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Effects of Hyperoxia on Pulmonary Inflammation and organ injury in a human in vivo model (HIPI): study protocol of a randomised, double-blind, placebo-controlled trial

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ABSTRACT

Introduction Liberal administration of supplemental oxygen (O_2) is ubiquitous across numerous healthcare settings. However, appropriate O_2 titration targets remain controversial and despite numerous large-scale randomised trials, there is an ongoing lack of consensus regarding optimal oxygenation strategies and the absence of high-quality mechanistic data pertaining to the potential proinflammatory effects of hyperoxia.

Methods and analysis We hypothesise that (1) short-term exposure to hyperoxia will induce mild pulmonary inflammation and cellular injury and that (2) hyperoxia will accentuate pulmonary inflammation and cellular injury in the setting of inhaled lipopolysaccharide challenge. To test our hypotheses, we will conduct a randomised, double-blind, placebo-controlled study of hyperoxia administered via a high-flow nasal O_2 delivery system (fractional inspired oxygen 1.0, 60 L/min flow rate) compared with synthetic medical air. Blocked randomisation will be undertaken by an independent clinical trials statistician. Healthy non-smoking adult volunteers (<45 years of age), taking no regular medications will be recruited. Bronchoalveolar lavage (BAL) will be performed at 6 hours. The study outcome measures will include BAL markers of inflammation and injury (including but not limited to interleukin (IL)-8, IL-6, tumour necrosis factor alpha), BAL differential cell counts, BAL markers of oxidative stress (superoxide dismutase and glutathione), alveolar epithelial cell injury (SP-D, vWF, RAGE) and markers of systemic inflammation (neutrophils and plasma C-reactive protein).

Ethics and dissemination Dissemination of the research findings will be achieved in the following ways: (1) Our findings will be presented at national and international meetings with open-access abstracts online and (2) in accordance with the open-access policies proposed by the leading research funding bodies we aim to publish the findings in high quality peer-reviewed open-access journals.

Trial registration number NCT05414370.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Numerous large randomised clinical trials have failed to elucidate the optimal oxygen (O_2) titration targets in the setting of critical care and the potential harmful effects of prolonged O_2 exposure remain poorly characterised.

WHAT THIS STUDY ADDS

⇒ Prospective mechanistic evaluation of the acute pulmonary and systemic inflammatory response to hyperoxia has not previously been studied in adults using randomised controlled trial methodology.

⇒ This study will significantly increase our understanding of the potential proinflammatory mechanistic effects of hyperoxia in the setting of acute lung injury.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The study will address a critical knowledge gap regarding whether hyperoxia contributes to excessive pulmonary and systemic inflammation potentially enabling the design of future therapeutic studies targeted at mitigating the proinflammatory effects of O_2.

INTRODUCTION

Hyperinflammatory mechanisms of oxygen

Oxygen (O_2) is the most common therapy in the setting of critical illness and is essential for the maintenance of physiological homeostasis, ATP synthesis and survival.[1 2] Alarmingly, this treatment is often administered in the absence of well-defined titration targets and may result in adverse effects including lung parenchymal injury, aberrant gas exchange,[3] absorptive atelectasis[4 5] and direct airway injury.[6] Hyperoxia has previously been shown to cause lethal lung injury in animals,[7–9]

however, the mechanisms of hyperoxia-mediated pulmonary inflammation and injury in humans remain poorly understood and there are only limited data pertaining to healthy subjects.[10] An important potential mechanism of hyperoxia-mediated pulmonary injury may be through oxidative stress. O_2 possesses injurious oxidising properties that are chiefly mediated via the generation of reactive oxygen species (ROS) including superoxide anions, hydroxyl radical and hydrogen peroxide.[11 12] ROS are an important by-product of inflammatory cell stimulation and contribute to the host antimicrobial defence but can also lead to tissue injury when the production of ROS exceeds host antioxidant defences.[13] ROS may react with essential intracellular molecules and impair their function resulting in dysregulated inflammatory response, tissue damage and eventual cell death via both apoptosis and necrosis.[14] Data supporting the direct toxic effects of ROS emanate from studies of transgenic mice with altered superoxide dismutase (SOD) activity.[15] Insulin-like growth factor 1[16] and angiopoietin-2[17] have also been shown to contribute to hyperoxia-mediated lung injury. Notably, murine models demonstrate that even moderate supplemental O_2 (fractional inspired oxygen (FiO_2) 60%) can significantly augment lipopolysaccharide (LPS)-induced lung injury resulting in increased histological injury, alveolar neutrophil infiltration and epithelial barrier permeability.[18] Interestingly, Aggarwal *et al* reported that antibody-mediated depletion of neutrophils substantially mitigated the effects of O_2-induced lung injury suggesting a critical role of these innate effector cells in O_2-mediated inflammation.[9]

