The Treatment of Acute Respiratory Distress Syndrome in Rats With a Peritoneal Dosing System[1]

Nathan Legband
Department of Mechanical and Material Engineering,
University of Nebraska–Lincoln,
Lincoln, NE 68588

Keely Buesing
Department of Surgery,
University of Nebraska Medical Center,
Omaha, NE 68198

Mark Borden
Department of Mechanical Engineering,
University of Colorado–Boulder,
Boulder, CO 80302

Benjamin Terry
Department of Mechanical and Material Engineering,
University of Nebraska–Lincoln,
Lincoln, NE 68588

1 Background

Current medical treatments for conditions involving an impaired respiratory system provide inefficient methods of delivering oxygen to the patient. Patients with these respiratory dysfunctions are often unable to get the required amount of oxygen to the brain and body. This can lead to delayed recovery from acute illness, multisystem organ failure, and ultimately death. The mortality rate for cases of respiratory dysfunctions has been recently reported in the range of 31–75% [1]. This is especially troubling considering there are many causes of respiratory failure leading to acute respiratory distress syndrome (ARDS), including pneumonia, sepsis, trauma, chemical inhalation, and bacterial or viral infection.

Methods of oxygenation and ventilation that bypass the lungs have been explored in an effort to promote lung recovery. So far, only extracorporeal membrane oxygenation (ECMO) has been approved for medical use. However, ECMO is an unsatisfactory treatment as it possesses many of its own risks, including hemorrhage, thrombosis, and cannula malfunction. These risks eliminate ECMO as a viable treatment alternative for many patients. Therefore, we have researched an alternative technology for delivering oxygen to the body through the peritoneal cavity.

We have developed a peritoneal dosing system (PDS) which delivers an infusate solution to the peritoneal cavity. In order to provide a patient with supplemental oxygenation, phospholipid shelled oxygen microbubbles (OMBs) would be administered to the patient. Previously, we have shown that peritoneal membrane oxygenation (PMO) with OMBs is a possible treatment method in acute lung injury models [2]. We are now extending the validity of PMO treatment by testing in long term disease models, which more accurately reflect clinical scenarios. ARDS is a medical condition that has been well established in animal models. Rat models of ARDS have been well established by tracheal delivery of the endotoxin, lipopolysaccharide (LPS) [3]. Our study involves two phases: (1) development and characterization of the ARDS disease model in rats; (2) design and validation of ambulatory infusion device.

2 Methods

In all phases, male Wistar rats are housed and cared for according to the University of Nebraska IACUC guidelines. Animals are allowed to acclimate for 4 days prior to the experiments. In phase 1, the model for ARDS is evaluated and characterized. Rats are sedated with ketamine–xylazine (18–2 mg/kg). Healthy baseline measurements of weight, chest radiographs (PRX 90, Bowie), pulse oximetry (PhysioSuite, Kent Scientific Corp.), and venous blood analysis are then done. Analysis of venous blood from the tail vein was performed with a handheld blood analyzer (VetScan iSTAT 1, Abaxis) to determine the animal's blood gases, *p*H, chemistry, and hematocrit. Intratracheal administration of 0.5 ml saline with LPS (7 mg/kg, Sigma-Aldrich) was performed with the MicroSprayer® Aerosolizer (Model IA-1B-R, PennCentury). An additional negative control (NC) group was not administered with any intratracheal solution. Observation and collection of daily blood samples and chest radiographs of each animal were performed until death or at 7 days post LPS administration. Upon completion of the observation period, living animals are then euthanized with sodium pentobarbital. Lung tissue was hematoxylin and eosin stained and sent to an independent pathologist for lung injury scoring [4].

Phase 2 is the development of an ambulatory delivery system that will infuse fluid to the rat's peritoneal cavity for treatment. The PDS must fulfill several design criteria: (1) continuously treat four rats simultaneously with the same or different infusates; (2) infusate needs to be stored at a temperature range of 2–8 °C; (3) warm the infusate to 37 °C immediately before delivery to the animal; (4) gently agitate solution to prevent dissociation of microbubbles; (5) prevent tubing from restraining the rat or being harmed by the rat; (6) be compatible with current facility housing and cages. The PDS will be validated by constant observation in trials with healthy animals to ensure the system fulfills the animal's safety and tubing requirements.

3 Results

For phase 1, trials have been completed for 7 day LPS and NC groups. Eight rats ($m = 541 \pm 60$ g) were successfully administered aerosolized LPS and all developed ARDS while two rats ($m = 556 \pm 96$ g) were selected for the NC group. The overall mortality rate was 37.5% for LPS trials and 0% for NC trials. All deaths occurred within 24 hr after administration of LPS. The remaining LPS rats began recovering over the course of the 7 day observation period. Chest radiographs taken of LPS rats show clear indications of bilateral infiltrates and interstitial edema in the lungs (Fig. 1). The LPS group showed a dramatic loss in lung function from the observed SpO_2 levels when compared to NCs (Fig. 2). Table 1 shows lung injury scores from LPS deaths at day 1, and NC and LPS trials euthanized on day 7.

