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# PEPTIDE NANOMEDICINES FOR TREATMENT OF ACUTE LUNG INJURY

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## Abstract

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) represent a heterogeneous group of lung disease in critically ill patients. Despite the increased understanding of the molecular pathogenesis of ARDS, the mortality remains unacceptably high, ranging from 34% to 64%. Hence, ARDS represents an unmet medical need with an urgency to develop effective pharmacotherapies. Several promising targets that have been identified as potential therapies for ARDS have been limited because of difficulty with delivery. In particular, delivery of peptides and proteins to the lung is an ongoing challenge. Nanobiotechnology and nanoscience are the basis of innovative techniques to deliver drugs targeted to the site of inflamed organs, such as the lungs. Nanoscale drug delivery systems have the ability to improve the pharmacokinetics and pharmacodynamics of agents allowing an increase in the biodistribution of therapeutic agents to target organs, resulting in improved efficacy with reduction in drug toxicity. These systems are exploited for therapeutic purpose to carry the drug in the body in a controlled manner from the site of administration to the therapeutic target. Hence, it is an attractive strategy to test potential targets

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for ALI/ARDS using nanotechnology. To this end, we have identified several potential targets and proposed the delivery of these agents using nanomicelles to improve the drug delivery.

## 1. INTRODUCTION

In recent years, nanomedicine has become an attractive concept for the targeted delivery of therapeutic and diagnostic compounds to the lung (Dames *et al.*, 2007; Foldvari and Elsabahy, 2011). Nanoscale drug delivery systems have the ability to improve the pharmacokinetics and increase the biodistribution of therapeutic agents to target organs, thereby resulting in improved efficacy and reduced drug toxicity (Kim *et al.*, 2010; Koo *et al.*, 2005; Singh, 2010). Nanocarriers are particularly designed to target inflammation and cancer that have a permeable vasculature. Among the various drug delivery systems considered for pulmonary application, the use of biodegradable polymeric nanoparticles represent several advantages for the treatment of respiratory diseases (Bailey and Berkland, 2009; Buxton, 2009). A number of different strategies have been proposed for modification of nanoparticle characteristics to control their behavior within biological environments, like cell-specific targeted drug delivery or modified biological distribution of drugs, both at the cellular and organ level. This method of delivery is particularly attractive for inflammatory conditions such as acute lung injury (ALI) and acute respiratory distress syndrome (ARDS).

Despite recent advances in diagnostic and therapeutic modalities, ALI and ARDS still represent an unmet medical need because it is associated with appreciable morbidity and mortality (30–40%) and substantial medical expenditure (Girard and Bernard, 2007; Matthay, 2008; Matthay and Ware, 2000; Rubinfeld and Herridge, 2007). Hence, there is an urgent need to develop and test new drugs to treat this devastating disorder. Unfortunately, contemporary drug development approaches to address this challenge that center on mono-metabolic pathway inhibitors are hindered by diverse mechanisms underlying ALI and ARDS pathogenesis and by nonselective drug effects that could predispose to serious adverse events.

To overcome both barriers, we emulated the clinical success of combination therapy in cancer and HIV (Shey *et al.*, 2009) by devising an innovative nanopharmacotherapeutic strategy consisting of a combination of three long-acting and safe nanomedicines that selectively target and inhibit three distinct key intracellular proinflammatory signaling cascades activated in ALI. Accordingly, we have harnessed unique attributes of three novel, long-acting, biocompatible, and biodegradable antiinflammatory nanomedicines. They consist of two amphipathic peptide drugs, human glucagon-like peptide-1(7–36) amide (GLP-1), triggering receptor expressed on myeloid cells (TREM-1)

peptide and 17-allylamino-17-demethoxygeldanamycin (17-AAG), a water-insoluble cytotoxic drug. This innovative approach consists of self-assembly of each drug with U.S. FDA-generally regarded as safe (GRAS) distearoylphosphatidylethanolamine covalently linked to polyethylene glycol of molecular weight 2000 (DSPE-PEG<sub>2000</sub>), a component of U.S.FDA-approved Doxil<sup>®</sup>, that forms long-acting, biocompatible, and biodegradable, sterically stabilized phospholipid nanomicelles (SSM) in aqueous milieu (size, ~ 15 nm; Rubinstein and Önyüksel, 2007a,b; Sadikot *et al.*, 2008a,b, 2009).