A further mechanism of O_2-induced lung injury may include inhibition of compensatory anti-inflammatory pathways.[8] Intriguingly, early exposure to hyperoxia during mechanical ventilation has previously been reported to correlate with rates of culture for *Pseudomonas aeruginosa*.[7] Murine studies demonstrate that O_2-induced lung dysbiosis contributes to the development of acute lung injury which may be partially attributable to alterations in the microbiota, which becomes enriched for O_2-resilient taxa such as *Staphylococcus aureus*.[19]

Supplemental O_2 in intensive care

Within the intensive care unit (ICU) arterial oxygenation must be kept in careful physiological balance to avoid the injurious effects of hypoxia while simultaneously avoiding supraphysiological partial pressures of O_2 (arterial oxygen pressure (PaO_2)), which have been shown to be associated with increased ICU mortality in a range of conditions including cardiac arrest,[20] stroke[21] and traumatic brain injury.[22] In an attempt to mitigate against pulmonary and systemic oxidative injury associated with sustained high FiO_2 and elevated arterial O_2 tension, numerous investigators have studied conservative O_2 as an intervention using a prospective randomised controlled trial (RCT) design. Girardis *et al* reported that conservative O_2 therapy (oxygen saturation (SpO_2) 94–98%) resulted in lower ICU mortality compared with more liberal O_2 therapy (SpO_2 >97%).[23] Notably, this study included a heterogeneous critical care population and was terminated early following interim analysis. More recent RCTs of conservative O_2 during mechanical ventilation have found no significant differences in either ventilator-free days[24] or 28-day mortality when comparing conservative O_2 therapy to either 'usual-oxygen' or 'high-oxygen' strategies respectively.[25] Importantly, a post hoc analysis of the ICU-ROX (Intensive Care Unit Randomized Trial Comparing Two Approaches to Oxygen Therapy) trial suggests the potential for clinically important harm in patients with sepsis receiving conservative O_2.[26] To date, there have been eight well-conducted RCTs attempting to elucidate the optimum O_2 titration strategy during mechanical ventilation.[23 25 27–29] However, there is currently insufficient evidence to recommend the use of more conservative O_2 titration targets in the setting of critical illness and consensus regarding the optimal oxygenation targets remains elusive.

Hyperoxia in intensive care

Hyperoxia was once considered a cornerstone in the immediate management of circulatory shock to counteract aberrant O_2 extraction and address the imbalance between O_2 demand and delivery.[30] Hyperoxia can lead to enhanced vasoconstrictor and antimicrobial properties[31] and has previously been shown to reduce surgical site infection in colorectal surgery.[32] The Surviving Sepsis Campaign Guidelines currently state that there is insufficient evidence to make recommendations on the use of conservative O_2 targets in adults with sepsis-induced hypoxaemic respiratory failure.[33] Notably, in patients with septic shock, the hyperoxia and hypertonic saline in patients with septic shock trial found that setting FiO_2 to 1.0 for induction of alveolar hyperoxia was associated with increased risk of mortality compared with normoxia.[34] Establishing optimal oxygenation targets is a priority in acute respiratory distress syndrome (ARDS) where higher FiO_2 settings are frequently mandated to facilitate low tidal volume ventilation. Interestingly, one study reported a U-shaped relationship between PaO_2 whereby mortality was lowest when PaO_2 was between 12.5–14 kPa suggesting that both conservative and excessively liberal O_2 targets may be associated with harm in ARDS.[35]

