Leukotrienes in Acute Lung Injury A Potential Therapeutic Target?

A rapidly growing body of literature has begun to clarify the role of plasma membrane–derived mediators, including metabolites of arachidonic acid, sphingomyelin, lysophospholipid, ceramide, and platelet-activating factor (PAF), in the pathogenesis of acute lung injury. For example, PAF causes increased permeability pulmonary edema by inducing ceramide synthesis and cyclooxygenase activity (1). Endothelial barrier integrity is maintained in experimental ventilator-induced lung injury (VILI) by sphingosine-1 phosphate (2). Injurious ventilation increases lung phospholipase A2 activity, a major source of arachidonic acid release from the plasma membrane (3).

The 5-lipoxygenase (5-LO) metabolite leukotriene (LT) D4 increases alveolar epithelial sodium transport by increasing sodium potassium ATPase activity and membrane abundance of the transporter (4). Although the precise mechanisms by which these interrelated pathways affect the balance of forces responsible for edema formation in acute lung injury have not been entirely resolved, it is clear that these lipid mediators play a larger role in lung endothelial and alveolar epithelial barrier function than previously recognized. Twenty years ago, it was reported that patients with acute lung injury had markedly elevated levels of LTs in pulmonary edema fluid compared with control patients with hydrostatic pulmonary edema, although the role of the LTs in the pathogenesis of lung injury was not clear at that time (5).

In this issue of the *Journal*, Caironi and colleagues (pp. 334–343) provide new information on the role of 5-LO metabolites in acute lung injury resulting from sustained high V_T ventilation (6). These studies were designed to determine the contribution of impaired hypoxic pulmonary vasoconstriction (HPV) to hypoxemia resulting from VILI and to determine the wider role of LTs in the pathogenesis of VILI. Vasoconstriction is the normal pulmonary vascular response to hypoxemia and results in the redirection of blood flow to areas with greater ventilation. This intrinsic vascular response is in part mediated through LTs. Using mice that lack 5-LO, these investigators found that ventilation with high V_T for 6 hours resulted in an impaired vasoconstriction response via a 5-LO–dependent pathway. HPV was measured as the change in pulmonary vascular resistance in response to occlusion of the left mainstem bronchus. Interestingly, impaired HPV was detected despite normal arterial oxygen partial pressures before the hypoxic challenge. Normal pulmonary vasoconstriction was preserved by pretreatment with LT inhibitors and was restored by infusion of angiotensin II.

In separate studies, high V_T ventilation for up to 10 hours resulted in increased alveolar epithelial permeability, elevated bronchoalveolar lavage levels of cysteinyl LTs (LTC4, LTD4, LTE4), and an influx of neutrophils into the airspaces. Importantly, 5-LO–deficient mice had better preservation of alveolar

epithelial permeability, higher arterial oxygen tensions, and improved survival over the 10-hour study period. Pharmacologic inhibition of 5-LO activity produced similar effects in wild-type mice, whereas specific inhibition of the cysteinyl LT 1 (cLT1) receptor prevented the increase in permeability, but had less effect on airspace neutrophil infiltration. This apparent discrepancy is perhaps explained by the differential signaling of the cLT1 and cLT2 receptors because others have reported that the cLT2 receptor is sufficient for cLT-mediated interleukin-8 secretion in mast cells (7).

The magnitude of the protective effect of cLT inhibition in this model of VILI is perhaps surprising in light of findings by numerous other groups, implicating a variety of seemingly unrelated cytokines, chemokines, neutrophils, and other mediators in the pathogenesis of endothelial and epithelial injury in VILI (8–10). However, it may be that 5-LO metabolites represent a common pathway in the cascade of inflammatory events during the development of VILI. Similarly, as these authors have previously reported, the effect of cLTs on HPV and lung injury is not specific to VILI, and cLT signaling may be a contributor to impaired HPV and increased lung permeability from a variety of causes, including endotoxin (11).

Data from the present study indicate that the cLT-dependent impairment in HPV is an early event and develops before overt lung injury. It is tempting to speculate how this aberrant physiologic response may contribute to the forces governing pulmonary edema formation in VILI. However, it is notable that hypoxemia *per se* is a poor marker of lung injury severity in clinical acute lung injury and acute respiratory distress syndrome (ARDS). In the ARDS Clinical Trials Network study of low V_T ventilation, patients ventilated with lower V_T had a lower Pa_{O_2} than patients ventilated with conventional V_T , despite lower mortality in the low V_T group (12). Although infusion of angiotensin II restored HPV in the present VILI model, the authors did not report the effect of angiotensin II on lung injury severity. The specific effects of cLT inhibition on permeability and vascular tone may be context-specific and the cellular sources of cLTs in the VILI model remain to be investigated.