## 2. STERICALLY STABILIZED PHOSPHOLIPID NANOMICELLES

SSM are a novel, long-acting, biocompatible, and biodegradable phospholipid-based drug delivery vehicles that acts as versatile carrier platform for peptide and water-insoluble drugs. This approach entails self-assembly of distearoylphosphatidylethanolamine covalently linked to polyethylene glycol of molecular weight 2000 (DSPE-PEG<sub>2000</sub>) with drugs to form SSM in aqueous milieu (size, ~ 15 nm in diameter) (Koo *et al.*, 2005; Sadikot *et al.*, 2009).

These nanomicelles are composed of a hydrophilic corona that houses amphipathic peptide drugs, such as TREM-1 peptide and GLP-1, and a hydrophobic core that accommodates water-insoluble drugs, such as 17-AAG. They are simple to prepare and, unlike liposomes, can be stored in lyophilized form without lyo- or cryo-protectants for extended periods of time. Nanomicelles stabilize TREM-1 peptide and GLP-1 in active biological form ( $\alpha$ -helix) which is preferred for ligand-receptor interactions and prevents rapid peptide degradation *in vivo*, thereby prolonging bioactivity (Al-Sabah and Donnelly, 2004; Alana *et al.*, 2006). In addition, SSM solubilize high concentrations of 17-AAG. Unlike surfactant micelles, the low critical micellar concentration (~ 1  $\mu$ M) of these nanoparticles prevents their disintegration upon dilution in biological fluids. Importantly, the PEG<sub>2000</sub> moiety of SSM confers steric hindrance in the circulation, while their nanosize mitigates renal clearance and extravasation from intact microvessels. This, in turn, prolongs the circulation time of drug-loaded nanomicelles and promotes preferential extravasation from hyperpermeable lung microcirculation, the hallmark of ALI, into the injured lung.

This innovative, passively targeted therapeutic strategy amplifies drug delivery to the lung, thereby maximizing efficacy and enhancing the resolution of inflammation while reducing collateral damage to innocent bystander organs. These nanomicelles are FDA-approved for human studies (Lim *et al.*, 2011; Sadikot *et al.*, 2009).

### 3. NANOTECHNOLOGY FOR DRUG DELIVERY TO THE LUNG

#### 3.1. Nanoparticle drug delivery

Systemic delivery of nanoparticles is based on the principle of passive targeting. Passive targeting occurs as a result of extravasation of the nanoparticles at the diseased site where the microvasculature is leaky (Pison *et al.*, 2006). Examples of diseases where passive targeting of nanocarriers can be achieved are tumor and inflamed tissues. Microvascular leakiness in ALI and ARDS is the result of increased permeability, and the presence of cytokines and other vasoactive factors that enhance permeability (Matthay and Ware, 2000). Thus, drugs used for treatment of ALI and ARDS can be administered systemically and will localize to the lungs by passive targeting. This innovative, passively targeted therapeutic strategy amplifies drug delivery to the lung thereby maximizing efficacy and enhancing resolution of inflammation while reducing collateral damage to innocent bystander organs as occurs in patients with ALI and ARDS.