Effects of hyperoxia in human in vivo studies

There are few in vivo studies of hyperoxia in human subjects and most previous studies have focused on physical and upper airway effects with no studies on pulmonary inflammation, cellular or distant organ injury. Tracheobronchitis is an early change due to hyperoxia which may be seen within hours of FiO_2 90–95%. A reduction in vital capacity may be noted following exposure to an FiO_2 of 100%. Postmortem studies of patients ventilated with an FiO_2 of 40–100%, in the absence of underlying lung disease, show alveolar type 1 epithelial cell

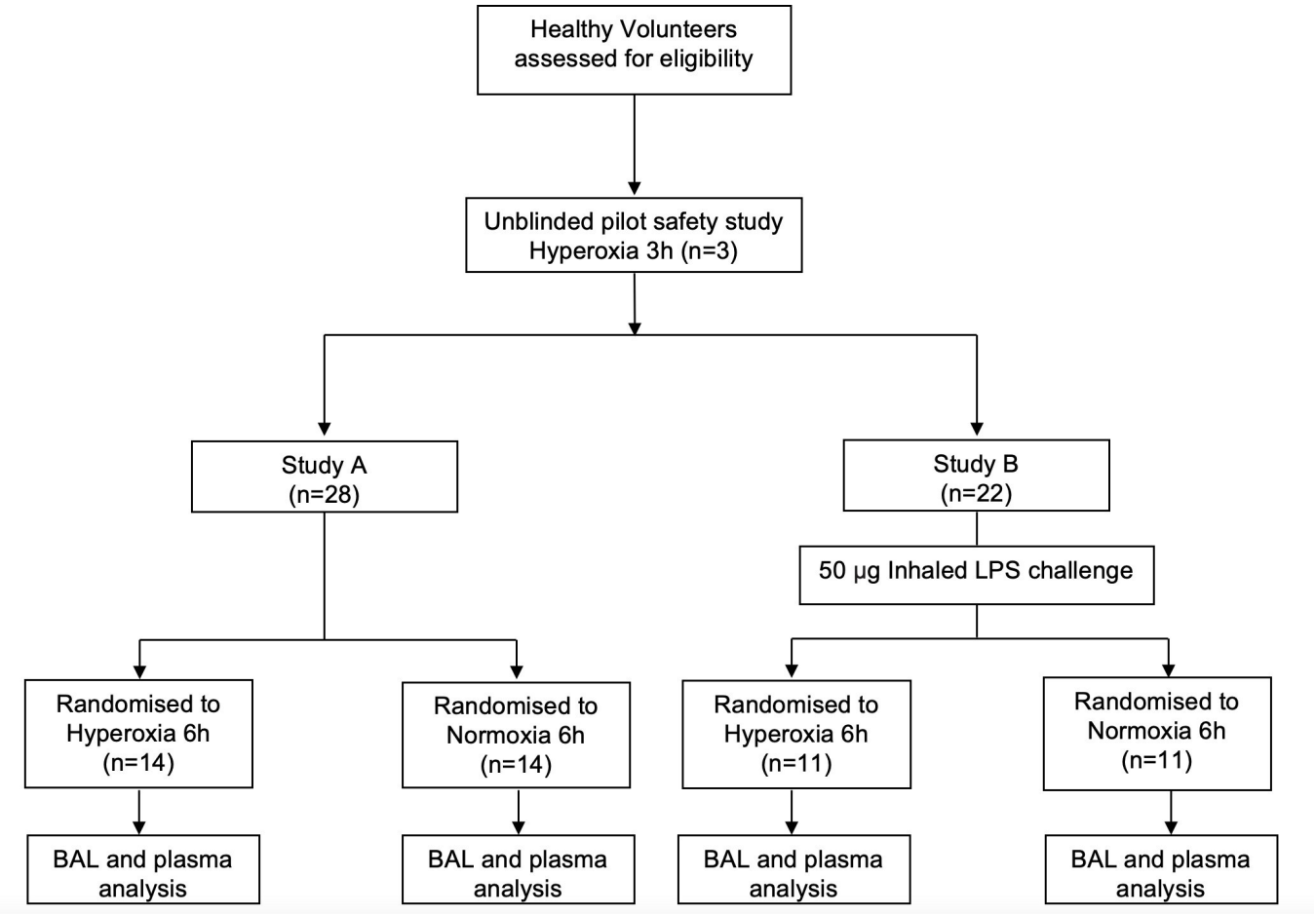

Figure 1 CONSORT diagram of healthy volunteer trial. BAL, bronchoalveolar lavage; CONSORT, Consolidated Standards of Reporting Trials; LPS, lipopolysaccharide.