In phase 2, final design of the PDS has been completed (Fig. 3). The PDS is comprised of a peristaltic pump, warm water bath, a spring pullbox for dynamic tubing restraint, and a LabVIEW control system. The PDS is compatible with current housing arrangements of the rats and will not restrict their mobility. Once the prototype of the PDS has been constructed, tests will be completed with healthy rats to validate the system and provide the rat with full mobility. We expect to be able to continuously dose saline, inert gas microbubbles (IMBs), and OMBs into the peritoneal cavity of four animals with the current PDS design.

Based upon our previous animal experiments with lung injuries, we anticipate improved pulse oximetry, blood gases, and survivability in rats provided OMBs compared to those administered saline or IMBs.

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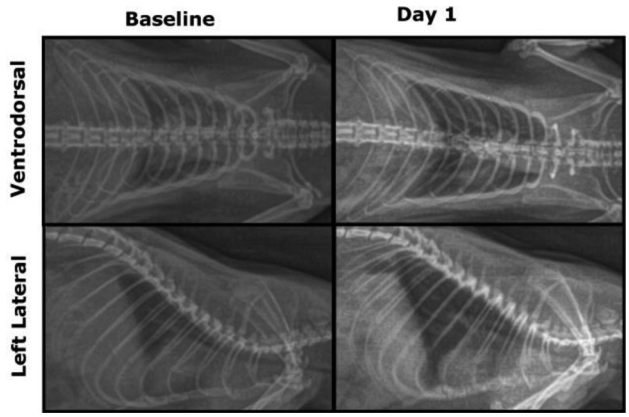

Fig. 1 Representative chest radiographs of rat given LPS

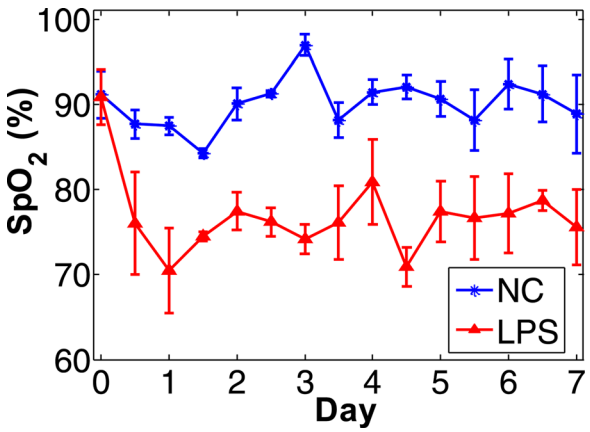

Fig. 2 Average peripheral oxygen saturation (SpO_2) levels of each group measured from pulse oximetry during observation period

4 Interpretation

We have developed an alternative extrapulmonary oxygenation method by delivering OMBs to the peritoneal cavity. In verifying the clinical benefits of PMO treatment we have devised the current study. Trials of phase 1 show that we are able to repeatedly induce ARDS in rats with our current methodology. Analysis of BAL specimens is pending, as we recently identified a lab able to conduct the analysis. The final design of the PDS has been completed and build of the prototype has begun. Upon completion of phase 1 and 2, PMO treatment will be evaluated.

The future direction of this work after validation will focus on reproducing results in large-animal models of moderate to severe ARDS, and commercializing the technology. We will begin development of large scale manufacture of OMBs. We believe PMO treatment will prove to be a safe and reliable lung bypass therapy for patients with severe ARDS who cannot tolerate the significant risk profile inherent to ECMO. As we move toward clinical translation, we foresee the implementation of PMO in intensive care units, military combat settings, and even space exploration vehicles.

Table 1 Lung injury scores (average ± standard deviation) from histology samples

	n	Edema	Hemorrhage	Inflammation
NC day 7[a]	2	0.8 ± 0.6	0.9 ± 0.4	1.3 ± 0.1
LPS day 1	3	2.3 ± 0.3	0.1 ± 0.3	2.9 ± 0.3
LPS day 7	5	0.0 ± 0.0	0.2 ± 0.3	0.2 ± 0.3

Scale ranges from no (0) and severe (3) injury.
[a]One rat suffered pulmonary injury during euthanasia, which artificially increased their score.

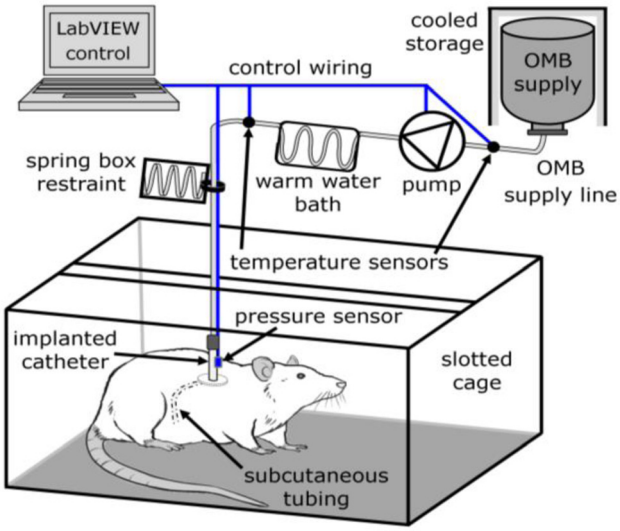

Fig. 3 Schematic of PDS for rat

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