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RESPIRATORY MECHANICS IN TRAUMATIC BRAIN INJURY (TBI) THE EFFECT OF INHALED NITRIC OXIDE

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14. ABSTRACT: **Background**: The etiology of post- traumatic brain injury (TBI) hypoxemia is multifactorial including ventilation/perfusion mismatch and loss of pulmonary hypoxic vasoconstriction. Inhaled nitric oxide (iNO) has been studied as an adjunct treatment to avoid the use of high positive end-expiratory pressure (PEEP) and inspired oxygen. We hypothesized that iNO after TBI would improve systemic and cerebral oxygenation via improved matching of pulmonary perfusion and ventilation. **Methods**: Thirteen human patients were enrolled following isolated TBI and were randomized to receive either placebo or iNO group with measurement of pulmonary parameters, blood gas data, and intracranial variables. To complement this study, a porcine model of TBI was utilized with bilateral blood flow and oxygenation intracerebral monitoring. Ventilatory parameter and blood gas data were also collected following period of administration and following drug removal and clearance. **Results**: There were no clinically significant changes in pulmonary parameters in either human or porcine arm following administration of iNO when compared to either placebo group (human arm), or internal control (porcine arm). Analysis of pooled human data demonstrated preservation of alveolar recruitment in TBI patients. There were no clinically significant changes in intracranial pressure (ICP) or cerebral perfusion pressure (CPP) following iNO administration compared to controls. **Discussion**: iNO had no significant effect on clinically relevant pulmonary parameters or intracranial pressures following TBI in both human patients and a porcine model. The pressure-based recruitment of human lungs following TBI was preserved. Further investigation will be needed to determine the degree of utility of iNO in the setting of hypoxia after polytrauma.

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1.0 SUMMARY/DISCLAIMER

The following final technical report provides results regarding a two-phase research effort which included a clinical trial and pre-clinical model research that explored the utilization of Inhaled Nitric Oxide (iNO) would improve gas exchange parameters with no alteration in Intracranial Pressure (ICP) or Cerebral Perfusion Pressure (CPP) in a randomized controlled human trial as well as improve pulmonary parameters, blood oxygen saturation, cerebral oxygenation, and cerebral blood flow in a porcine model. The University of Cincinnati Institutional Review Board (UC IRB # 2017-3618) and the United States Air Force Human Research Protection Office approved the clinical trial (FWR20180117X). The pre-clinical model research was approved by the University of Cincinnati Institutional Animal Care and Use Committee (IACUC) (protocol # 20-04-20-01) and USAF Animal Use Oversight and Compliance (AFOSR-2020-0012A)

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2.0 BACKGROUND

Traumatic Brain Injury (TBI) represents a significant source of morbidity and mortality among critically injured trauma patients. While neurologic dysfunction represents an obvious source of such morbidity, non-neurologic organ system failure occurs in up to 89 percent (%) of severe TBI patients and is associated with worse outcomes. Up to 35% of TBI patients experience some degree of lung injury and concomitant hypoxemia with a proportion progressing to Acute Respiratory Distress Syndrome (ARDS), making respiratory failure the most common form of TBI-associated non-neurologic organ system failure.[1–4] The exact mechanism of TBI leading to lung injury/ARDS and associated hypoxemia is thought to be multifactorial with contribution from neurogenic edema[5–7] and damage to the alveolar epithelium via inflammatory lung injury.[8,9] Further evidence supports loss of the ventilation/perfusion (V/Q) mismatch regulatory mechanisms as a contributing factor to hypoxemia in this population.[3,10]

Respiratory parameters of mechanically ventilated TBI patients have been well-studied with respect to the treatment of concomitant ARDS and associated neurologic insult. However, the balance between preservation of adequate blood oxygenation and the avoidance of ICP elevation represents a significant clinical challenge. While elevated Positive End-Expiratory Pressure

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(PEEP) is widely used in the treatment of severe ARDS, elevated PEEP used to preserve a normal cerebral oxygen level remains controversial given its potential role in ICP elevation.[3] iNO, used as a selective pulmonary vasodilating agent, was initially hypothesized as a treatment for isolated ARDS under the supposition that it would improve V/Q mismatch via vasodilation of blood vessels associated only with functional alveoli.[11] The use of iNO, however has not demonstrated benefits in mortality or time on ventilatory support despite short-term, transient improvements in oxygenation in mechanically ventilated patients.[12,13]

There is evidence to suggest that therapeutic iNO may serve a role in the regulation of cerebral blood flow and prevention of secondary brain injury in mechanically ventilated TBI patients.[14,15] In this study, we hypothesized that administration of iNO following a TBI would improve gas exchange parameters with no alteration in ICP or CPP in a randomized controlled human trial as well as improve pulmonary parameters, blood oxygen saturation, cerebral oxygenation and cerebral blood flow in a porcine model.

