

## Reducing and replacing animal models of blast and chemical lung injury using computational simulation

**Scientific importance:** Primary blast lung injury (PBLI) and chemical lung injury are increasingly common features of military conflict and terrorist attacks on civilian populations. PBLI occurred in 7% of UK casualties in the most recent conflict in Afghanistan despite the rudimentary nature of the opposition forces, and it is likely that PBLI will be more widely encountered in future, more industrialised, conflicts. The disease is more prevalent in civilian casualties resulting from terrorist attacks on transport infrastructure with over 150 PBLI casualties caused by the train bombings suffered in Madrid in 2004 and half of the serious casualties seen in the London underground and bus bombings affected. Military and civilian casualties exposed to PBLI are increasingly likely to survive to reach hospital, as improvements in personal protective equipment and prehospital care have reduced immediate fatalities due to penetrating injury. The toxic industrial chemicals (TIC's) Phosgene ( $\text{COCl}_2$ ) and Chlorine ( $\text{Cl}_2$ ) are both ubiquitous within the plastics, agricultural and other chemical industries. Whilst a well-recognised risk to health following industrial and transportation accidents, their ease of production combined with their lethality has resulted in their use as chemical weapons, most notably against civilian populations in the Middle East over recent years. Both agents cause a severe chemical lung injury that leads to acute respiratory distress syndrome (ARDS) and frequently death. The unpredictable and sporadic nature of human lung injury from both blast or TIC exposure makes clinical study in human casualties practically impossible, and research on improving treatment strategies is therefore almost wholly reliant on the use of rodent as well as large animal models. We propose a new approach, based on the use of validated high-fidelity computer simulations, with the dual aims of radically reducing the current dependence of researchers in this field on animal models, whilst increasing the scope, complexity and duration of studies that can be performed into novel treatment strategies.

**Current use of animal models:** Animal models are the mainstay of research into the pathophysiology and treatment of acute lung injury [1,2]. Whole animal models are very widely used, with a noticeable recent trend for the increased use of large animal models [3,4]. Acute lung injury is produced typically by forced inhalation of acid (e.g. oleic acid) and the animal is then subjected to mechanical ventilation of the lungs via a tube placed in the trachea via the mouth or through the front of the neck. The effect of the proposed treatment strategy on the lung is ascertained after a period of lung ventilation by measuring key clinical parameters using a variety of invasive and non-invasive monitoring devices. Subsequently, the animal is killed, and its lungs dissected to measure markers of lung injury chemically or microscopically. Animals most widely used in this context are rats, mice, rabbits, sheep, dogs and pigs. The number of whole animals used is difficult to ascertain (because many studies are not published and because many animals are used setting up the studies); our best estimate of the worldwide use of such whole animal models in this research is 2,000 - 3,000 animals per year. Isolated organ models are also used. This involves killing the animal and dissecting the lungs and/or chest wall and performing repeated mechanical inflation and deflation with subsequent examination of the lungs. Smaller numbers of animals are used in this scenario - we estimate 500 - 1,000 animals per year, worldwide, mostly rats, pigs, sheep and dogs.

**Limitations of animal models of acute lung injury:** It is widely acknowledged that no single animal model replicates the complex pathophysiological changes seen in acute lung injury in human patients [1]. In the case of ARDS, for example, alveolar neutrophilia (indicating inflammation), hyaline membrane deposition (indicating disruption of the alveolar/capillary barrier) and microthrombi (indicating endothelial injury) all feature prominently. In contrast, in most experimental models of ARDS only one feature predominates: alveolar neutrophilia in LPS instillation, epithelial injury in acid aspiration, etc. Therefore, no single animal model is the "best" model of lung injury, and instead the best model will be that which best reproduces the features that will be tested by the investigator's hypothesis. This leads to the need for large numbers of animals to be used in order to study the various different aspects of the disease. The nature of this research is inherently unpleasant, and requires significant doses of anaesthetic agent to facilitate. The use of high-dose anaesthetic and analgesic agents confounds the interpretation of such work, as all such drugs exhibit additional physiological effects. The limited relevance of any animal model to human pathophysiology critically limits the confidence of clinicians in novel therapeutic strategies proposed from such studies, as acknowledged by researchers themselves: "we cannot directly extrapolate these experimental findings to patients, who have longer time constants than pigs and might not tolerate RRs (respiratory rates) as high" [5]. This situation represents a severe limitation that has severely impaired scientific and therapeutic progress in the field. In the case of PBLI, the benchmark modelling work undertaken by Bowen and colleagues in 1968 has significant weaknesses, including its use of a broad range of large and small animal species, the mixing of long and short duration blasts and the mixing of blast overpressure measuring modalities (reflected and incident measurements differ significantly for any given explosion introducing significant differences in recorded overpressure). This work suggested exposure injury and lethality thresholds, but having been undertaken almost 50 years ago does not reflect the significant advances in medical care achieved over this period. It also does not describe the severity of injury in survivors and the likely requirement for, and duration of, intensive care management. More recent work using porcine models undertaken by Garner et al at the Defence Science and Technology Laboratories (DSTL) in Porton Down, demonstrated a significant increase in mortality when haemorrhagic shock and blast exposure are combined, which subsequently lead to a change in resuscitation protocols. However, the four arms of this study were limited to six to eight subjects and so could only accommodate the study of an immediately life-threatening combination of injuries (ie, coarse data) and not the intermediate-term and more subtle outcomes normally sought in medical intervention research.