Among various drug delivery systems considered for pulmonary application, nanoparticles demonstrate several advantages for the treatment of respiratory diseases, including prolonged drug release, cell-specific targeted drug delivery, or modified biological distribution of drugs, both at the cellular and organ level (Azarmi *et al.*, 2008; Roy and Vij, 2010; Rudolph *et al.*, 2010; Yang *et al.*, 2008). Nanoparticles composed of biodegradable lipid-based nanomicelles and polymers fulfill many requirements placed on these delivery systems, such as the ability to be transferred into an aerosol, stability against forces generated during aerosolization, biocompatibility, targeting of specific sites, or cell populations in the lung, release of the drug in a predetermined manner, and degradation within an acceptable period of time (Roy and Vij, 2010; Yacobi *et al.*, 2008). Clearly, further studies are warranted to establish the role of aerosolized nanopreparations for inhalational therapy.

#### 3.2. Nanoparticle delivery to the lung

We have used nanomicellar preparations for the delivery of targeted drugs to the lungs using a systemic approach. Micelles are self-assemblies of amphiphiles that form supramolecular core-shell structures in the aqueous environment (Koo *et al.*, 2005). Hydrophobic interactions are the predominant driving force in the assembly of the amphiphiles in the aqueous medium when their concentrations exceed the CMC. SSM were developed and patented in our laboratory as versatile carriers for peptide and

water-insoluble drugs (Rubinstein and Önyüksel, 2007a,b; Sadikot *et al.*, 2008a,b, 2009).

Systemic delivery of these agents is based on the principle of passive targeting (Kim *et al.*, 2010; Koo *et al.*, 2005). Passive targeting occurs due to extravasation of the nanoparticles at the diseased site where the microvasculature is leaky (Liu *et al.*, 2009). Examples of diseases where passive targeting of nanocarriers can be achieved are tumor and inflamed tissues. Leakiness in ALI/ARDS is the result of increased permeability and the presence of cytokines and other vasoactive factors that enhance permeability. Thus, agents used for treatment of ALI/ARDS can be administered systemically and will localize to the lungs by passive targeting.

We hypothesize that this innovative, passively targeted therapeutic strategy amplifies drug delivery to lung thereby maximizing efficacy and enhancing resolution of inflammation while reducing collateral damage to innocent bystander organs that occurs in patients with sepsis and ARDS.

Controlled drug delivery systems have also become increasingly attractive options for inhalation therapies (Klingler *et al.*, 2009; Mansour *et al.*, 2009; Roy and Vij, 2010). The large surface area of the lungs and the minimal barriers impeding access to the lung periphery make this organ a suitable portal for a variety of therapeutic interventions (Beck-Broichsitter *et al.*, 2010; Bur *et al.*, 2009; Buxton, 2009). The blood-barrier between the alveolar space and the pulmonary capillaries is very thin to allow for rapid gas exchange. Alveoli are small and there are approximately 300 million of them in each lung. Although alveoli are tiny structures, they have a very large surface area in total ( $\sim 100 \text{ m}^2$ ) for performing efficient gas exchange, making it an attractive organ for direct drug delivery (Rudolph *et al.*, 2010; Smola *et al.*, 2008). Among the various drug delivery systems considered for pulmonary application, nanoparticles demonstrate several advantages for the treatment of respiratory diseases, such as prolonged drug release, cell-specific targeted drug delivery, or modified biological distribution of drugs, both at the cellular and organ level (Azarri *et al.*, 2008; Bailey and Berklund, 2009; Pison *et al.*, 2006; Sung *et al.*, 2007; Yang *et al.*, 2008). Nanoparticles composed of biodegradable polymers fulfill many requirements placed on these delivery systems, such as ability to be transferred into an aerosol, stability against forces generated during aerosolization, biocompatibility, targeting of specific sites or cell populations in the lung, release of the drug in a predetermined manner, and degradation within an acceptable period of time (Rytting *et al.*, 2008; Schleh *et al.*, 2011; Yacobi *et al.*, 2008). Further studies are needed to establish the role of aerosolized nanopreparations for inhalational therapy.