3.0 METHODS

3.1 Human Study Arm

Recruitment and Inclusion/Exclusion Criteria: All subjects 18-75 years of age who were admitted to the University of Cincinnati Medical Center with traumatic brain injury requiring mechanical ventilation within 24 hours of admission were screened for enrollment over a 48-month period with a recruitment goal of 43 patients. Inclusion criteria for study enrollment included TBI with head Computed Tomography (CT) imaging findings of injury via penetrating or blunt mechanism, required mechanical ventilation within 24 hours of admission, and had a negative pregnancy test when applicable. Exclusion criteria included brain death, expected survival less than 48 hours, pneumothorax requiring chest tube placement, elevated fraction of inspired oxygen concentration (FiO_2) greater than 0.65 at assessment, hemodynamic instability defined by systolic blood pressure (SBP) less than 100 Millimeters of Mercury (mmHg), cardiac arrhythmia, uncontrolled ICP (greater then 20mmHg or requiring hypertonic saline bolus therapy), pupil asymmetry, spinal cord injury with hypotension, severe ARDS (Partial Pressure of Oxygen (PaO_2):FiO_2 ratio < 100), flail sternum or unilateral flail chest segment (defined as 2 fractures on 3 or more consecutive ribs), pulmonary contusion or lobar infiltrate visible on admission chest radiograph, chronic lung disease (Partial Pressure of Carbon Dioxide (PCO_2) greater than 60mmHg, Bicarbonate (HCO_3^-) greater than 32 Millimoles per Liter (mmol/L)), known heart failure (defined as left ventricular ejection fraction of less than 20%), and central venous pressure (CVP) greater than 20 mmHg with associated SBP less than 100mmHg. Enrollment was ultimately determined according to the discretion of the trauma attending and the consulting intensivist. At the end of study enrollment, 13 patients met appropriate criteria and were entered into the trial. Informed consent was obtained for all enrolled patients via legally authorized representative or next-of-kin.

Randomization and iNO administration: Patients underwent blind randomization to receive either treatment with nitrogen and oxygen (placebo group) or iNO. Ultimately, 6 patients received iNO treatment and 7 received placebo. If randomized to the iNO treatment group, patients received dose of 20 parts per million (PPM) for 2 hours -/+ 30 minutes pending tolerance.

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PEEP Titration and Measurement of Respiratory Parameters: Following informed consent, patients were randomized to undergo serial monitoring through administration of study agent (placebo vs. iNO) via existing monitors within the mechanical ventilator, as well as an NM3 respiratory monitor (Philips Respironics, PA) and airway sensor. Pulmonary function parameters as well as arterial blood gas values were measured at intervals including baseline (prior to administration of study agent), 90 minutes after administration of the study agent, following a predetermined increase in PEEP and observation period of 10 minutes, following a second predetermined increase in PEEP and observation period of 10 minutes, and following the return to baseline ventilator settings with an additional observation period of 10 minutes. Measured pulmonary parameter endpoints included functional residual capacity (FRC), peak inspiratory pressure (PIP), mean airway pressure, and PaO_2:FiO_2 ratio (P:F ratio). FRC was measured using a modified nitrogen washout by using a step change in FiO2 over a predefined time period. Blood samples were analyzed for blood gas and pH values. Pulmonary parameters were measured non-invasively using the integral monitoring of the mechanical ventilator (GE CareStation, GE Medical). Oxygenation metrics included PaO_2 and P:F ratio and were obtained via arterial blood gas (ABG), which was collected at three time points in the experiment including baseline, following treatment with the study agent, and following the return to baseline settings after two pre-determined PEEP increases. PEEP was increased twice throughout the study period from baseline (determined according to patient-specific oxygenation requirements) up to maximum of 15 Centimeters (cm) water (H_2O) (in 5 Millimeters (mm) H_2O increments) while monitoring previously outlined, dependent endpoints following 10-minute observation at each of the two increased PEEP levels. Final measurements were taken following a 10-minute observation period after returning to baseline PEEP. Finally, both patient groups were pooled (n=13) for an analysis of pulmonary recruitment parameters in the setting of serial PEEP increase in the post-TBI patient.

Intracranial Monitoring: Invasive intracranial monitoring was not initiated solely for the purposes of this study. However, patients with a clinical course that dictated the use of ICP and CPP monitoring were included in the study and therefore these values were observed as endpoints when available. Patients with recorded ICP and CPP monitoring represented 4 of the 13 total enrolled patients, including 2 patients randomized to the iNO treatment group and 2 patients randomized to the placebo group.

Of note, subject recruitment was severely limited throughout the 48-month recruitment period due to injury and non-injury related comorbidities requiring exclusion, concurrent Caronavirus Disease (COVID) research restrictions during period of enrollment, and competing interventional treatment trials for TBI patients preventing co-enrollment. Given low enrollment in the human study, a supplementary controlled porcine study was designed to include a standardized injury, measurement of cerebral blood flow and oxygenation via brain parenchymal probes, and exposure of all porcine models to iNO with use of internal controls.