Other investigators recently reported that inhibition of cyclooxygenase 2 (COX-2) delayed the resolution of acute lung injury in a mouse model of acid-aspiration–induced acute lung injury (13). Inhibition of COX-2 prolonged airspace neutrophilia and the increase in alveolar epithelial permeability. Interpreted in the context of the present study, these data support a complex relationship among both pro- and antiinflammatory derivatives of arachidonic acid.

Nitric oxide (NO) may be an important contributor to pulmonary edema and neutrophil recruitment in acute lung injury and VILI. For example, the nonspecific NO synthase (NOS) inhibitor L-NAME prevented the increase in permeability associated with high V_T ventilation in isolated rabbit lungs (14). Presumably, inhibition of NO signaling would have some influence on HPV in VILI, although this has not been reported. Several of the authors of the present study recently reported that NO was required for the impaired HPV response in endotoxin-treated mice (15). In addition, inducible NOS (iNOS)–deficient mice

have fewer neutrophils sequestered in the pulmonary circulation, but more neutrophils in the airspaces than wild-type mice after cecal ligation and puncture (16). High V_T ventilation can induce iNOS expression and activity in the airspace, and reactive nitrogen species contribute to impaired alveolar epithelial fluid transport and pulmonary edema in VILI (17). The mechanisms of cLT-mediated lung permeability are uncertain, and how cLTs may influence NO-mediated alteration in barrier function and neutrophil trafficking remains to be investigated.

Caironi and colleagues (6) have further clarified our understanding of the pathogenesis of VILI. Early events, such as mechanical disruption of the basement membrane, disruption of the plasma membrane, or mechanically triggered chemical signaling, initiate an inflammatory program that culminates in neutrophil recruitment and activation that amplifies lung injury. The effects of lipid mediators, including arachidonic acid metabolites, in the pathogenesis of VILI appear to be greater than previously recognized and should stimulate renewed interest in 5-LO inhibitors for the treatment of acute lung injury.

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A Urinary Test for Pulmonary Arterial Hypertension?

Abnormality of nitric oxide (NO) production has long been a primary hypothesis for the pathophysiology of pulmonary arterial hypertension (PAH). Animal models of pulmonary hypertension, mice genetically deficient in NO synthases (NOS), and complementation studies with gene transfer of NOS provide abundant evidence for the concept that NO is a critical determinant of pulmonary vascular tone (1, 2). However, human studies measuring exhaled NO have provided conflicting results on whether or not NO in patients with PAH is less than in healthy control subjects. In this issue of the *Journal*, Girgis and colleagues (pp. 352–357) provide definitive evidence that patients with PAH have reduced NO in the lung and that successful therapy increases exhaled NO (3). In elegant studies based on measurement of exhaled NO at varying airflow rates and the two-compartment model of pulmonary NO exchange in the lung, they provide new information on how NO production is affected in select regions of the lung, and localize impaired NO production to the airway wall compartment. Similar to previously noted effects of prostacyclin therapy on exhaled NO (4), exhaled NO in patients with PAH increased after endothelin-A receptor

blockade with bosentan, therapy that enhances the release of NO and may also reduce oxidative consumption of NO (5, 6). In this context, one important limitation of exhaled NO studies is the inability to determine the mechanisms producing the observed effects, because reduced NO levels do not necessarily identify decrease in NO synthesis. NO levels reflect a steady state between synthesis and consumption of NO, and several studies have identified increased oxidant reactions in PAH lungs, which almost certainly lead to increased NO consumption (7, 8). Nevertheless, improvement in exhaled NO values in patients on therapy is clearly related to improvements in pulmonary vascular resistance (3, 4, 8).

An important distinction from previous studies is the complete evaluation in Girgis and colleagues' experiments of whole-body NO metabolism in patients with PAH. Exhaled NO, stable oxidation end-products of NO in serum and urine, endogenous inhibitors of NOS, and the NOS substrate arginine were determined at baseline before therapy, and 3 months after initiation of therapy. The NOS enzymes use the terminal guanidine nitrogen of L-arginine as the precursor for NO, which is oxidized to