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REVIEW

**Thomas Jaecklin**
**Gail Otulakowski**
**Brian P. Kavanagh**

# Do soluble mediators cause ventilator-induced lung injury and multi-organ failure?

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T. Jaecklin · G. Otulakowski
Physiology and Experimental Medicine Program, Hospital for Sick Children, University of Toronto, Toronto, Canada

B. P. Kavanagh (✉)
Department of Critical Care Medicine, Hospital for Sick Children, 555 University Avenue, Toronto, ON M5G 1X8, Canada
e-mail: brian.kavanagh@sickkids.ca
Tel.: +1-416-8136068

**Abstract** *Background:* Significant advances in the management of patients with acute respiratory distress syndrome have been few in the recent past despite considerable efforts in clinical testing and experimental work. The biotrauma hypothesis of ventilator-associated lung injury (VALI), suggesting that mechanical ventilation induces the release of injurious mediators from the lung, implies that pharmaceutical interventions targeting these circulating pathogenic mediators would be clinically beneficial. Among the commonly reported classes of ventilation-associated mediators are cytokines, coagulation factors, hormones (e.g., angiotensin-II), lipid-derived mediators and oxidants, yet proof of their pathogenicity is lacking. *Discussion:* This review discusses evidence surrounding the roles of these mediators in VALI and describes how definitive proof could be provided based on Koch's postulates, using an isolated perfused lung model. According to this experimental concept, candidate mediators would fulfill certain criteria, including increased accumulation in perfusate during injurious ventilation and induction of injury during non-injurious ventilation. Accumulation of mediators in the perfusate would facilitate isolation and characterization by standard biochemical means, from broad determination of physical and chemical properties to precise identification of individual molecules (e.g., by modern "omic" approaches such as mass spectrometry). Finally, confirmation by exogenous administration of mediators or antagonists can assess effects on injury and its mechanisms such as cell permeability or cytotoxicity. *Conclusions:* Adaptation of Koch's postulates to the biotrauma hypothesis of VALI could provide important insights. Translation of the acquired knowledge into clinical testing is challenged by the heterogeneity of the patient population (e.g., etiology, co-morbidity, genetics or concomitant therapy) and the specificity and efficacy of the therapeutic intervention on the cellular/molecular level.

**Keywords** Mechanical ventilation · ARDS · Cytokines · Isolated organ model

## Introduction

The therapeutic arsenal for acute lung injury is limited. Its most severe form, the acute respiratory distress syndrome (ARDS), has been the object of a large number of randomized controlled trials (almost 8,000 patients) [1] that have yielded only a single large-scale positive result [2], and for decades mortality has not decreased [1]. Patients

with ARDS are believed to have two types of insult: one from the underlying illness (e.g., sepsis, trauma) and an additional contribution from injurious mechanical ventilation (i.e., ventilator-associated lung injury, VALI). In contrast, ventilator-induced lung injury (VILI) represents lung injury caused by mechanical ventilation in experimental models. Any potential mechanism that explains how mechanical ventilation injures lungs would require rapid testing, as it would be an important candidate for novel therapy. But before being tested in patients it would need to be understood. Over the last decade, the biotrauma hypothesis [3] postulated that mechanical ventilation causes the release of soluble mediators from the lungs into the bloodstream and that these circulating mediators cause injury in the lungs and in other organs. This hypothesis is attractive and plausible (see below), but has not been proved.

Circulating mediators make an attractive target for therapy in VALI, given that beyond avoidance of large tidal volumes, little else in ventilator management has been proved to increase survival with routine use, despite multiple studies (e.g., prone positioning, positive end-expiratory pressure, surfactant, inhaled nitric oxide). Before a mediator can be considered a therapeutic target, one needs to know that it causes—or worsens—lung injury, i.e., that a lung-derived compound contributes to lung injury and induces damage in healthy lungs. This distinguishes pathogenic mediators from biomarkers or surrogate outcome measures. The current review outlines the evidence—among the major classes of associated mediators—that supports the biotrauma hypothesis, discusses experimental approaches to proving it and suggests potential therapeutic interventions.

Apart from contributing to lung injury, the biotrauma hypothesis also postulates that lung-borne mediators might be implicated in the development of distant organ dysfunction. Although this review primarily focuses on the poof that circulating factors affect lung injury, extension of the proposed experimental construct to provide evidence for the systemic effects of lung-derived compounds would be theoretically straightforward.

