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Rapamycin nanoparticles localize in diseased lung vasculature and prevent pulmonary arterial hypertension

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ABSTRACT

Vascular remodeling resulting from pulmonary arterial hypertension (PAH) leads to endothelial fenestrations. This feature can be exploited by nanoparticles (NP), allowing them to extravasate from circulation and accumulate in remodeled pulmonary vessels. Hyperactivation of the mTOR pathway in PAH drives pulmonary arterial smooth muscle cell proliferation. We hypothesized that rapamycin (RAP)-loaded NPs, an mTOR inhibitor, would accumulate in diseased lungs, selectively targeting vascular mTOR and preventing PAH progression. RAP poly(ethylene glycol)-*block*-poly(ε-caprolactone) (PEG-PCL) NPs were fabricated. NP accumulation and efficacy were examined in a rat monocrotaline model of PAH. Following intravenous (IV) administration, NP accumulation in diseased lungs was verified via LC/MS analysis and confocal imaging. Pulmonary arteriole thickness, right ventricular systolic pressures, and ventricular remodeling were determined to assess the therapeutic potential of RAP NPs. Monocrotaline-exposed rats showed increased NP accumulation within lungs compared to healthy controls, with NPs present to a high extent within pulmonary perivascular regions. RAP, in both free and NP form, attenuated PAH development, with histological analysis revealing minimal changes in pulmonary arteriole thickness and no ventricular remodeling. Importantly, NP-treated rats showed reduced systemic side effects compared to free RAP. This study demonstrates the potential for nanoparticles to significantly impact PAH through site-specific delivery of therapeutics.

1. Introduction

PAH is fatal disease that ultimately results in right heart failure (Pauwaa et al., 2011). Mortality rates from PAH in the US have increased by >65% in older aged patients, while hospitalization rates have increased by 44% (George et al., 2014), warranting the need for improved therapies for PAH patients. PAH is defined hemodynamically by elevated mean pulmonary arterial pressures (mPAP) > 25 mm Hg in the presence of normal pulmonary artery occlusive pressure and elevated pulmonary vascular resistance. It is characterized by pathophysiological changes that include endothelial dysfunction, proliferation of pulmonary arterial endothelial cells (PAECs) and smooth muscle cells (PASMCs) (Farber and Loscalzo, 2004). Drugs used to treat PAH have

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principally focused on direct regulation of vascular tone and indirectly affect proliferation (Sahni et al., 2016). While these drugs improve hemodynamics, quality of life, and patient survival (Montani et al., 2014), present-day PAH pharmacological therapies suffer from considerable shortcomings. As an example, epoprostenol, the first prostanoid used in PAH, has a severely short half-life (on the order of minutes), requiring continuous intravenous administration that lessens patient compliance and increases the risk of sepsis (Delcroix and Howard, 2015; Kitterman et al., 2012). These drugs also have off-target effects that limit their clinical applicability. Several agents have been shown to promote left heart disease and parenchymal lung diseases (Galie et al., 2009), with PAH progressing despite combination therapy. Lung transplantation remains the only effective therapeutic option in the majority of PAH patients (George et al., 2011), but with a limited availability of donor lungs (Valapour et al., 2016), there is a pressing need to develop more effective pharmacological interventions in PAH that extend beyond current strategies.

Positive outcomes in PAH rely on the ability to site-specifically direct drugs to target cells in lung vasculature such as PAECs and PASMCs. Nanoparticles (NPs) have emerged as suitable drug delivery vehicles that have significantly impacted the field of chemotherapy, resulting in improved patient outcomes and morbidity (Blanco et al., 2011). A large part of the success of NPs in cancer is owed to enhanced permeability in tumor tissue, wherein NPs effectively extravasate through fenestrations in blood vessels, significantly increasing their accumulation (Maeda et al., 2013). This phenomenon is also present in PAH, however current treatments available clinically do not exploit this. Inflammation and hypoxia lead to focal disruptions in endothelial cell basement membranes, consequently enhancing vascular permeability (Guignabert et al., 2013; McLaughlin and McGoon, 2006; Stenmark et al., 2006). Additionally, increased vascular pressure induces mechanical and shear stress, resulting in increases in the vascular surface area, leading to stretching, loss of endothelial tight junctions, and formation of fenestrations (Fig. 1) (Zhou et al., 2016). Lastly, bone morphogenetic protein receptor 2 (BMPR2) mutations, prevalent in ~70% of patients with heritable disease and 25% of idiopathic cases, lead to dysregulation of TGF-β signaling, contributing to loss of endothelial function and increased vascular permeability (Morrell, 2006).