injury and endothelial cell injury.[36] In another study in patients ventilated for brain injury, ventilation with pure O_2 was associated with a significant reduction in PaO2/FiO2 (P/F) ratio when compared with ventilation with air and a sharp decline in P/F was seen after 30 hours of ventilation with O_2.[37]

Research importance for the study

Despite accumulating evidence indicating the potential detrimental effects of excessive O_2 exposure, its liberal administration remains commonplace in the acute care setting. This may be partially attributable to insufficient understanding regarding the proinflammatory effects of hyperoxia. Our current understanding of the mechanisms of O_2 toxicity is largely derived from animal experimental models. There are only a limited number of human studies which were conducted in the late 20th century with the absence of measurements of inflammatory cytokines, markers of cellular injury and oxidative stress. Furthermore, there are currently no human in vivo studies exploring the relationship between hyperoxia and direct pulmonary injury and inflammation as well as distant organ injury. Consequently, prospective human studies are an essential unmet need because it is otherwise impossible to differentiate hyperoxia-mediated injurious effects from potential confounding factors such as the underlying lung injury process or concurrent respiratory comorbidities when studying critically ill patients.[38] Furthermore, human studies may enable the identification of potential pharmacotherapeutic targets and will allow the administration of high concentrations of O_2 in situations of clinical need while avoiding hyperoxia-mediated injury.

METHODS AND ANALYSIS

Hypothesis

The aim of this study is to test the following hypotheses:

1. Short-term exposure to hyperoxia induces mild detectable inflammation and cellular injury.
2. Short-term exposure to hyperoxia accentuates LPS-induced inflammation in a clinically relevant human model that replicates the pathophysiological mechanisms of ARDS development.

Study objectives

The primary objective is to (1) Investigate pulmonary inflammation and injury in response to hyperoxia, (2)

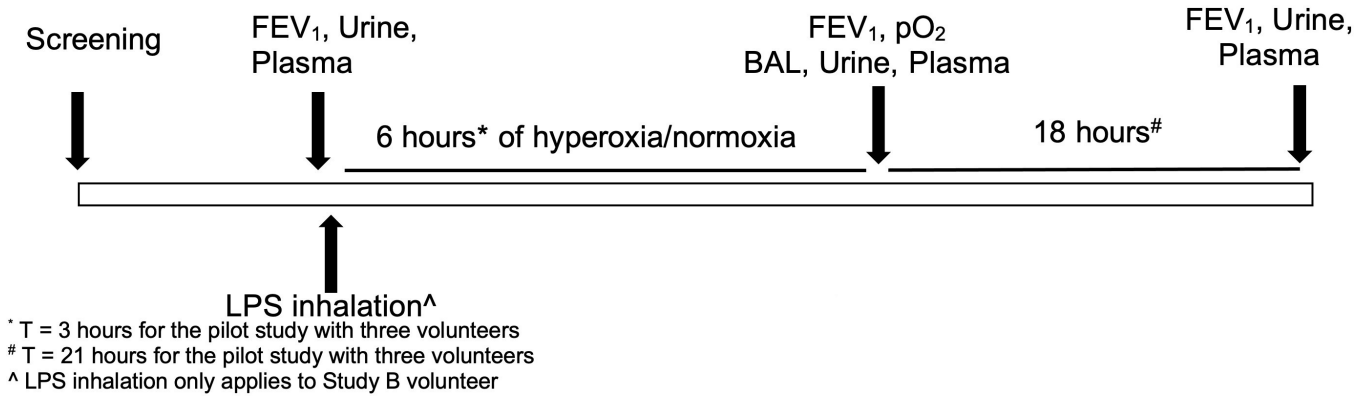

Figure 2 Schematic of study schedule of events. BAL, bronchoalveolar lavage; FEV1, forced expiratory volume in 1 s; LPS, lipopolysaccharide; PO_2, partial pressure of oxygen.

Investigate the superadded effect of supplemental O_2 on LPS-induced inflammation and injury.