## Circulating mediators in ventilator-induced lung injury

Among the most frequently reported mediators are cytokines, hormones, coagulation factors and lipid-derived mediators.

### Cytokines

Cytokines [e.g., tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), macrophage inflammation protein-2 (MIP-2) and pre-B-cell colony enhancing factor] were among the first inflammatory mediators thought to contribute to VILI. Elevation in cytokine levels is associated with lung injury in laboratory [4, 5] and clinical [2, 6, 7] studies, and is associated with worse morbidity and mortality [8, 9]. Anti-cytokine strategies such as antibodies (e.g., against TNF-α [10], MIP-2 [11] and pre-B-cell colony-enhancing factor [12]), blockade with soluble receptors (e.g., soluble TNF-α receptors [13]) or receptor antagonists (e.g., recombinant interleukin-1 receptor antagonists [14]), antibodies to nuclear factor κB (NFκB) [15] and steroids [16] have all conferred protection in experimental models. However, such associations do not prove causation, and there is substantial evidence against a categorical or simple pathogenic role.

Several laboratory studies report minimal elevations in cytokine level despite severe lung injury [11, 17–19], which together with the finding that exogenous cytokines do not necessarily produce lung injury [20] argues against an obligate pathogenic role.

In the clinical setting, the issue is also confounded. Some patients undergoing elective surgery have similar levels of circulating cytokines whether the ventilation parameters are injurious or 'protective' [21]; in addition, profound elevation in circulating cytokines can occur despite the use of 'protective' ventilation and the absence of structural lung injury [22]. Such lack of correlation—in a variety clinical and experimental settings—suggests that these molecules may be markers of tissue inflammation or repair rather than pathogenic mediators.

An exemplar cytokine is TNF-α. It is an important early pro-inflammatory mediator, is expressed and secreted very early in the course of VILI, and is biologically active (confirmed by cytotoxicity assay) in concentrations that are below detection limits of commonly used assays [4]. TNF-α acts on two receptors: p55 and p75, which have opposing effects [23]. The former promotes pulmonary edema, but the latter is protective and has a higher binding affinity; thus, TNF-α can have contrasting effects depending on its concentration [23].

There is a vast redundancy among cytokines. Some bind to several receptors causing a large number of effects; conversely, a single effect can be caused by several different cytokines [24]. In addition, there is a fine balance between pro- and anti-inflammatory mediators and between ligands and their receptors; both pro- and anti-inflammatory mediators may be increased in acute lung injury and by mechanical ventilation [25–28]. In addition, localized inflammation can be accompanied by systemic immune modulation [29]. Thus, levels of circulating mediators might not reflect the net effect on tissue or cellular injury, and a simple correlation of a specific circulating cytokine level with the degree of injury cannot prove—and might not indicate—pathogenic effect. This also explains why mediator inhibition would have multiple and unpredictable effects.

### Coagulation factors

Intra-alveolar fibrin is common in injured lungs [30]. Pro-inflammatory mediators can stimulate tissue factor expression (activates coagulation [31]), suppress activation of protein C (APC) and promote the secretion of both thrombomodulin and plasminogen activator inhibitor-1 (PAI-1) [32]. Increased levels of the latter exist in ARDS and correlate with outcome [32]. Consistent with these effects, injurious ventilation results in similar alterations in coagulation [33], and protective ventilation attenuates such effects [32, 34].

Because of these associations, circulating components of the coagulation system have been identified as potential therapeutic targets. Heparin, initially promising because of anti-inflammatory as well as anti-coagulant properties [35], has however had little net benefit in clinical trials [36]. Other interventions have disappointed also: tissue factor pathway inhibitor (i.e., tifacogin) did not attenuate lung injury in patients with sepsis [37], and APC—commonly used in severe sepsis—has uncertain effects in patients with acute lung injury. Finally, thrombomodulin, an important cofactor in fibrin formation and activation of protein C, has a complex relationship with lung injury and is being evaluated as a potential therapeutic target [36, 38].

### Hormones

A prototypic hormone related to lung injury is angiotensin-II. This hormone increases tissue vascular permeability via production of eicosanoids and vascular endothelial growth factor, as well as by alteration of cytoskeletal proteins [39]. In addition, by enhancing inter-cellular adhesion and chemokine expression, angiotensin-II augments migration of inflammatory cells and promotes tissue repair [39].