In light of the physiological characteristics that can favor enhanced accumulation of nanotherapeutics in the vasculature of lungs with PAH, our objective was to demonstrate the potential of polymeric NPs as a viable treatment strategy in PAH. Given that aberrant activation of mTOR has been implicated in pulmonary vascular remodeling in PAH (Goncharova, 2013), the mTOR inhibitor rapamycin (RAP) was encapsulated within NPs for PAH treatment. We hypothesized that enhanced permeability of pulmonary arterioles in PAH would result in heightened accumulation of NPs in remodeled vasculature, with a RAP NP formulation consequently resulting in effective, site-specific therapy. Our findings indicate that the use of NPs that allow for localized therapy, combined with RAP, a molecule targeting the mTOR pathway, is effective at treating PAH and lowers potential side effects. These results open new avenues for the exploration of nanotherapeutics for treatment of PAH and other pulmonary diseases.

2. Materials and methods

2.1. Materials

Poly(ethylene glycol)-*block*-poly(ε-caprolactone) (PEG 5k-*b*-PCL 5k) was obtained from Advanced Polymer Materials (Montreal, Canada). Monocrotaline (MCT) was purchased from Sigma-Aldrich (St. Louis, MO). RAP used in this study was purchased from LC Laboratories (Woburn, MA). The fluorophore BODIPY 630/650 was obtained from Life Technologies (Waltham, MA). All organic solvents were purchased from Thermo Fisher Scientific (Waltham, MA).

2.2. Methods

2.2.1. NP fabrication

Polymer NPs were fabricated using a previously established solvent evaporation procedure (Blanco et al., 2014). Briefly, poly (ethylene glycol)-*block*-poly(ε-caprolactone) and either RAP or

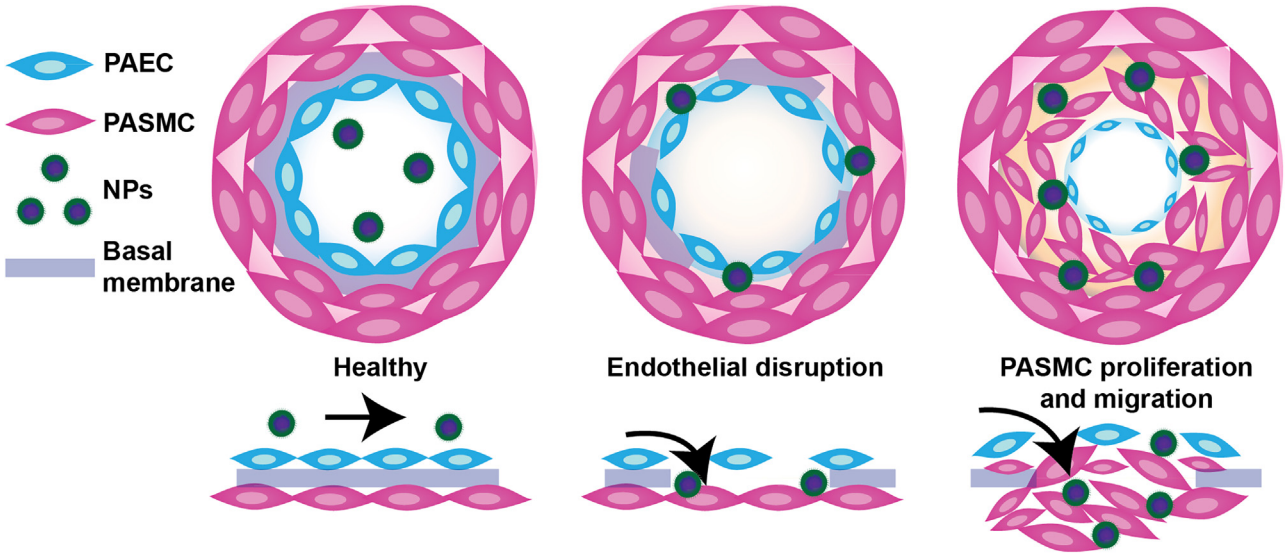

Fig. 1. Schematic representation of endothelial dysfunction in PAH and the potential for nanoparticle extravasation into pulmonary vasculature. In healthy pulmonary vasculature, the endothelium is a tight, semipermeable membrane barrier. In lungs undergoing PAH, the inflammatory response caused by dysregulation of vascular effectors along with hypoxia leads to endothelial dysfunction. This results in loss of tight junctions, discontinuous endothelia, and disrupted basal membranes. Continued PAH progression is accompanied by neointimal hyperplasia and the generation of plexiform lesions, further damaging endothelial linings. The disrupted vasculature and fenestrations present in PAH was hypothesized to result in enhanced accumulation of nanoparticles in pulmonary vasculature.