3.2 Porcine Study Arm

Animal Model: This study was reviewed and approved by the University of Cincinnati Institutional Animal Care and Use Committee, as well as the United States Air Force Medical Support Agency Office of Research Oversight and Compliance. Animals were cared for by a program approved by the Association for Assessment and Accreditation of Laboratory Animal Care International and in compliance with the National Research Council's 2011 Guide for the Care and Use of Laboratory

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Animals, as well as Department of Defense Instruction 3216.1. Female Yorkshire pigs were obtained from Isler Genetics (Prospect, OH) and were acclimated in our animal facility for 48-72 h before experimentation. Animals were housed alone or in pairs and provided with food and water without restriction, except for the night prior to study initiation, to prevent aspiration during induction of anesthesia. Pigs were sedated with tiletamine hydrochloride (Telazol) and xylazine hydrochloride (both given 5 mg/kg intramuscularly; Henry Schein Animal Health, Dublin, OH). Sedated pigs were placed in a supine position and orotracheally intubated. Pigs were maintained on a ventilator (Carescape R860; GE Healthcare, Chicago, IL) in pressure control mode throughout experimental period.

TBI and intracerebral monitor placement: Ten pigs underwent general anesthesia and mechanical ventilation prior to induction of TBI via controlled cortical injury (CCI). Following excision of scalp tissue overlaying the crown of the frontal bone via electrocautery, a right-sided 2cm craniotomy was made 16mm anterior to the coronal suture and 12mm lateral to the sagittal suture leaving the underlaying dura intact. A 5.3 mm craniotomy was made on both the ipsilateral (1cm anterior to large craniotomy) and contralateral side of the skull for placement of intracranial monitors. CCI was then induced via a 15 mm impactor at 4 meters/second for 100 millisecond dwell time and 13mm depth of impact onto exposed dura and underlying parenchyma. Quad lumen bolts were placed into adjacent craniotomy sites. OxyLite tissue oxygenation probe and OxyFlo blood flow probe (Oxford Optronix, Milton, United Kingdom) were inserted into brain parenchyma via the two quad lumen bolts. Given the use of the craniotomy sites for probe placement and monitoring throughout the experiment, the craniotomy sites were not closed.

Porcine iNO administration and respiratory parameters: Following a 2-hour monitoring period after induction of TBI, iNO was administered at a dose of 20 PPM for 30 minutes. Hemodynamics, mechanical ventilation parameters, serial arterial blood gases, brain tissue perfusion, and brain tissue oxygenation were assessed at baseline prior to TBI, immediately following TBI, two hours post-TBI, after 30 minutes of iNO treatment, and 30 minutes following cessation of iNO administration. Endpoints of pulmonary mechanics measured included FRC, PIP, and lung compliance (mL/cm H2O). Oxygenation metrics were measured with an arterial blood gas to determine the PaO_2 and P:F ratio.

3.3 Data Analysis

All statistical analysis was performed with SAS Statistical Analysis Software (SAS, Cary North Carolina) and figures generated with Prism 9 (GraphPad Software, La Jolla, California). In the human arm, equal-variance T-tests were used to compare treatment and control groups at each individual timepoint. Within-subject change scores (percent change) were calculated for each timepoint relative to the timepoint immediately preceding it. In the porcine arm, within-subject change scores (percent change) were computed between each timepoint and the timepoint immediately preceding it. Additionally, when assessing blood flow specifically, the injured half of the brain was compared to the uninjured half at each timepoint. Paired T-tests were used to compare each timepoint with the previous one and to compare the injured and uninjured halves of the brain. When measures were observed to be very non-normally distributed, a nonparametric test (signed rank) was used to confirm stability of results.

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4.0 RESULTS

4.1 Patient Demographics

The total number included in human arm of study was 13. There was no significant difference in mean age (49.9 ± 20.3 years placebo vs. 63.1 ± 17.4 years iNO, p=0.23), sex (placebo 42.9% M/57.1% F vs. iNO 83.3% M/16.7% F, p=0.27), race (placebo 100% Caucasian/0% African American vs. 83.3% Caucasian/16.7% African American, p=0.46), GCS (8.1 ± 1.7 years placebo vs. 6.8 ± 2.8 iNO, p=0.33), 30-day mortality (placebo 66.7% alive/33.3% deceased vs. iNO 40.0% alive/60.0% deceased, p=0.57), or time (hours) from admission to enrollment between the two groups (25 ± 9.0 hours placebo vs. 30.8 ± 6.5 hours iNO, p=0.29) (Figure 1).

Figure 1.