Several effects point to a potential role in VALI. First, injurious ventilation upregulates the renin-angiotensin system and increases expression of angiotensin receptors [40]. Second, the genotype (i.e., DD) coding for more active angiotensin-converting enzyme (ACE) is more prevalent in critically ill patients who have ARDS (vs. those who do not); among those with ARDS, the DD genotype is associated with higher mortality [41]. Third, pre-treatment with the ACE inhibitor captopril—or the angiotensin-II receptor antagonist, losartan—attenuates VILI in vivo [40]. Finally, ACE-2 is a captopril insensitive ACE homolog that inactivates angiotensin-II; it protects against experimental lung injury [42]. Thus, circulating angiotensin-II represents a candidate mediator. However, research continues to better understand the complex renin-angiotensin system. For example, several angiotensin-II receptors have been described, and while one receptor type ($AT_1a$) promotes lung injury, another ($AT_2$) appears to protect [42].

### Lipid-derived mediators

Several lipid-derived mediators have been identified as candidate mediators. Eicosanoid levels correlate with lung injury in various animal models [43, 44], and they are potent modulators of vascular tone, permeability, inflammation and platelet activation. The interactions are complex however, and the net effects may be pro- or anti-inflammatory depending on the context. For example, inhibition of the proximal regulatory enzyme (cytosolic phospholipase $A_2$) attenuates capillary permeability induced by injurious ventilation [44], but inhibition of all downstream enzymes (i.e., cyclooxygenase, lipoxygenase and cytochrome P450) is required for effective protection; this suggests that many of the effects overlap [44].

Platelet activating factor (PAF) increases platelet activation, leukocyte attraction and microvascular permeability in part via synthesis of prostaglandins and ceramides [43]. While a pre-clinical study of PAF receptor blockade (i.e., lexipafant) was promising [45], clinical testing reduced neither the incidence [46] nor the severity [47] of lung injury.

Ceramides and other sphingolipids have recently been recognized as potentially important in VILI [48]; they appear to regulate apoptosis and maintain the integrity of intercellular junctions. Increased acid sphingomyelinase activity (resulting in elevated ceramide levels) occurs in the lungs of patients with ARDS [49], and in sepsis the circulating levels correlate with mortality [50, 51]. Finally, inhibition of acid sphingomyelinase stabilizes surfactant [52] and attenuates pulmonary inflammation in in vivo lung injury [48].

## Proof of pathogenicity

Proof matters in this setting. Without proof that lung-derived mediators are pathogenic, there is little basis for exploration of mediator-directed therapies. In addition, because the prospects of major further advances in bedside ventilator management (e.g. tidal volume or PEEP) seem modest, the search for alternative approaches—based on (proven) mechanisms—is imperative. In the nineteenth century, Robert Koch and Friedrich Loeffler described a framework to prove that microorganisms are responsible for diphtheria and tuberculosis [53]. Their concept has been applied in modern research to prove the link between a possible cause and a disease (e.g., heart failure [54], atherosclerosis [55]) and could readily be applied to the biotrauma hypothesis in VILI.

Table 1 outlines how the postulates could be adapted to provide a framework for proof of causation between circulating mediators and VILI, and relates landmark studies to individual postulates. Injurious ventilation causes release of lung-derived factors into the circulation

**Table 1** Koch's postulates for proving causation with infectious diseases applied to ventilator-induced lung injury

| Koch's postulate | Adaptation to VILI | Landmark studies | Study description |
|---|---|---|---|
| Microbes must be found in disease but not in health | Circulating factors increased by injurious ventilation, but not by non-injurious ventilation | [6, 56, 57] | Injurious mechanical ventilation increases circulating inflammatory mediators in humans and in experimental settings |
| Microbes isolatable from a diseased organism and grown in culture | Soluble candidate mediators are derived from the lung and can be identified in the circulation | [4–6, 56, 57] | A vast array of candidate mediators are described in the context of VILI and VALI with plausible causative link to lung injury |
| Cultured microbes cause disease when introduced to healthy organisms | (A) Perfusate from injured lungs injure healthy lungs during non-injurious ventilation | None | Construct for proof of concept developed in the current review |
| | (B) Exogenous mediators induce injury in healthy lungs | [20, 43] | Exogenous TNF-α, synthetic ceramide and PAF each caused lung injury |
| Same microbe isolated from the inoculated, diseased experimental host | Increased candidate mediators found in lung injury caused by the transfer of perfusate | None | Construct for proof of concept developed in the current review |

*VILI* ventilator-induced lung injury, *VALI* ventilator-associated lung injury, *TNF* tumor necrosis factor, *PAF* platelet activating factor

[2, 6, 56], and these lungs develop injury; the 'biotrauma' hypothesis links these observations and suggests that the link is causal. The isolated non-perfused lung model was instrumental in formulating the hypothesis [57], and by adapting Koch's postulates, the isolated perfused lung could prove it.