## 4. ALI AND NANOMEDICINE

### 4.1. Acute lung injury/acute respiratory distress syndrome

ALI and ARDS arise from direct and indirect injury to the lungs and result in a life-threatening form of respiratory failure with diffuse, bilateral lung injury, and severe hypoxemia caused by noncardiogenic pulmonary edema which affects approximately 1 million people worldwide annually (Girard and Bernard, 2007; Rubenfeld and Herridge, 2007). As with inflammatory processes elsewhere in the body, lung inflammation is accompanied by many cellular and biochemical processes; some of them specific to the syndrome and includes injury to both the pulmonary capillary endothelium and the alveolar epithelium. The importance of ALI has been highlighted by the emergence of SARS (severe acute respiratory syndrome). ALI and ARDS are a leading cause of morbidity and mortality in the United States (Matthay and Ware, 2000). The major reason underlying the lag in improvement in outcome is the lack of novel and specific therapies for ALI and ARDS (Matthay, 2008). Thus, ARDS represents an unmet medical need and there is an urgent need to develop novel therapies for this condition.

The molecular pathobiology of ARDS is being extensively defined and the role of several molecules including pattern recognition receptors present on the immune cells, such as Toll-like receptors and downstream signaling molecules, such as NF- $\kappa$ B and effector molecules, such as TNF- $\alpha$  and IL-1 $\beta$  are being investigated in the pathogenesis and treatment of ALI and ARDS. Targeting central molecules such as NF- $\kappa$ B attenuates lung inflammation but has major limitations, because inhibition of NF- $\kappa$ B is immunosuppressive and compromises host defense (Sadikot *et al.*, 2006). However, because of the complex nature of the disease targeting single cytokine or chemokine has also failed to attenuate lung inflammation as these are not sufficient singly to attenuate lung inflammation in ARDS.

Thus, we propose innovative approaches that involve: (1) targeting multiple upstream molecules TREM-1 (Bouchon *et al.*, 2000, 2001), reactive oxygen species (ROS), and Hsp90 that lead to activation of NF- $\kappa$ B, ultimately leading to ALI and ARDS, with poor outcomes in many cases; (2) developing a novel approach to deliver inhibitors of these molecules *in vivo*. We have previously shown that these individual nanoformulations are effective at attenuating lung inflammation; and (3) using combination therapeutic approach that involves three distinct intracellular metabolic pathways likely to be successful as it is in patients with cancer (Sadikot and Rubinstein, 2009; Sadikot *et al.*, 2004).

## 4.2. Nanomedicine therapy for acute lung injury

Nanoparticles have potential application in medical field including diagnostics and therapeutics. Nanoscale drug delivery systems have the ability to improve the pharmacokinetics and increase the biodistribution of therapeutic agents to target organs which results in improved efficacy and reduces drug toxicity (Koo *et al.*, 2005; Rudolph *et al.*, 2010). Nanocarriers are particularly designed to target inflammation and cancer that have permeable vasculature. Additionally many nanocarriers have the desirable advantage of improving solubility of hydrophobic compounds in the aqueous media to render them suitable for parenteral administration. In particular, delivery systems have shown to increase the stability of a wide variety of therapeutic agents, such as hydrophobic molecules, peptides, and oligonucleotides (Koo *et al.*, 2005).

These systems are exploited for diagnostic and therapeutic purposes to carry the drug in the body in a controlled manner from the site of administration to the therapeutic target (Mazzone, 2009). This implies the passage of the drug molecules and drug delivery system across numerous physiological barriers, which represents the most challenging goal in drug targeting. Nanoparticles can be constructed by various methodology so that effect can be targeted at desired site (Koo *et al.*, 2005).