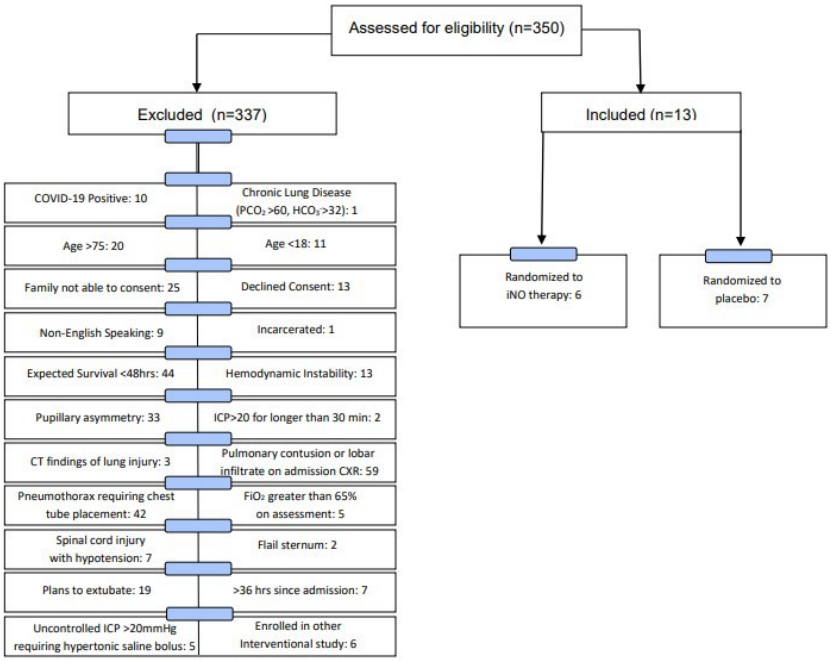

Figure 1. CONSORT flow diagram demonstrating exclusion and randomization in the human arm of the study.

4.2 Human Pulmonary Function Parameters

There were no significant differences in FRC, PIP, or Mean airway pressure between the placebo group or the iNO group at any of the observed timepoints. There was a noted significant increase in Mean Airway Pressure both with the first and second increase in PEEP ($p<0.05$) with return to baseline following restoration of baseline ventilatory settings, however this change was seen in both the placebo and iNO group and no significant differences were demonstrated. (Figure 2A-C) Taken together, these data suggest fairly normal respiratory mechanics with an expected increase in mean airway pressure with the PEEP challenge.

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Figure 2.

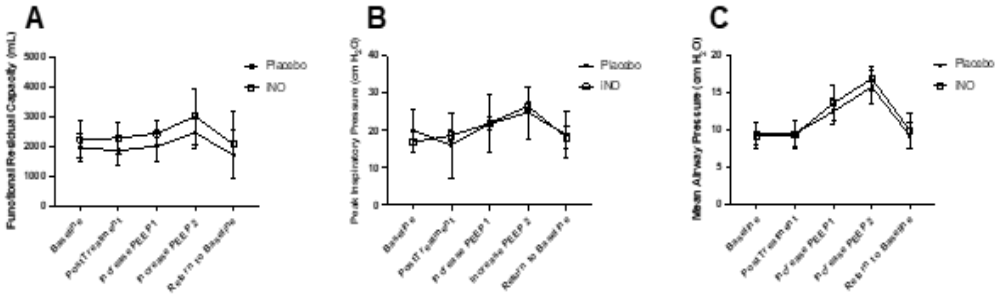

Figure 2. Effect of inhaled NO following TBI compared to administration of placebo on the respiratory parameters of human subjects. (iNO n=6, placebo n=7). (A) FRC (B) PIP (C) Mean Airway Pressure. No significant differences were noted between treatment groups.

4.3 Human Systemic Oxygenation Metrics

There were no significant differences in PaO_2 or P:F Ratio between the placebo group or the iNO group at any of the observed timepoints. (Figure 3A-B)

Figure 3.

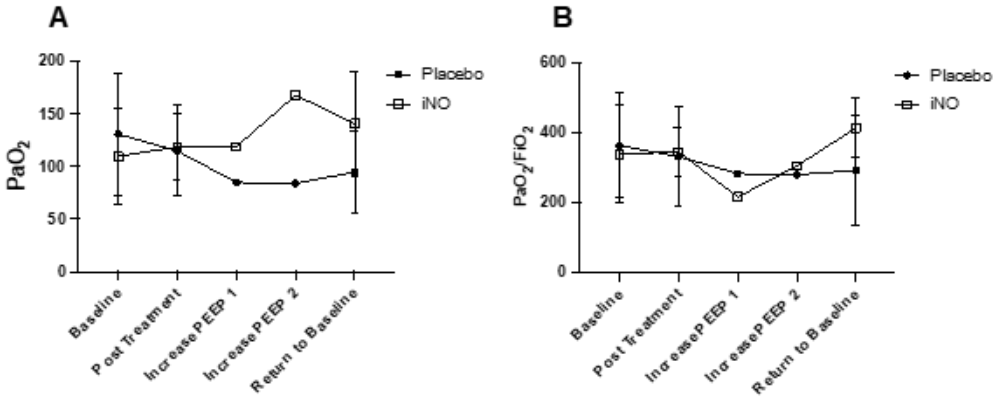

Figure 3. Effect of inhaled NO following TBI compared to administration of placebo on the oxygenation metrics of human subjects. (iNO n=6, placebo n=7). (A) PaO2 (B) P/F Ratio. No significant differences were noted between treatment groups.