Study design

This will be a randomised, double-blind, placebo-controlled, allocation-concealed clinical study comparing hyperoxia to synthetic medical air. Healthy non-smoking adult volunteers (<45 years of age), taking no regular medications will be invited to take part in this study through local advertising and randomised (1:1) to either hyperoxia or synthetic medical air. The study is designed in two parts mapped to the objectives (figure 1) and adheres to the Standard Protocol Items: Recommendations for Interventional Trials 2013 checklist (online supplemental appendix 1).

Study A is a RCT designed to characterise the pulmonary and systemic inflammatory response to a 6-hour exposure to hyperoxia. Study A will also incorporate a pilot study for the first three volunteers. The pilot study volunteers will be exposed to hyperoxia individually at an interval of at least 1 week to ensure safety. Blood will be collected at t=0 hours immediately before each participant commences the study intervention and again at 6 and 24 hours. Bronchoalveolar lavage (BAL) will be performed at 6 hours following the initiation of high-flow O_2/medical air. The study schedule is summarised in figure 2.

Study B is a RCT designed to investigate the additional effect of a 6-hour exposure to hyperoxia on inhaled LPS-induced pulmonary and systemic inflammation. Study B participants will inhale 50 µg of LPS (026:B6, Sigma-Aldrich) immediately prior to commencing the study intervention using an automated breath-activated dosimeter (Dosimeter MB3, Air-Liquide, France). No volunteer will be permitted to take part in the study more than once. Blood will be collected at t=0 hours immediately before each participant commences the study intervention and again at 6 and 24 hours. BAL will be performed at 6 hours following the study intervention. A summary of the study procedures is shown in table 1.

Study setting

This will be a UK single-centre trial conducted in the Wellcome-Wolfson Northern Ireland Clinical Research Facility (NICRF) and The Mater Hospital Belfast.

The study sponsor is The Belfast Health and Social Care Trust.

Participant eligibility criteria

Participants will be eligible for the study if they fulfil the following eligibility criteria.

Inclusion criteria

1. Healthy adults <45 years of age.
2. Non-smoker.
3. Body mass index <29 kg/m².

Exclusion criteria

1. Age <18 years.
2. Taking concomitant medications including over-the-counter medications excluding oral contraception and paracetamol.
3. Previous adverse reactions to LPS, lignocaine or sedative agents.
4. Pregnant or breastfeeding.
5. Participation in a clinical trial of an investigational medicinal product within 30 days.
6. Consent declined.
7. History of asthma or other respiratory conditions.
8. Smoking or use of e-cigarettes/vapes.
9. Marijuana or other inhaled product use with/without nicotine in the last 3 months.
10. Alcohol abuse, as defined by the Alcohol Use Disorders Identification Test.
11. Subjects with a history of prior conventional cigarette (> 100 cigarettes lifetime and smoking within 6 months) or electronic cigarette use.

Screening assessment

The following will be performed at study screening visits:

Table 1 demonstrates the assessments and procedures to be performed at the given time points

TIME	Screening	0 hours	0–6 hours*	6 hours*	24 hours
Inclusion criteria	*				
Exclusion criteria	*				
Informed consent	*				
Demographics/questionnaire	*				
Safety bloods	*				*
LPS inhalation†		*			
Specialised blood tests/urine		*		*	*
Blood gas‡				*	
Bronchoalveolar lavage (BAL)				*	
Symptom assessment and temperature	*	*	*	*	*
Lung function (FEV_1, FVC and FEV_1/FVC ratio)	*	*		*	*
Adverse event assessment		*	*	*	*

*T=3 hours for the pilot study with three volunteers.
†Study B participants.
‡Following hyperoxia (or sham-hyperoxia) exposure and at T=6 hours.
FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; LPS, lipopolysaccharide.

1. Participant demographics (date of birth, gender, height and weight, medical history, recreational and prescribed medication history).
2. Tobacco smoking and electronic cigarette history.
3. Vital signs—heart rate, blood pressure, respiratory rate, SpO_2 and temperature.
4. Laboratory assessments: haematological and biochemical parameters.
5. Pulmonary function—forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), FEV_1/FVC ratio.
6. Review of inclusion and exclusion criteria.

The screening period should not exceed 30 days. Inclusion and exclusion criteria will be verified and confirmed again if 30 days is exceeded. For volunteers who are female, a urine pregnancy test will be performed on the day of intervention.

