A Cocktail of Superoxide Dismutase and Fasudil Encapsulated in Targeted Inhalable Liposomes Prevent PAH Progression at a Reduced Dosing Frequency

N. Gupta, H. M. Ibrahim, F. Ahsan
Texas Tech University Health Sciences Center

**Purpose**

Pulmonary arterial hypertension (PAH) is a multifaceted chronic disorder of the cardiopulmonary system. Currently approved therapies, monotherapy or combination, provide modest hemodynamic relief but fail to restrain disease progression, and thus patient mortality and morbidity remains disappointingly high. We hypothesize that a cocktail of superoxide dismutase (SOD), a reactive oxygen species scavenger, and fasudil, a specific rho-kinase inhibitor, encapsulated in inhalable targeted liposomes provide hemodynamic relief and ameliorate chronic symptoms of PAH such as occlusion and remodeling.

**Methods**

We prepared and characterized CAR, a lung homing peptide, conjugated nanosized liposomes for the inhalational delivery of SOD and fasudil. Optimized formulations were tested for the in vivo drug absorption studies after intratracheal administration and acute hemodynamic efficacy in monocrotaline (MCT) induced PAH rats. Long term efficacy was evaluated in a chronic model: SU-5416/hypoxia induced PAH rats. Plain drugs/comboination or formulations were administered every 48 or 72 hours, respectively, for 3 weeks after the development of PAH. Pulmonary hemodynamics and histopathological studies were performed to evaluate the effect of formulation on PAH progression. Bronchoalveolar lavage (BAL) was done to assess the safety of formulations.

**Results**

We successfully prepared nanosized CAR-liposomes containing both drugs and optimized for favorable physicochemical properties (Fig. 1). CAR-liposomes increased biological half-lives of SOD and fasudil by~3 fold. Formulation produced pulmonary specific vasodilation in MCT-induced PAH rats as lung targeting index of CAR liposomes was found to be ~10-fold higher than plain cocktail. Chronic studies revealed that formulation, at a reduced dosing frequency, showed significant hemodynamic relief (>50% reduction in pulmonary arterial pressure) and prevented right ventricular hypertrophy and degree of occlusion in PAH rats (Fig. 2). Further, CAR-liposomes reduced the extent of collagen deposition and muscularization of arteries and, modulated expression of molecular biomarkers such as eNOS (increased) and p-MYPT1 (decreased) as compared to plain drugs/comboination (Fig. 3). Finally, BAL studies of formulation showed reduction in wet lung weight and levels of lung injury markers such as alkaline phosphatase and lactate dehydrogenase after intratracheal administration.

**Conclusion**

Our preclinical study suggests that CAR liposomes containing SOD and fasudil, at a reduced dosing frequency, has better efficacy against various PAH symptoms than plain drugs/comboination.