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4.4 Human Intracranial Monitoring

There were no significant differences in ICP or CPP between the placebo group or the iNO group at any of the observed timepoints. There were no significant changes noted over time within either group throughout all observed timepoints. As previously described, only 4 subjects in the study had invasive intracranial monitoring dictated by clinical course (placebo n=2, iNO n=2).

Human pulmonary recruitment parameters: Patients from both treatment and placebo group pooled for recruitment analysis (n=13). There was a significant increase in FRC noted with second PEEP increase (2196 ± 504mL PEEP1 vs 2722 ± 771mL PEEP2 p=0.05), and a subsequent significant decrease following return to baseline PEEP (2722 ± 771mL PEEP2 vs 1873 ± 941mLreturn to baseline, p=0.02). Significant increase in plateau pressure noted with both first increase in PEEP (15.6 ± 3.2cmH_2O post treatment vs 19.0 ± 2.5cmH_2O PEEP1, p=0.03), as well as the second increase in PEEP (19.0 ± 2.5cmH_2O PEEP1 vs 23.0 ± 2.6cmH_2O PEEP2, p=0.01). A significant decrease in plateau pressure was observed following return to baseline PEEP (23.0 ± 2.6cmH_2O PEEP2 vs 16.6 ± 5.5cmH_2O return to baseline, p=0.01). (Figure 4A-D). Significant increase in FRC noted with second PEEP increase (p=0.05), subsequent significant decrease following return to baseline PEEP (p=0.02). Significant increase in plateau pressure noted with both first and second PEEP increase (p=0.03, p=0.01, respectively). Significant decrease in plateau pressure following return to baseline PEEP (p=0.01).

Figure 4.

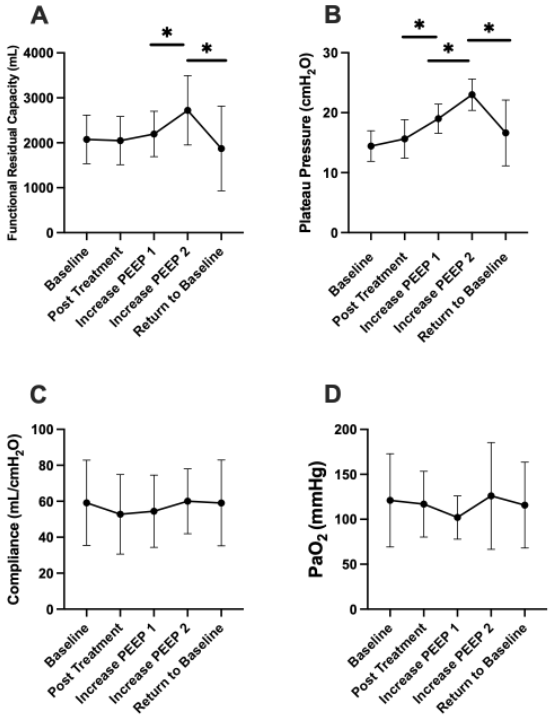

Figure 4. Pooled human data including both placebo group and iNO group (n=13) examining pulmonary parameters of recruitment with serial PEEP increase. (A) FRC (B) Plateau pressure (C) Compliance (D) PaO2.

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4.5 Porcine Pulmonary Parameters

There was a significant increase in PIP after TBI compared to baseline (21.2 ± 2.0 cmH_2O TBI vs. 19.8 ± 1.7 cmH_2O Baseline, p=0.02). Following the treatment with iNO after TBI, lung compliance was significantly increased (37 ± 5.6 mL/H_2O iNO vs. 35.8 ± 5.8 mL/H_2O TBI, p=0.01). Following iNO removal (internal control) after treatment, FRC and PIP were significantly different (FRC 995 ± 186 mL iNO vs. 950 ± 194mL iNO removed, p=0.004; PIP 20.9 ± 2.0 cmH_2O iNO vs. 21.4 ± 1.8 cmH_2O iNO removed, p=0.02). (Figure 5A-C) However, these changes are considered to be clinically insignificant. Figure 5 shows the effect of inhaled NO following TBI with subsequent removal of iNO (internal control) on porcine respiratory parameters. (n=10 for all groups). (A) FRC (B) PIP (C) Compliance. *p<0.05 FRC at timepoint following iNO treatment versus timepoint following removal of iNO (internal control). **p<0.05 PIP at baseline versus timepoint following TBI. ***p<0.05 PIP at timepoint following iNO treatment versus timepoint following removal of iNO (internal control). ****p<0.05 compliance following timepoint post-TBI versus timepoint following iNO treatment.

Figure 5.