1. Injurious ventilation increases circulating factors (necessary 'positive' association).
2. Non-injurious ventilation does not increase circulating factors (necessary 'negative' association').
3. Perfusate from injured lungs causes injury during non-injurious ventilation, or worsens injury caused by injurious ventilation (necessary 'positive' association, proof of 'concept').
4. Candidate factors (i.e., mediators) can be isolated from perfusate and characterized (proof of candidacy, biological plausibility).

In order to prove that the soluble mediators are responsible, recirculating (vs. not recirculating) the perfusate in order to concentrate the mediators would result in more injury. In addition, preservation of the effect after filtration of the perfusate would ensure that the effects were not due to artifacts (e.g., coagulate, tissue debris).

The following steps would confirm the pathogenic role of candidate mediators:

1. Administration of purified or synthesized mediators would reproduce (or worsen) injury.
2. Antagonism of the mediator effect(s) would attenuate injury.

The experimental concept can be extended to demonstrate that lung-derived circulating factors induce distant organ dysfunction. Perfusate from ventilated lungs could be 'donated' to isolated perfused systemic organs (e.g., liver, kidney) or incubated with excised tissue or isolated cells from distal organs; in such situations perfusate from injuriously ventilated lungs would be expected to induce injury (e.g., apoptosis, necrosis, altered gene expression, increased permeability).

The lung perfusate could be blood-based or blood-free. Blood-free perfusate is a simpler biological system that virtually eliminates white blood cells as confounders of lung-derived mediators; this might be the preferred choice for establishing proof of concept. In contrast, blood-based perfusate might be preferred if investigating pathophysiologic applicability.

## Characterization of the responsible factor(s)

There are two basic approaches in searching for a responsible factor. In one case, there is no a priori knowledge (i.e., no suspicion) of the nature of the candidate mediator; this means that the mediator must be purified from the perfusate. In the other scenario, the identity of the factor is suspected (e.g., a 'known' cytokine), in which case the aim would be 'confirmation.'

### Factor identification

Characterization of the physicochemical characteristics is an important initial step. This involves determination of whether the factor is a protein, a lipid or a polysaccharide, as well as an estimate of its size. Protein inactivation, by treating the perfusate with proteolytic enzyme (e.g., trypsin) or by heat denaturation, would—if it reduced the biological activity of the perfusate—suggest that at least some of the activity resides in a protein. Similarly, reduction of the activity of the perfusate following lipid extraction [58], or transfer of the activity through the

extracted lipids, would imply that at least some of the activity resides in a lipid. The nature of what is being sought will determine the nature of the purification approach used.

Direct analysis of experimental perfusate could be supplemented by study of tissue, including protein or mRNA extraction, which would allow investigation of associated inflammatory pathways activated, signal transduction (e.g., phosphorylation), and changes in gene expression by microarray or RT-PCR methods. Such evidence could lend support to the role of individual candidate mediators.

To determine molecular size, fractionation through filters or columns and testing the residual activity of the filtered and retained fractions could narrow the search to molecules of a certain size range, thereby ruling out (but not positively identifying) candidate molecule classes. Estimates of molecular size are complicated by post-translational modification (e.g., dimerization, conjugation).

Novel high throughput approaches allow comparisons of perfusates (e.g., from injured vs. non-injured lungs), using proteomics [59] and, more recently, lipidomics [60]; these approaches permit identification of the overall spectrum of proteins or lipids in a complex biological mixture, using either two-dimensional gels (for protein mixtures) or high-performance liquid chromatography (HPLC, for lipid mixtures). Mass spectrometry can identify the isolated components. Here, the 'candidate mediators' are those compounds present (or more abundant) in the 'injurious' (vs. the non-injurious) perfusate.