**Work leading up to this application:** While sophisticated mathematical models of the lung now exist, their exploitation in clinical practice has to date been limited and they have not been widely accepted by clinicians as valid alternatives to animal models. This situation is now beginning to change however, with recent simulation-based work by the applicants appearing in leading clinical journals [6,7,8]. In collaboration with our proposed project partner, Surgeon Commander Tim Scott (Intensive Care Unit, University Hospital of North Staffordshire NHS Trust and Academic Department of Military Anaesthesia and Critical Care, Ministry of Defence) we have recently adapted our simulator to develop the first computational model of PBLI [9]. In the proposed project, we will develop this model to a stage where it can provide a credible long-term alternative to studies in animals, as follows:

**Research Programme:** The project naturally breaks down into three interlinked work packages, as follows:

**WP1 - Validation of the PBLI simulator:** To enable its use as the primary research tool in future studies of novel treatment strategies in PBLI, it is essential that we fully validate the model's ability to predict the responses of individual PBLI patients to varying modes of mechanical ventilation. This work will be carried out in collaboration with a number of clinicians who have agreed to supply novel (pre-existing) data for the development of this aspect of the model. We will then develop our simulator so that it can be demonstrated to robustly match data on the responses and outcomes detailed in the largest database of individual casualties suffering from PBLI collected to date. This work will involve blast-dosing each casualty in the database via voxel analysis of their initial CT scans. The database describes the arterial blood gases, serum electrolytes, cardiovascular and mechanical ventilation settings for each casualty throughout their intensive care stay.

**WP2 - Modelling pharmacological treatments:** This work will require significant further development of the model to introduce trans-membrane receptors coupled with a pharmacokinetic module. Whilst we can currently evaluate the effect of physical interventions such as changes in airway pressure within the lungs, the medical profession also urgently needs the ability to explore potential drug therapies. The pharmacology and physiology of drug treatment in the context of PBLI is very amenable to modelling, and is the natural next step in the development of this research tool. Modelling the physiology around activation of ligand operated membrane receptors will be the most challenging task, as we will need to determine the distribution and concentration of the molecular targets of interest and super-impose a physiological effect that results from their stimulation. On completion of this development phase, we will evaluate the potential of two candidate therapeutic drugs. N-acetylcysteine amide (NACA) is a relatively novel modification of N-acetylcysteine (NAC) in which a negatively charged carboxyl group has been replaced with an amide group resulting in an electrochemically neutral compound. This facilitates much greater penetration into the intra-cellular space, across the blood brain barrier and into mitochondria with a resulting significantly improved bioavailability. NACA is a potent antioxidant and reducing agent. It is five times more potent an anti-oxidant than NAC and replenishes depleted mitochondrial glutathione levels to over 90% of normal compared to 15% for NAC. It is also non-toxic to humans. The ion channel transient receptor potential vanilloid 4 (TRPV4) was also recently identified as a major mediator of pulmonary oedema in animal models of acute lung injury. It is a non-selective calcium ion channel activated by pressure, stretch, heat and toxin exposure. This makes it an ideal target for inhibition in the treatment of blast lung as well as toxic acute lung injury. Phase II trials are currently on-going evaluating its use in the management of heart failure related pulmonary oedema. We will model the effects of these drugs in both PBLI and Phosgene generated lung injury, both individually and in combination (bundled care) both as intravenous preparations and as nebulised agents. We will also study their use in spontaneously breathing casualties and in mechanically ventilated casualties.

**WP3 - Creation of a Phosgene/Chlorine chemical lung injury model:** This work will be carried out using data supplied by our collaborators at DSTL Porton Down. A large animal database of Phosgene exposed animals under a variety of conditions is already available. The database details a comprehensive set of cardiovascular parameters, arterial and mixed venous blood gas results measured regularly throughout the trial period. A similar database for Chlorine exposed animals is currently being generated. The process would replicate the construction of our blast lung injury simulator in that the average dose-injury relationship would be delineated and subsequently represented in the model. The model would then run injury scenario's and the physiological outcome will be compared to the source data. Our collaborators here share our interest in reducing dependence on live animal modelling for future work in this area.

**References (applicants' papers in bold):** [1] Animal models of acute lung injury, Matute-Bello & Matthay, American Thoracic Society, 2011. [2] Development of animal models for the acute respiratory distress syndrome, Bastarache & Blackwell, Disease Models and Mechanisms, 2009. [3] Acute respiratory distress syndrome induction by pulmonary ischemia-reperfusion injury in large animal models, Fard et al, Journal of Surgical Research, 2014. [4] Large-animal models of acute respiratory distress syndrome, Ballard-Croft et al, Annals of Thoracic Surgery, 2012. [5] Moderately high frequency ventilation with a conventional ventilator allows reduction of tidal volume without increasing mean airway pressure, Cordioli et al, Intensive Care Medicine Experimental, 2014. [6] Computational simulation indicates that moderately high frequency ventilation can allow safe reduction of tidal volumes and airway pressures in ARDS patients, W. Wang et al, Intensive Care Medicine Experimental, 2015. [7] Evaluation of lung recruitment maneuvers in Acute Respiratory Distress Syndrome using computer simulation, Das et al, Critical Care, 2015. [8] High PEEP in ARDS: evaluating the trade-off between improved oxygenation and decreased oxygen delivery", Chikhani et al, British Journal of Anaesthesia, 2016. [9] The primary blast lung injury simulator: A new computerised model, Haque et al, Journal of the Royal Army Medical Corps, 2018.