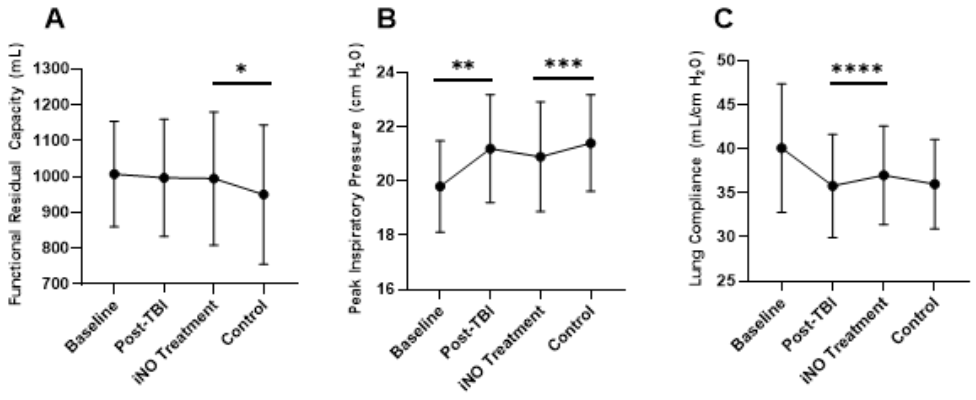

Figure 5. Effect of inhaled NO following TBI with subsequent removal of iNO (internal control) on porcine respiratory parameters.

4.6 Porcine Systemic Oxygenation Metrics

Baseline was compared to timepoint following TBI, demonstrating a significant difference in P:F ratio and PaO2 (P:F ratio 435 ± 42 baseline vs. 480 ± 38 TBI, p=0.005; PaO2 131 ± 13 mmHg baseline vs. 144 ± 11mmHg TBI, p=0.005). (Figure 6A-B) However, the baseline oxygenation metrics were measured with the animals supine during vascular cannulation and the subsequent measures were taken with the animals prone after craniotomy, intracranial monitor placement, and TBI induction. Figure 6 shows the effect of inhaled NO following TBI with subsequent removal of iNO (internal control) on porcine oxygenation metrics. (n=10 for all groups). (A) PaO2 (B) P/F

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Ratio. #p<0.05 PaO2 at baseline versus timepoint following TBI. ##p<0.05 P/F ratio at baseline versus timepoint following TBI.

Figure 6.

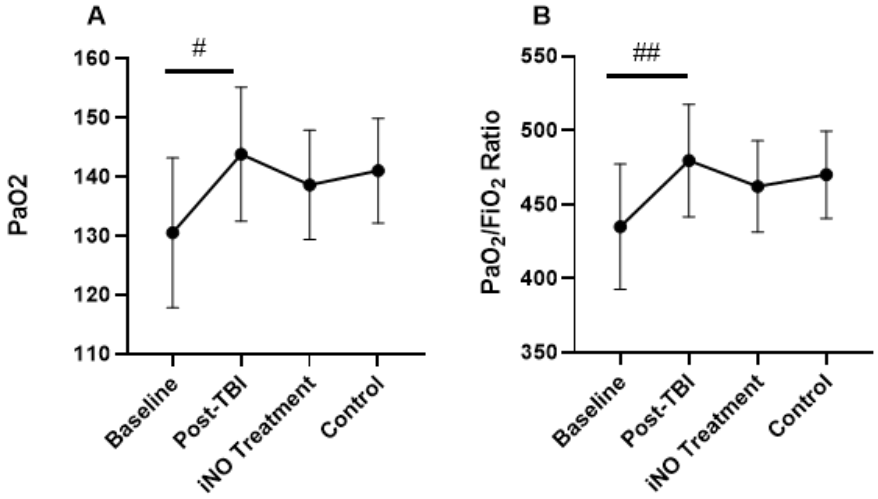

Figure 6. Effect of inhaled NO following TBI with subsequent removal of iNO (internal control) on porcine oxygenation metrics.

4.7 Porcine Intracranial Monitoring

There were no significant differences in cerebral parenchymal blood flow comparing the ipsilateral and contralateral sides of injury. In addition, there were no changes in the cerebral blood flow in either side of the brain over experimental progression through TBI, iNO initiation, and iNO treatment cessation. Similarly, there were no significant differences in brain tissue oxygenation between sides of the brain or over time with TBI induction or iNO treatment.

5.0 DISCUSSION

This study aimed to determine the clinical benefit of the use of a selective pulmonary vasodilating agent to improve pulmonary parameters and reduce the impact of TBI-associated hypoxia after head injury. In the human arm of the study, there were no clinically meaningful differences in respiratory parameters or oxygenation metrics between the iNO and the placebo group. Importantly, there were no significant changes noted in the ICP or CPP between the iNO and placebo groups throughout all observed timepoints. In the porcine arm, while there were significant differences among the pulmonary function parameters at various timepoints, those including PIP, PaO_2, and P:F ratio following TBI represented clinical improvement rather than pulmonary decompensation. Although other findings demonstrated statistically significant changes, they remain modest in clinical context and in the absence of acute pulmonary decompensation following TBI in the porcine arm.