To begin to address the potential of nanotechnology for treatment of ARDS, we developed novel, long-acting, biocompatible, and biodegradable phospholipid micelles (size,  $\sim 15$  nm) to modulate key signaling molecules that are critical to the inflammatory response in ALI and ARDS (Matthay and Ware, 2000). We selected molecules that initiate and propagate inflammatory response by distinct mechanisms so that multiple pathways can be targeted either singly or by a combinatorial approach as in diseases like HIV (Shey *et al.*, 2009). Among these TREM-1 (Bouchon *et al.*, 2000, 2001), ROS, and Hsp90 were initially selected to modulate the inflammatory response in the lung (Sadikot *et al.*, 2004, 2008a,b).

Realizing the short half-life of peptide drugs (min) hampers their clinical use, we invented micellar TREM-1 peptide and GLP-1 where each peptide drug is stabilized in its active form ( $\alpha$ -helix) and its bioactivity is prolonged for hours *in vivo*. Likewise, water-insolubility of 17-AAG, a selective Hsp90 inhibitor, constrains its use in humans. Accordingly, self-association of 17-AAG with these micelles overcomes this limitation while at the same time increasing its stability and bioavailability. These long-acting micellar drugs provide significant advancement in the treatment of experimental of ALI which could then be extended to critically ill patients. Nanoparticles can be introduced by systemic administration (oral, dermal, intravenous, etc.) or directly introduced into the lung through inhalation, intranasal, or oropharyngeal aspiration.



In a recent study, we tested the efficacy of GLP-1 nanomicelles in a mouse model of lipopolysaccharide (LPS) induced lung injury (Lim *et al.*, 2011; Sadikot *et al.*, 2008a,b). *In vivo* administration of GLP1-SSM to LPS-induced ALI mice resulted in significant downregulation of lung inflammation, with dose-dependent antiinflammatory activity observed. Similar therapeutic activity was not detected for GLP-1 in saline, indicating that the SSM nanocarriers played a critical role in protecting the enzyme-labile GLP-1 and delivering it to inflamed tissues *in vivo*. This study demonstrated for the first time that the lipid-based nanoformulation of GLP-1 is effective at attenuating inflammation in ALI/ARDS (Lim *et al.*, 2011). We have also tested the efficacy of TREM-1 nanomiceller peptide in a model of LPS-induced sepsis and lung injury and shown that TREM-1 nanomicelles are more efficacious than the naked peptide at abrogating inflammation (Sadikot *et al.*, 2008a,b). Studies with other nanomiceller preparations such as 17-AAG for treatment of ALI/ARDS are currently ongoing in our laboratory. Together, our studies demonstrate the feasibility of translating the use of these nanomiceller preparations for translational human studies to the clinics to treat this devastating disease. We hypothesize that combinatorial administration of nanomicelles that modulate distinct signaling pathways will prove to be more potent, however, will need further studies to optimize the administration of the nanopreparations.

## 5. SUMMARY

Nanotechnology is an emerging science involving manipulation of materials at the nanometer scale. Nanomedicine, the medical application of nanotechnology, promises an endless range of applications from biomedical imaging to drug delivery and therapeutics. These novel approaches using nanotechnology are revolutionizing the future of medicine. Advances in nanotechnology are proving to be beneficial in therapeutic field such as drug discovery, drug delivery, and gene/protein delivery. Nanoparticulate drug delivery systems, designed as multifunctional engineered nanoparticles, appear to be particularly attractive and promising for drug delivery to organs such as the lung since they combine several opportunities like uniform distribution of drug dose among all ventilated alveoli allowing for uniform cellular drug internalization. Besides sustained release of drugs in plasma and organs, other potential advantages of the system include the possibility of reduction in drug dosage, adverse effects, and drug interactions. Although the field of nanomedicine offers multiple opportunities, it still is in its infancy and the research has to proceed in order to obtain a specific targeting of the drug combined with minimum side effects. Ongoing studies offer several exciting prospects for the application of engineered nanomaterials for drug delivery in years to come.

## ACKNOWLEDGMENT

Supported by the Department of Veterans Affairs (VA Merit Grant).

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