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Systemic oxygen delivery by peritoneal perfusion of oxygen microbubbles

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ABSTRACT

Severe hypoxemia refractory to pulmonary mechanical ventilation remains life-threatening in critically ill patients. Peritoneal ventilation has long been desired for extrapulmonary oxygenation owing to easy access of the peritoneal cavity for catheterization and the relative safety compared to an extracorporeal circuit. Unfortunately, prior attempts involving direct oxygen ventilation or aqueous perfusates of fluorocarbons or hemoglobin carriers have failed, leading many researchers to abandon the method. We attribute these prior failures to limited mass transfer of oxygen to the peritoneum and have designed an oxygen formulation that overcomes this limitation. Using phospholipid-coated oxygen microbubbles (OMBs), we demonstrate 100% survival for rats experiencing acute lung trauma to at least 2 h. In contrast, all untreated rats and rats treated with peritoneal oxygenated saline died within 30 min. For rats treated with OMBs, hemoglobin saturation and heart rate were at normal levels over the 2-h timeframe. Peritoneal oxygenation with OMBs was therefore shown to be safe and effective, and the method requires less equipment and technical expertise than initiating and maintaining an extracorporeal circuit. Further translation of peritoneal oxygenation with OMBs may provide therapy for acute respiratory distress syndrome arising from trauma, sepsis, pneumonia, aspiration, burns and other pulmonary diseases.

1. Introduction

Severe hypoxemia arising from lung injury is life-threatening in critically ill patients. Mechanical ventilation may be inadequate owing to limited mass transfer in the injured lung; overinflation, barotrauma and cyclic closing and reopening of the alveoli may further damage the lung and trigger a pulmonary and systemic inflammatory reaction that may lead to multiple system organ failure [1]. Researchers have long sought a safe and effective method for extrapulmonary oxygenation to treat these patients.

Currently, the last resort for treating pulmonary failure uses extracorporeal membrane oxygenation (ECMO), a temporary artificial extracorporeal support of the respiratory system and/or cardiac system [2]. ECMO was first used in an adult in 1972 to treat severe respiratory failure [3] and in 1974 on the first newborn [4]. Innovations in ECMO include the introduction of polymethylpentene hollow fibers with nonthrombogenic coatings and thin wire-reinforced cannula walls. ECMO use has historically centered on neonatal care [5]. Recently, the H1N1 flu pandemic led to wider use of ECMO, proving its utility in hypoxemic emergencies.

Alternative therapies are required, however, because ECMO is correlated with significant complications associated with the mechanically powered external blood circuit [2,6]. Thrombosis and other deleterious factors are common results. To mitigate these problems, anticoagulants (heparin) are administered to the patient, often with additional adverse effects. Intracranial brain hemorrhage is an unpredictable and lethal complication of ECMO that occurs in ~5% of patients [2,7,8]. Additionally, ECMO is expensive and complex to operate, limiting its accessibility for emergency care.

Thus, researchers have focused on other methods of extrapulmonary respiration, such as peritoneal oxygenation. Peritoneal oxygenation uses the large surface area of the peritoneum, the membrane that lines the abdominal cavity, as a gas exchanger. The main advantages of peritoneal oxygenation are easy access for catheterization; relative safety of circulating oxygen through the peritoneal cavity, in which the mesothelium acts as a gas-permeable barrier between the perfusate and the blood; and avoidance of extracting and reintroducing blood. The technique is analogous to peritoneal dialysis, a simple, low-tech form of renal replacement therapy that is less expensive and complex than conventional in-center hemodialysis [9].

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The most obvious method is to mechanically ventilate the peritoneal cavity with pure oxygen gas. This method was first proposed almost a century ago [10]. Perhaps the most successful application of mechanical ventilation of the peritoneal cavity was reported by Barr et al. [11,12], who showed an increase in arterial oxygen partial pressure (PaO_2) and decrease in mortality rate for a lung injury model compared to pulmonary mechanical ventilation alone. The same group later showed improved outcome from peritoneal mechanical ventilation therapy in a hemorrhagic shock model [13]. In a subsequent large animal study, however, peritoneal mechanical ventilation showed no increase in mixed venous PO_2 [14]. A more recent study in rabbits suffering a tracheal clamp showed only a moderate increase in survival time from 5.0 min for no treatment to 6.5 min for peritoneal mechanical ventilation [15], supporting claims of low clinical utility.