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ARDS represents a well-established, non-neurologic sequela of TBI that leads to substantial morbidity and mortality in critically ill trauma patients.[1–3] A retrospective study by Solenki, et al. demonstrated that non-neurologic medical complications represent half of the mortality suffered in subarachnoid hemorrhage patients, the majority of which were pulmonary in origin.[16] This relationship was supported by Zygun, et al., demonstrating 23% prevalence of respiratory failure among TBI patients requiring intensive care.[4] Despite a large body of literature, the mechanism of respiratory failure in the setting of isolated TBI remains unclear. Studies have demonstrated an increased incidence of pulmonary edema following traumatic neurologic insult compared to non-TBI patients at time of autopsy, with the phenomenon of trauma-related neurogenic pulmonary edema reported as early as 1968.[17–19] Further pre-clinical studies have shown intracellular edema, cell component distortion, and malfunction in Type II pneumocytes in a rat model following isolated TBI. Studies also demonstrate pulmonary neutrophil infiltrates with decreased compliance in a murine TBI model .[20,21] Further, Robertson et al. demonstrated a relationship between preservation of CPP to prevent secondary ischemic brain injury following TBI and a five-fold increase in the frequency of ARDS.[22] The combined effect of surfactant depletion secondary, type II pneumocyte malfunction, and V/Q mismatch secondary to pulmonary edema and subsequent ARDS are the thought to be the leading contributors to hypoxemia in TBI patients.[3] In our study, there were no acute abnormalities noted in pulmonary function or blood oxygen saturation among the human
TBI patients prior to intervention. In the porcine arm, no pulmonary decompensation or hypoxemia were noted following TBI when compared to baseline.

Given the lack of significant difference in pulmonary parameters between placebo and iNO groups, including alveolar recruitment as observed in FRC comparison, we sought to examine the effect of PEEP increase and subsequent ability to increase alveolar recruitment following TBI. We pooled data from both placebo and iNO groups and examined the difference in pulmonary recruitment parameters over the course of the observed experiment, including both pre-determined PEEP increases and return to baseline PEEP. This analysis demonstrated overall preserved recruitment of the lung in the post-TBI population, with increases in both FRC as well as plateau pressure with PEEP increase, and subsequent decrease in FRC and plateau pressure when PEEP was returned to baseline. Throughout this time, there were no significant differences in compliance or overall oxygenation as measured by PaO_2. This analysis demonstrates that there was no clinically significant structural change in the lung that would affect the parameters of recruitment, with unchanged structural expansion potential (as measured by compliance) as well as expected changes to FRC and plateau pressure with PEEP increases. A lack of response in PaO_2 with increased PEEP is expected given overall normal PaO_2 prior to PEEP manipulation and lack of observed ARDS.

From a therapeutic perspective, the hallmark of treatment in ARDS is relieving hypoxemia with incrementally increased PEEP and increasing FiO2. The use of high PEEP settings in the clinical context of TBI is controversial, however, given the mixed data that indicates possible increased ICP and decreased CPP associated with PEEP-induced increases in intra-thoracic pressure causing decreases in systemic arterial pressure and impaired thoracic venous return.[23,24] A study by McGuire et al. demonstrated an increase in ICP following an increase in PEEP, though a lack of associated drop in CPP calls into question the clinical significance of such ICP changes.[25] Our

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study demonstrated that human patients with TBI undergoing a moderate step-wise PEEP increase and return to baseline experienced no significant change in either ICP or CPP. The porcine arm of our study noted no significant changes in cerebral blood flow or tissue oxygenation following TBI, treatment with iNO, or removal of iNO therapy. It is important to note, however, that the porcine measurements in this study were completed in the setting of an open craniotomy, which could dampen ICP and CPP effects on cerebral blood flow and brain parenchymal oxygenation in the setting of TBI.

iNO is a selective pulmonary vasodilator that has been shown to decrease pulmonary vascular resistance, pulmonary arterial pressure, and right ventricular afterload. The efficacy of iNO in treating hypoxemia related to ARDS secondary to V/Q mismatch is due to its propensity as an inhaled agent to selectively target vessels associated with functional alveoli. Most commonly delivered via mechanical ventilator, the typical dose for iNO is less than 40ppm and the dose used in both arms of our study was 20 PPM.[11] The clinical use of iNO in the setting of non-neurologic respiratory failure secondary to ARDS remains controversial, as it has demonstrated temporary increases in oxygenation without significant effect on duration of required ventilatory support or mortality.[12,13] Given the mechanism of iNO and its demonstrated effect on improving hypoxemia, Terpolilli et al. completed a study utilizing iNO and demonstrated improvements in ischemic secondary brain damage following TBI. Their group demonstrated a significant decrease in several markers of cellular brain injury severity and improved overall neurologic function without adverse effects on cerebral autoregulation or systemic blood pressure.[14] In our study, we aimed to measure the change in pulmonary function parameters and systemic as well as cerebral oxygenation with the use of iNO following TBI. Our data did not support any significant differences in pulmonary parameters between human patients following TBI that were randomized to either an iNO therapy group or placebo group at any observed timepoints. Of note, these timepoints included two incremental increases in PEEP and subsequent return to baseline to determine if iNO could mitigate intracerebral pressure changes induced by an increase in PEEP. In the porcine arm of our study, several statistically significant changes occurred in pulmonary function parameters, including an increase in oxygenation and P:F ratio following TBI, a decrease in FRC following iNO cessation, PIP increase both with TBI and following cessation of iNO, and increase in lung compliance with induction of iNO treatment. Taken together, these differences do not represent a clinically significant impact of iNO on pulmonary function parameters following TBI.

