Local Lung Co-Delivery of siRNA and Drug

Vera Ivanova\textsuperscript{1}, Olga B. Garbuzenko\textsuperscript{1}, Ruslan Rustamov\textsuperscript{1}, Gediminas Mainelis\textsuperscript{2}, Tamara Minko\textsuperscript{1}

\textsuperscript{1}Department of Pharmaceutics, Rutgers, the State University of New Jersey, Piscataway, NJ, 08854, USA
\textsuperscript{2}Department of Environmental Sciences, Rutgers, The State University of New Jersey, New Brunswick, NJ, 08901
vivanova@eden.rutgers.edu

\textbf{ABSTRACT SUMMARY:}
Complex delivery system (DS) was developed for inhalation treatment of idiopathic pulmonary fibrosis (IPF). DS was based on lipid nanoparticles (LN) as carriers and contained prostaglandin E2 (PGE2) and siRNAs targeted to MMP3, CCL12, and HIF1A mRNAs. The DS was tested on mouse experimental model of IPF. Administration was performed by inhalation and intravenously (IV). The data obtained showed that proposed combinatorial treatment significantly suppressed inflammation, fibrosis, apoptosis, lung injury and finally decreased the mortality of experimental animals with IPF. Positive effects of the complex DS were more pronounced after the delivery by inhalation when compared to IV treatment with the same DS or its components applied separately.

\textbf{INTRODUCTION:}
Inhaled therapeutics has recently played an important role in local therapy of various lung diseases including IPF. However, the efficiency of therapeutic agents delivered via inhalation is limited by the complexity of devices used and difficulties in formulation of suitable dosage forms [1-3]. Furthermore, unlike asthma drugs, biological therapeutics including siRNA cannot be administered without an appropriate dosage form due to their fast degradation and poor cellular penetration. PGE2, a cyclooxygenase-derived lipid mediator, has attracted considerable attention for its role in the development and progression of IPF and as a possible therapeutic agent for this disease [4,5]. Previously, we found that chemokine CCL12, matrix metalloproteinase MMP3 and hypoxia inducible factor HIF1A are key proteins responsible for the development of inflammation and IPF [4]. We hypothesize that inhibition of these proteins by the local inhalation co-delivery of PGE2 and multiple siRNAs targeted to mRNA encoding these proteins can be successfully used for effective treatment of IPF. The present investigation is aimed at developing an appropriate nanoscale-based complex of drug and siRNAs for such inhalation co-delivery and verifying the hypothesis.

\textbf{EXPERIMENTAL METHODS:}
LN were used as a vehicle to deliver PGE2 and siRNAs. Precirol® ATO5, PGE2, and lecithin were dissolved in methanol with addition of butanol as cosurfactant and preheated to 70 °C under stirring. Then temperature was decreased to 37 °C and aqueous solution was added. Aqueous solution was prepared by mixing DOTAP with siRNA in 10 ml of Tween 80/MeOH/H\textsubscript{2}O. The mixture was homogenized, sonicated and allowed to stir for 8 h for precipitation. Nanoparticles were characterized by atomic force, transmission electron and fluorescence microscopes (AFM, TEM and FM, respectively) and differential scanning calorimetry (DSC). Size and charge of LN were also measured. Cyto- and genotoxicity of nanoparticles were assessed using MTT and Comet assays, respectively. \textit{In vivo} experiments were carried out on SKH1 mice. Bleomycin was administered intratracheally to the mice in 1.5 U/kg dose. Mice were treated for three weeks with complex DS by inhalation or IV injection. Mice were sacrificed and lungs, liver, kidney, spleen, heart, brain, and serum were collected for histological analysis. In addition, lungs were used for the histopathological evaluation, hydroxyproline and collagen measurement, TEM, immunohistochemistry, TUNEL apoptosis assay. RNA was isolated and quantitative PCR analysis of 84 key genes involved in the development of lung inflammation and fibrosis was carried out. Distribution of LN in lungs and other organs after intravenous or inhalation administrations
was examined using an IVIS imaging system. Magnetic resonance imaging (MRI) was used to visualize fibrotic tissues.

**RESULTS AND DISCUSSION:**

The developed LN had a spherical form with a narrow size distribution (Fig.1). A 4-port nose-only exposure chamber was used for inhalation of small laboratory animals as described [6]. It was found that the chamber provided a uniform distribution of nanoparticles between the ports and did not influence significantly on the size and shape of LN (Fig.1). Data showed that the developed nanoparticles were non cytotoxic and genotoxic (Fig.2) and provided for an efficient protection of active components from the degradation during the nebulization. The development of IPF and lung injury in experimental animals after the instillation of bleomycin was confirmed by the histopathological evaluation, and collagen concentration measurements. In addition, MRI revealed a dense tissue in the lungs (Fig. 3). Development of IPF also led to the overexpression of inflammatory and mesenchymal transition genes and protein that destabilized homeostasis and induced apoptosis in lung tissues. Mice with IPF were treated by the developed DS via inhalation and IV injections. Biodistribution studies and TEM clearly showed that LN-based DS was predominately accumulated in the lungs and internalized by lung cells after inhalation. Combinatorial treatment of mice with IPF by LN containing PGE2 and siRNAs had synergistic effect in attenuating lung fibrosis by downregulating inflammatory factors, preventing alveoli collapse through synthesis of surfactant, and downregulated myofibroblast proliferation. These positive effects were significantly more pronounced after inhalation when compared with IV treatment. Finally, inhalation treatment by PGE2 and siRNAs significantly decreased mortality of mice with IPF.

**CONCLUSION:**

Our results demonstrated that IPF can be effectively treated by local inhalation lung delivery of PGE2 and several specific siRNAs by LN. The data provided evidence that dual drug/siRNA nanoparticle-based inhalation treatment led to the overcoming of fibrogenesis making this composition an attractive for treatment of inflammation and IPF.

**REFERENCES:**


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