Prior peritoneal oxygenation studies introducing aqueous perfusates, such as perfluorocarbons (PFCs) and red blood cells (RBCs), into the intraperitoneal space have reported no effect or only a mild increase in PaO_2 [15–20]. Prior to our study, the most successful application of peritoneal oxygenation used liposomal synthetic hemoglobin carrier TRM-645, which produced a mean increase in rat cardiac arrest time following a right pneumothorax from 9 to 33 min [20].

Here we report on a new method of peritoneal oxygenation, in which we use OMBs designed for high oxygen carrying capacity, high oxygen delivery rate and sufficient stability for storage and transport. This follows a recent study by Kheir et al. [21], which showed a limited but significant therapeutic benefit from the intravenous injection of OMBs in rabbit hypoxic ventilation and tracheal clamp asphyxiation studies. The intravenous route appears to be a promising method for short-term rescue, but the prolonged continuous infusion of oxygen microbubbles into the bloodstream poses significant challenges for clinical translation, including the potential for embolism, thrombosis, immunogenicity and toxicities of lipid and saline load.

Our approach of direct systemic oxygenation by injecting OMBs into the peritoneal space is a radical change from existing oxygen delivery platforms. The procedure and apparatus for circulating OMBs through the peritoneal cavity is simple and straightforward, and the therapy precludes the need for an extracorporeal loop to circulate blood, thus potentially circumventing the complications from thrombosis and intracranial hemorrhage presented by ECMO. In this study, we examined the utility of peritoneal oxygenation with OMBs to increase mean survival time of rats experiencing a right pneumothorax lung injury, a common model for pulmonary failure that has been used in prior studies to examine the efficacy of peritoneal oxygenation.

2. Materials and methods

2.1. Synthesis of oxygen microbubbles

All solutions were prepared using filtered, 18 MΩ cm^{-1} deionized water (Direct-Q, Millipore, Billerica, MA). Glassware was cleaned with 70 vol% ethyl alcohol solution (Sigma–Aldrich; St. Louis, MO) and rinsed with deionized water. Phospholipid 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) was purchased from NOF (Tokyo, Japan), and polyoxyethylene-40 stearate (PEG-40S) was purchased from Sigma–Aldrich (St. Louis, MO). Phosphate buffered saline used for the control experiments was assumed to have neutral PaO_2 tension relative to atmosphere, and thus was "oxygenated" considering the PaO_2 in venous and arterial blood.

DSPC and PEG-40S were weighed in dry form, combined to a 9:1 M ratio, and dissolved into a 0.2-μm filtered solution of 0.15 M phosphate buffered saline (PBS) to a final lipid concentration of 12 mg/mL. The mixture was heated to 65 °C and dispersed using a Branson 450 sonifier (Danbury, CT) with an output power of 5/10 until the solution was translucent. The resulting solution was stored in a refrigerator prior to microbubble synthesis. Oxygen microfoam was prepared by adapting the process design developed by Swanson et al. [22] for synthesizing large volumes of oxygen microbubbles. The process consisted of an ultrasonic horn reactor enclosed in a water-cooled, continuous-flow chamber (Branson, Danbury, CT). The lipid suspension was fed at 5 °C and combined with room temperature oxygen gas in the reactor at roughly equal volumetric flow rates. The oxygen gas was emulsified at full sonication power and then fed to a flotation column to separate the oxygen microbubbles (bottom) from the macrofoam (top). Oxygen microbubbles were immediately collected from the bottom of the column using 60-mL syringes and centrifuged (Eppendorf, Hauppauge, NY) at 110 relative centrifugal force (RCF) for 4 min to form a ~70 vol% concentrate. The concentrated oxygen microfoam was then transferred to 500-mL Wheaton serum bottles (Pasadena, TX), sealed under an oxygen atmosphere, and stored in the refrigerator. The infranatant lipid suspension from the centrifugal wash was collected and recycled into the sonication process to produce more microfoam. The process was repeated until the desired volume of 70 vol% oxygen microfoam was produced. Oxygen microbubble size distribution was determined using the electrozone sensing method (Coulter Multisizer III, Beckman Coulter, Opa Locka, FL). Oxygen gas volume fraction was determined by subtracting the weight of a fixed volume of microfoam from the weight of the same volume of aqueous medium (10 mg/mL lipid in PBS), and dividing this difference by the weight of the same volume of aqueous medium. Gas headspace purity in the serum bottle was determined using a model 6600 precision headspace gas analyzer (Illinois Instruments Inc, Johnsburg, IL).

