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Editorial View: If we ask a mouse about biotrauma, will it give us a sensible answer?

Masao Takata, MD PhD FRCA and Michael R Wilson, PhD

Section of Anaesthetics, Pain Medicine and Intensive Care, Faculty of Medicine, Imperial College London, Chelsea and Westminster Hospital, 369 Fulham Road, London, SW10 9NH, UK. Tel: +44 20 3315 8816, Fax: +44 20 3315 5109

Acute respiratory distress syndrome (ARDS) is a major cause of mortality in critical care, but to date no specific treatment exists. There is growing concern about our failure to translate from bench to bedside within the acute lung injury research community, and the crucial importance of better modelling in preclinical studies to identify targets with more predictive power is increasingly appreciated. Mechanical ventilation, while being a vital tool for support of ARDS patients, produces or worsens lung injury. This ‘ventilator-induced lung injury’ (VILI) has substantive negative impact on the outcome of ARDS. Increasing tidal volumes are associated with enhanced release of local and systemic inflammatory mediators in patients, and animal models demonstrated that excessive tidal volumes induce lung inflammation, edema and physiological dysfunction. Such findings have lent support to the ‘biotrauma’ hypothesis, i.e. VILI promotes the release of inflammatory mediators, which play a critical role in the progression of injury of the lungs as well as other systemic organs [1]. In this issue of *Anesthesiology*, Lex and Uhlig [2] investigate whether this biotrauma can be studied in so-called ‘one-hit’ models of VILI in mice. Their results provide useful information for physiologists to better design mouse VILI experiments, but more importantly, provoke a series of important questions that are essential for clinicians desiring to interpret animal VILI models for future clinical translation.

Within the study, the authors performed experiments using a one-hit model of VILI, i.e. ventilating healthy mice with a series of increasing tidal volumes/plateau pressures (Pplat). Having done an extraordinary job in maintaining these fragile animals stably for 7 hours, they identified a Pplat (between 24 and 27cmH₂O) below which there was only mild lung inflammation with no signs of physiological injury, while above this inflammation was dramatically increased and animals eventually developed catastrophic lung failure. Importantly, tidal volumes corresponding to this threshold pressure are much higher than those established as ‘injurious’ in human ARDS patients (i.e. 10-15ml/kg). This finding, consistent with a previous report [3], highlights a crucial issue often overlooked in preclinical studies: animal models are by their very nature extreme constructs, designed to mimic certain aspects of human pathophysiologies within logistically attainable time-frames. Hence, our focus must remain on what aspects of the human condition are actually

Original article: ‘One-hit models of ventilator-induced lung injury: benign inflammation versus inflammation as a by-product’, by Dennis Lex and Stefan Uhlig.

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being modeled, rather than whether experimental conditions (e.g. absolute values of tidal volume) are directly translatable to humans. The authors showed clearly that tidal volumes which induce $P_{plat} < 27 \text{ cmH}_2\text{O}$ would not produce a stretch-mediated lung inflammation or injury, even within 7 hours, in healthy mice whose lungs are more compliant than humans with injured/inflamed lungs. Indeed, a number of one-hit mouse VILI studies in the literature utilize such lower 'clinically-relevant' tidal volumes for shorter periods of time to investigate inflammatory processes during VILI. It is now clear that those studies are modelling something other than a 'stretch'-induced inflammation.

The other major conclusion of the study relates to that of 'biotrauma' expressed in its simplest (or as the authors term, strongest) form; i.e. that the inflammation induced by ventilation directly causes the subsequent pulmonary edema and physiological dysfunction. The authors conclude that this is not the case, based on the finding that dexamethasone treatment only delayed but did not prevent the onset of injury. In addition, the observed profile of random-onset, rapidly-progressing catastrophic lung failure is interpreted as more consistent with fatigue failure of lung structural materials. This assertion by the authors may be deemed debatable, but points us to another crucially important aspect of animal studies; what are the most relevant outcomes/end-points, and what should we expect from the mouse models?

The authors acknowledge that if they had stopped their experiments sooner, they could have come to different conclusions regarding the impact of dexamethasone. Once pulmonary edema starts to develop in a model of VILI using a constant tidal volume, regardless of the initiating mechanism (inflammatory or otherwise) lungs inevitably experience more and more strain as the same volume is delivered to a continually decreasing aeratable space, and injury not surprisingly proceeds exponentially. In such an ultimately lethal model, should we realistically expect any intervention to 'completely' prevent all injury? Or is a delay in the onset of injury sufficient to inform us that the pathways being manipulated are involved in the pathophysiology? In our opinion, this is a matter of careful interpretation – a 30-min delay in a process that develops over many hours/days is probably less relevant than a similar delay in a process that would otherwise be lethal in an hour. Furthermore, why should we expect that dexamethasone must prevent the injury when inflammation is involved? This is just one method to attenuate inflammation, and may not be very effective to inhibit key inflammatory pathways in VILI. Corticosteroid treatment has been found not very efficacious to reduce mortality in sepsis or ARDS patients [4, 5], so we might argue that the model accurately reflects the human situation in this aspect.

We feel the authors assertion that biotrauma cannot be studied in one-hit mouse models of VILI is something of a 'glass half empty' conclusion. Certainly, some degree of inflammation is present during VILI, and ventilation alone can very rapidly initiate inflammatory pathway activation in lung cells, suggesting that inflammation is not merely a consequence of injury from the chronological viewpoint. Although the magnitude of inflammation may not be so impressive as seen in some multi-hit models using inflammatory stimulants such as lipopolysaccharide, one-hit models have an advantage to be capable of clearly dissecting out the effects of ventilation per se. Indeed, many previous studies have shown that interfering with inflammatory pathways prevents (or maybe 'only'

delays) the onset of pulmonary edema even in one-hit models in mice. Particularly compelling evidence for involvement of biological mediators during VILI comes from studies in which perfusate collected from isolated lungs ventilated with injurious tidal volumes provoked injury in recipient lungs ventilated in a non-injurious manner [6].

Overall, this study beautifully and thoroughly illustrates intrinsic difficulties in animal models of complex in vivo diseases such as VILI, particularly in the case of one-hit mouse models due to the nature of injury and fragility of the species. As with any model there are pros and cons, and it would not be appropriate to believe that one-hit VILI models can address all possible mechanistic explorations and be predictive of treatment efficacy. This would lead to a suggestion of a more multifaceted approach using multiple-hit or chronic models of VILI in addition to one-hit models, which we fully agree with and seems to be becoming a consensus of the acute lung injury research field. However, the key will always be appropriate interpretation in both one-hit or multi-hit models, cautious of the limitations of each model itself. We must carefully assess if positive effects are truly significant/physiologically relevant and sustainable, or if negative results are solid enough to entirely exclude involvement of particular pathways. Interpreting the word 'biotrauma' with a narrow or broader definition may therefore not be so important, but critical reappraisal of animal preclinical studies is paramount for clinicians, in co-operation with scientists, to identify promising targets for effective clinical translation. Ultimately, a mouse will certainly give you an answer - we just need to understand their language to know what it means.

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