Study schedule

The study start date was 2 December 2022. The planned study end date, including completion of laboratory measurements and analysis, is December 2025.

Informed consent

Consenting processes are standardised and are reinforced via training prior to study start-up. Consent will be performed by a medically trained doctor in accordance with the ethical principles that have their origin in the Declaration of Helsinki. Participants will have all questions regarding the study answered to their satisfaction. Following a detailed discussion of the study, written, informed consent will be documented by the participant using the REC-approved study information sheet (online supplemental appendix 2). A copy of the consent form will be given to each participant while the original consent form is filed in the trial master file. A further photocopy of the consent form will be filed in the participant's medical notes.

Withdrawal from the study

Study participants may withdraw from the study without giving a reason at any time without prejudice. If a participant requests to withdraw, then no further data will be collected. In the event that a participant requests to withdraw from the study, the option to withdraw from part or all of the study including data destruction will be offered.

Intervention

Participants randomised to hyperoxia will receive liquid medical O_2 administered using a high-flow nasal O_2 cannula (Airvo 2 Optiflow, Fisher & Paykel Healthcare, UK) with an FiO_2 setting of 100% and flow rate setting of 60 litres per minute for a duration of 6 hours. The temperature setting will be maintained at 31 C.

Comparator

Participants who are randomised to normoxia or 'sham-oxygen' will receive synthetic medical air administered using an identical high-flow nasal cannula (Airvo 2 Optiflow, Fisher & Paykel Healthcare, UK) with a flow rate setting of 60 litres per minute for a duration of 6 hours. The temperature setting will be maintained at 31 C.

Randomisation

Blocked randomisation will be undertaken by an independent trials statistician from the Northern Ireland Clinical Trials Unit (NICTU) using the statistical software nQuery Advisor (V.7.0). Fixed block sizes and no

stratification factors will be used. Following participant randomisation, the study administrator from NICTU will send the treatment allocation via email to a designated unblinded NICRF research nurse to maintain allocation concealment and blinding of the study intervention.

Blinding

The intervention will be started by an unblinded NICRF research nurse independent of the research team. Blinding of the study intervention will be achieved by sham administration of synthetic medical air through high-flow nasal cannula. Liquid medical O_2 or synthetic medical air will be entrained through a high-flow nasal O_2 cannula (Airvo 2 Optiflow, Fisher & Paykel Healthcare, UK). The same apparatus will be used throughout the conduct of the study. Blinding will be achieved by the placement of a privacy screen and masking to conceal the Airvo 2 system and O_2/medical air port from both the participants and the research team.

Study procedures

Pilot safety study

The duration of exposure to hyperoxia will be informed by the unblinded pilot study of the first three healthy volunteers. A duration of 3 hours is chosen based on historical studies where a reduction in mucociliary transport is demonstrated after 3 hours of hyperoxic challenge. The pilot study will ensure that the duration of the hyperoxic challenge is kept to the minimum required to detect a subclinical inflammatory response. The duration of the hyperoxic response will not exceed 6 hours to ensure that any inflammatory response is subclinical.

Study A

Participants recruited to Study A (n=28) will undergo exposure to hyperoxia for a total duration of 6 hours. Blood will be collected at t=0 hours immediately before each participant commences the study intervention and again at 6 and 24 hours. BAL will be performed at 6 hours following the initiation of high-flow O_2/medical air.

LPS inhaled challenge: Study B

Participants recruited to Study B will inhale 50 µg of LPS (026:B6, Sigma-Aldrich) immediately prior to commencing the study intervention using a breath-activated dosimeter (Dosimeter MB3, Air-Liquide, France). This dose of LPS has been used extensively in healthy volunteer trials as a model of acute pulmonary inflammation in a model that recapitulates the pathophysiological mechanisms of ARDS. LPS will be reconstituted for nebulisation immediately prior to administration in a clinical room designated for research purposes in the hospital Day Procedure Unit. In addition to the provision and use of appropriate personal protective equipment (PPE), all steps involving the handling of LPS will be carried out in a well-ventilated neutral pressure side room with an aseptic non-touch technique using a sterile dressing pack.