One significant limitation of our study was low enrollment in the human arm. Low enrollment was primarily due to low incidence of isolated head injury in the absence of concurrent exclusion criteria as outlined previously. This degree of low enrollment led to a particularly low sample size when considering intracranial monitoring, as only two patients randomized to each group underwent invasive intracranial monitoring determined by their respective clinical courses. The use of a porcine model was then added to the study to compensate for unplanned low enrollment. An additional limitation of our study was the use of only female pigs in the porcine arm. We chose to only include only females due to the known sex differences in the functional outcomes following TBI.[26] By studying only one sex, we were able to isolate differences in outcomes based on treatment alone. A third limitation of the study was the use of internal control within the porcine arm, rather than randomization to a treatment and placebo group. We felt this was an appropriate way to maximize resources and minimize the use of a non-survival large animal model given the short half-life of iNO once administered (2-6 seconds in presence of appropriate physiologic

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hemoglobin concentration). The cessation of iNO was followed by a 30-minute clearance period prior to data collection for the final timepoint. A fourth limitation to our study was the use of TBI in the absence of space-occupying lesion to replicate elevated ICP in the porcine model. Although our CCI protocol appropriately models the condition of TBI in relation to parenchymal injury, the craniotomy remains open throughout experimental period for the purposes of monitor placement. The open craniotomy in the absence of a space-occupying lesion does not allow for the increase in ICP and decrease in CPP associated with TBI and that may impact pulmonary parameters. The final and perhaps most significant limitation of our study was the lack of acute pulmonary decompensation in both the human and porcine arms prior to administration of iNO. This was demonstrated most clearly by the normal range P:F ratio observed both in human patients at first time point (post-TBI) and in the porcine arm at the timepoint immediately following TBI. The lack of pulmonary decompensation induced by TBI was perhaps the most limiting factor in evaluating the efficacy of iNO on post-TBI respiratory failure and consideration could be given to a future porcine model of iNO treatment in the setting of TBI with induced intentional post-traumatic ARDS.

In conclusion, our study demonstrated that iNO had no significant effect on clinically relevant pulmonary parameters following TBI in both human patients and a porcine model. As previously mentioned, however, these observations were made in the absence of profound neurogenic respiratory failure in both arms of the study. Our study successfully demonstrated that iNO did not cause a significant increase in ICP or decrease in CPP as compared to placebo and across several incremental increases in PEEP. Furthermore, there were no significant changes in brain tissue oxygenation or blood flow induced by introduction of iNO in our porcine model. However, it was importantly noted that there were no significant pulmonary or intracranial changes in either the human or porcine models, suggesting that the lack of iNO effect was consistent across both a clinical and pre-clinical model of TBI. Finally, the challenge of under-enrollment significantly impacted the initial intention of our study. Given ongoing challenges with recruitment in human trials, we have established a novel approach in which the addition of a large animal study arm with treatment equivalents can be used to supplement an under-powered human study in the setting of low enrollment.

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

%: Percent
-: Minus
+: Plus
<: Less Than
=: Equals
ARDS: Acute Respiratory Distress Syndrome
CCI: Controlled Cortical Injury
cm H2O: Centimeters of Water
cm: Centimeters
COVID: Caronavirus Disease
CPP: Cerebral Perfusion Pressure
CT: Computed Tomography
CVP: Central Venous Pressure
FiO2: Fraction of Inspired Oxygen Concentration
FRC: Functional Residual Capacity
HCO3: Bicarbonate
HPW: Human Performance Wing
IACUC: Institutional Animal Care and Use Committee
ICP: Intracranial Pressure
iNO: Inhaled Nitric Oxide
mm H2O: Millimeters of Water
mm: Millimeters
mmHg: Millimeters of Mercury
mmol/L: Millimoles per Liter
p: Statistical P Value
P:F Ratio: PaO2:FiO2 Ratio
PaO2: Partial Pressure of Oxygen
PCO2: Partial Pressure of Carbon Dioxide
PEEP: Positive End-Expiratory Pressure
PIP: Peak Inspiratory Pressure
PPM: Parts Per Million
SAS: Statistical Analysis Software
SBP: Systolic Blood Pressure
TBI: Traumatic Brain Injury
Telazol: Tiletamine Hydrochloride
V/Q: Ventilation/Perfusion

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