2.2. Right pneumothorax lung injury model

The objective of this study was to test our hypothesis that oxygen microbubbles delivered by intraperitoneal infusion can significantly increase the mean survival time of an animal experiencing acute lung injury, in comparison to an intraperitoneal infusion of oxygenated saline solution. The research subjects were male Wistar rats ($n = 10$, mass = 430 ± 15 g, Charles River), housed 2 per cage and acclimated for 4 days with free access to food and water. We performed a controlled laboratory study to test the therapeutic benefit of an intraperitoneal infusion of oxygen microbubbles in rats experiencing acute lung injury. Right pneumothorax was chosen as an appropriate model for acute lung injury based on prior work reported in the literature [20]. Two treatments were applied: oxygen microbubbles or oxygenated saline control. Following induction of pneumothorax, we observed vital signs and measured intraperitoneal pressure, infused volume, rectal temperature, heart rate and oxygen saturation (SaO_2). The animals were randomly assigned to cages by the caretakers, and the animals were randomly chosen for treatments. No additional blinding was done in the study. The number of animals used in this study was determined from work by Matsutani et al. [20] and a preliminary feasibility study. In Matsutani, a synthetic oxygen carrier was injected into the intraperitoneal (IP) space of rats for the purpose of systemic oxygenation. The mean standard deviation and mean effective size were 6.9 and 19.9, respectively [20]. We assumed the same standard deviation, but half the effective size ($\mu - \mu_0 = 10$). Using these values, a target Type I error of 0.05 and a sample size of 6 rats per group yielded a power of 0.81. In our preliminary feasibility study, a sample size of 4 rats was used in the oxygen microfoam group, and 3 rats were used in the control group. Using the Log-rank test for comparison of the survival distribution ($\alpha = 0.05$), a significant difference was found between the two groups ($X^2 = 6.624$, $P = 0.010$). Based on these results, it was decided that a sample size of 5 rats per group would be adequate for the lung injury study. Data collection was stopped due to either cardiac arrest or achievement of the primary endpoint. Additional criteria were established to allow for the exclusion of samples where complications arose. Criteria for exclusion included the occurrence of unsuccessful pneumothorax or infrequent data points being collected by the veterinary monitor. One rat from the control group was excluded from the study because both exclusion criteria occurred in the trial. No other data were excluded. No test for outliers was used; all data were included in the study. A primary endpoint of 2 h following induction of pneumothorax was selected to avoid complications arising from animal recovery from anesthesia and the initial lung injury. We sometimes observed these complications in animals living more than 2 h in our preliminary feasibility study. We calculated that the 2-h endpoint would be sufficiently long to demonstrate a statistically significant increase in mean survival time between animal receiving our therapy and the control group. Prior to our study, the longest mean survival time for an intraperitoneal oxygenation therapy following pneumothorax reported in the literature was ~33 min [20]; thus, a mean survival time exceeding 2 h was deemed a significant improvement over the current state of the art and of high clinical utility. No other endpoints were specified. No replicates were performed. Each animal received only one pneumothorax and one treatment.

All animals were housed and underwent procedures approved by the University of Nebraska, Lincoln, Institutional Animal Care and Use Committee. Male Wistar rats ($n = 10$, mass = 430 ± 15 g, Charles River) were anesthetized in an induction chamber with 5% isoflurane to effect and maintained on 2% isoflurane. Each rat was weighed, given an IP injection of sodium pentobarbital solution (50 mg/kg dose), and then placed in the supine position on a warming pad (T/pump Classic, Gaymar) set at 38 °C to maintain body temperature. After the rat was fully sedated and unresponsive to pain delivered by paw pinches, a small incision into the skin was made to expose the fascia of the abdominal wall. A 12-gauge indwelling catheter was then inserted into the IP cavity and fitted to tubing (3.2 mm inner diameter, Tygon) by a Luer lock for subsequent infusions. A 22-gauge indwelling catheter was also inserted into the IP cavity and connected to two pressure transducers (4426-005G,