The initial biochemical characterization allows both a narrowed search for specific mediators within a particular class and could reveal a requirement for multiple classes of mediators, for example, if both proteins and lipids were implicated, or if application of specific combinations of mediators has additive or synergistic effects. The notion that a single compound is responsible for lung injury in the context of VILI is not realistic, as injury likely results from a highly complex interplay among a vast array of mediators. Nevertheless, rigorous application of Koch's postulates could significantly advance the process of identifying key specific mediators and integrate evidence for the interplay of multiple compounds in the perfusate.

## Mediator confirmation

If a specific protein or lipid is suspected, its concentration can be measured provided a validated assay is available, noting that assays may be most available for murine models. In addition, blockade of a candidate mediator could confirm its role, such as with an antibody or a soluble receptor in the case of a protein, or inhibition of a key synthetic enzyme or use of an antagonist in the case of a lipid mediator.

## Which endpoints are appropriate?

The testing can be done by administration of the candidate mediator—or serum extract—in vivo, or into surrogate models of lung injury, such as isolated organ systems or cell cultures, allowing assessment of the effects on known mechanisms that are relevant to acute lung injury, such as permeability [61], cytotoxicity [4], apoptosis [62] or cell stretch [63]. Similarly, in vitro experiments could be used to measure the impact from lung-derived mediators on tissue or cells of other organs to investigate distal effects of the biotrauma hypothesis.

## Translation: developing therapies

Development of a mediator-directed therapy assumes that the candidate molecule is responsible for mediating the injury, at least to the extent that its antagonism will significantly attenuate the injury. Many studies have examined the inhibition of inflammatory pathways in critically ill patients, using global (e.g., corticosteroids) or specific (e.g., soluble receptors, antibodies) approaches, and lessons regarding the risk–benefit balance of such approaches have been learned [64, 65].

The two basic problems in translating from mediator identification to effective intervention are the specificity of the mediator and heterogeneity of the patient population. There must be an effective biological blockade available for the candidate mediator, and there must be certainty that with effective blockade, a sufficient proportion of the pathogenic pathways will be inhibited. The diagnosis of most critical illness states is based on epidemiological—not biological—criteria, posing an obvious hurdle for pathway-directed inhibitor therapies. Beyond the diagnostic criteria, the nature of most critical illness is extremely heterogeneous; indeed, lung injury is a perfect example of this. For example, among any cohort of patients with lung injury, there is usually a broad spectrum of etiology (e.g., sepsis, trauma, ischemia, immunologic rejection), clinical presentation, co-morbidity and concomitant therapy.

Many different therapeutic strategies could modulate the effect of circulating soluble compounds, including targeting the mediator itself (e.g., antibodies, binding proteins), its cell surface receptors (e.g., antagonists) or the intracellular signalling pathways (e.g., inhibition of key enzymes or transcription factors). It is important to note that while circulating factors may mediate the bulk of the injury burden, such mediators invariably act via multiple cell-specific signalling pathways; thus, targeting specific mediators—even if all were identified—would likely be an incomplete therapy because of the inherent limitations of chemical antagonism and the presence of multiple cellular receptors and intracellular pathways.

Limitations

The experimental approach we are suggesting has major limitations. For example, in small rodent models lung injury is induced over a few hours in previously healthy animals using ventilation parameters that not necessarily clinically applicable. These models (and the methods suggested in this review) might therefore study a phenomenon that differs substantially from that observed in larger animals or humans. In addition, the more significant the physiologic interactions, the more difficult it is to dissect individual mechanisms, making definitive in vivo proof challenging. Yet the simplified system of an isolated perfused organ and use of a mouse model, for which the widest variety of biochemical assays and genetic approaches are available, allow a systematic approach to 'proof of concept' of the biotrauma hypothesis. Although the evidence is critically limited to the specific system and cannot replicate the full complexity of human patients, this proof of concept would be invaluable to guide subsequent large animal experiments and clinical investigations.

## Conclusion

The idea that mechanical ventilation results in the release of circulating mediators, which cause or worsen lung injury (and perhaps systemic organ failure), is provable. The leap from observation to hypothesis has been made previously, and the approaches needed to prove the hypothesis exist and are outlined in the current review. Evolving molecular methodology will facilitate subsequent steps, including identification of candidate mediators. As further refinements in bedside ventilator management are associated with diminishing returns, the ultimate development and testing of mediator-directed therapy will assume greater urgency.

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