Bronchoscopy and BAL

Bronchoscopy and BAL will be performed in accordance with national guidelines.[39] In summary, the bronchoscope will be wedged within the right middle lobe (or lingula), 180 mL of 0.9% normal saline instilled via the bronchoscope and aspirated, as previously described.[40–43] The BAL fluid and blood samples will then be immediately transported on ice to the research laboratory where they will be separated into cellular and soluble fractions and separated for further analysis (online supplemental appendix 3). Volunteers will be observed post-bronchoscopy in compliance with the current standard of care practised in the Belfast Health and Social Care Trust. The duration of observation will be not less than 60 min postprocedure and the patients will need to be accompanied by a responsible adult. Patients will be discharged only when their vital signs (pulse, blood pressure, peripheral O_2 saturation) are within normal limits. Postprocedure advice will be provided and a contact number will be provided for postprocedural follow-up.

Primary outcome measure

The primary outcome of this study is BAL interleukin-8 (IL-8) concentration at 6 hours following the initiation of high-flow O_2 (or 'sham-oxygen').

BAL IL-8 has been chosen as the primary outcome as this is a key biomarker of the acute pulmonary inflammatory response and is closely associated with neutrophil chemotaxis and influx into the alveolar compartment.

Secondary outcome measures

BAL total and differential white cell count.

BAL cytokine markers of alveolar inflammatory response, including tumour necrosis factor alpha (TNFα), IL-1β, IL-6, IL-1Ra and MCP-1.

BAL markers of alveolar proteolytic activity including MMP-7, MMP-8, MMP-9, neutrophil elastase and TIMP-1 and TIMP-2.

Indices of oxidative stress in live BAL cells.

Indices of oxidative stress in BAL fluid, erythrocytes and plasma may include SOD, catalase, hydrogen peroxide, myeloperoxidase, NO2-expression, NOX-1 and NOX-4 expression, advanced glycation end products, malondialdehyde, ratio of reduced glutathione to oxidised glutathione (GSH/GSSG), 8-hydroxy-2-deoxyguanosine, DNA methylation and acetylation and cell-free mitochondrial DNA, xanthine oxidase, thioredoxin, oxidised low-density lipoprotein, F2 isoprostanes, lipid oxidation products (trans-4-hydroxy-2-nonenal nitrotyrosine.

Plasma inflammatory biomarkers (TNFα, IL-1β, IL-6, IL-8, C reactive protein, neutrophil count), proteases and antiproteases (MMPs, NE, TIMPs).

Indices of alveolar epithelial and endothelial function and injury in plasma and BAL including RAGE, Ang I/II, SP-D, vWF, Procollagen III N-terminal peptide as well as total protein, plasma albumin and protein permeability.

Emergency unblinding

A doctor from the research team will be present for the duration of the intervention. In the event of the need for emergency unblinding, the medically trained research team member will have the opportunity to discontinue the intervention immediately if needed. The site researcher will be able to remove the masking to unblind the intervention. The study sites are equipped with a resuscitation trolley. Following the study visit, volunteers will be given a study card including 24-hour contact details of the researcher who will liaise with the chief investigator (CI) if necessary. Where unblinding has occurred this should be fully documented by the site, sponsor and NICTU.

AE reporting

The CI or their delegated investigator is responsible for recording adverse events (AEs) observed during the study period. The investigator should attempt, if possible, to establish a diagnosis based on the subject's signs and symptoms. When a diagnosis for the reported signs or symptoms is known, the investigator should report the diagnosis as an AE, rather than reporting the individual symptoms.

The investigator should follow all AEs observed during the study until they are resolved or stabilised or the events are otherwise explained. All AEs should be treated appropriately. Treatment may include one or more of the following: no action taken (ie, further observation only); non-drug therapy given; subject hospitalised. The action taken to treat the AE will be recorded in the case report form (CRF).

The CI must assess the seriousness, causality and expectedness of any AEs in keeping with regulatory requirements. The investigator must record the AEs, seriousness as well as duration (start and end dates). AEs are recorded at each study time point and tabulated for inclusion in an annual safety report to the sponsor, Medicines and Healthcare products Regulatory Authority (MHRA) and the Research Ethics Committee.

Patient and public involvement

The patient and public involvement (PPI) for this study included discussion and review of the trial design, study procedures, measurements and review of patient-facing documents. Ongoing PPI input will be sought for any substantial amendments to the study procedures. Where appropriate, research details will also be posted on institutional websites available to the general public. In addition, the most significant results of the study will be communicated to the public through press releases.

