induce macrophage influx on day 7. SOD/PK3 particles co-injected with bleomycin exhibited the fibrotic condition to a lesser degree than the positive control, marked by decreased macrophagal infiltration, decreased matrix collagen deposition and overall histological examination.

Conclusions: Polyketal microparticles are a superior biomaterial for drug delivery into the periphery of the lung, as shown by a significantly lesser degree of inflammation due to particulate instillilation as compared with PLGA, a commonly used biomaterial. We also report that antioxidant delivery, in this case SOD, effectively reduced inflammation and progression of fibrosis due to bleomycin, as shown by histological analysis and hydroxyproline analysis (collagen deposition).

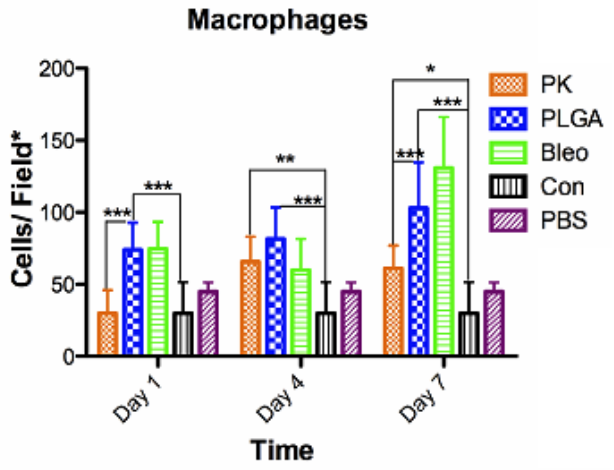

Figure 1. Bronchoalveolar lavage analysis after intratracheal particle injections (*p<0.05, **p<0.01, ***p<0.001).

References:

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