Statistical considerations

Sample size calculation

Study A

Based on data from our prior study demonstrating BAL IL-8 level of 23.03 (7.9) pg/mL in controls, a cohort of 14 hyperoxia users and 14 controls will be able to detect a minimal difference of 40% between groups with 80% power at a two-tailed significance level of 0.05.

Study B

Based on data from our previous inhaled LPS studies demonstrating BAL IL-8 level of 389 (94) pg/mL 6 hours after inhaling LPS, a cohort of 11 hyperoxia and 11 controls will be able to detect a minimal difference of 40% between groups with 80% power.

Analysis population

Primary analysis will be conducted on all outcome data obtained from all participants as randomised and regardless of protocol adherence, that is, intention-to-treat analysis. Per-protocol analysis will also be conducted which will involve a comparison of treatment groups that includes only those who completed the treatment originally allocated. Participants who fail to complete the study that is defined as the BAL at 6 hours will be excluded.

Statistical analysis

Descriptive statistics will be used to define the hyperoxia and normoxia cohorts respectively. Continuous variables will be analysed by comparing hyperoxia and normoxia groups using an unpaired t-test or Mann-Whitney U test. Data will be reported as mean±SD or median (IQR). Data will be analysed using GraphPad Prism (GraphPad Software; San Diego, California, USA). A p value of <0.05 will be considered significant. There is no plan to adjust for multiple comparisons.

The results from each study cohort may be reported separately.

Ethics

End of study

The study will end when the completed number of patients have been recruited and completed follow-up.

The trial will be stopped prematurely if:

- Mandated by the ethics committee.
- Mandated by the MHRA.
- Mandated by the sponsor, for example, following data monitoring and ethics committee (DMEC) recommendation.
- Funding for the trial ceases.

The Research Ethics Committee and the MHRA will be notified in writing if the trial has been concluded or terminated early.

Patient confidentiality

Patient confidentiality will be maintained at every stage and compliance with the Data Protection Act (2018).

Good clinical practice

The study will be carried out in accordance with the principles of the International Conference on Harmonisation Good Clinical Practice guidelines (www. ich.org).

Sponsorship

Belfast Health and Social Care Trust will act as a sponsor and will provide indemnity for the study.

Data collection management

All data for individual subjects will be collected by the CI or by a delegated investigator and recorded in the CRF. Participant identification in the CRF will be through their unique trial identifier and initials. No interim analysis is currently planned.

Data monitoring and ethics committee

An independent DMEC has been appointed to safeguard the safety and well-being of participants. The first DMEC meeting will take place after the pilot safety study and thereafter after the recruitment of every 10 participants. The DMEC will also meet in the event of an unexpected serious adverse reaction. A DMEC report will be drawn up by the trial statistician to include information on any severe AEs, suspected unexpected serious adverse reactions (SUSARs), AEs, recruitment, outcomes and any other data requested.

Dissemination

The current trial will be reported in accordance with the Consolidated Standards of Reporting Trials guidelines (www.consort-statement.org). Dissemination will be achieved in the following ways: (1) the findings will be presented at national and international meetings with open-access abstracts online and (2) in accordance with the open-access policies proposed by the leading research funding bodies we aim to publish the findings in high-quality peer-reviewed open-access journals. Written requests for data sharing will be reviewed on an individual basis by the CI.

Current trial status

The study was given sponsor approval on 2 December 2022 and recruitment commenced in January 2023. The pilot safety study (n=3) was completed in February 2023 and to date a combined total of 24 participants have been recruited to Study A and Study B. The planned study end date is December 2025.

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Contributors All authors have reviewed and approved the manuscript. DL, CMO, DFM conceived the idea for the trial and wrote the protocol. DL and DD drafted the manuscript. CCT, JK commented on the intellectual content and reviewed the manuscript. DL is responsible for the overall content (guarantor).

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Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by Office for Research Ethics Committees Northern Ireland (ORECNI) Business Services Organisation Unit 4, Lissue Industrial Estate, West Rathdown Walk, Moira Road, Lisburn, BT28 2RF REC Reference Number: 19/NI/0181. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data sharing not applicable as no data sets generated and/or analysed for this study.

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