

Plasma Membrane Stress Failure in Ventilator-Injured Lungs

A Hypothesis about Osmoregulation and the Pharmacologic Protection of the Lungs against Deformation Injury

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Key Words

Osmolar concentration · Cell membrane · Mechanical stress · Lung injury

Abstract

Cell injury and repair are invariable consequences of mechanical ventilation with large tidal volumes. Rate and amplitude of deforming stress affect numerous cell metabolic functions including host defense and wound repair. Recently, we have focused on the role of plasma membrane stress failure as a trigger for a pro-inflammatory response in mechanically ventilated lungs. We have developed both cell- and organ-based models to study this problem. Alveolar epithelial cells that are exposed to deforming stresses seek to maintain sublytic plasma membrane tension and may activate mechanisms of cell surface area regulation to control membrane tension. Interventions which either increase the amount of excess plasma membrane or enhance lipid trafficking should be cytoprotective against deformation induced injury. Osmotic manipulation may be one such intervention. Preconditioning the lungs with anisotonic solutions may allow the cells to recruit excess plasma membrane and thus be more resistant to ventilator-induced lung injury.

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Rate and amplitude of deforming stress affect numerous cell metabolic functions and consequently modulate transcellular as well as intercellular fluid transport, mechanisms of inflammation, host defense and wound repair [1–7]. In recent years, we have focused on the role of plasma membrane stress failure as a trigger of pro-inflammatory responses in mechanically ventilated lungs and have developed cell and organ based models to study this problem [8–12]. Specifically, we have demonstrated that cell injury and repair are invariable consequences of mechanical ventilation with large tidal volumes and that both may be manipulated by pharmacologic means [9].

Alveolar epithelial cells that are exposed to deforming stresses seek to maintain sublytic plasma membrane tension primarily by two mechanisms: (a) unfolding of excess plasma membrane, and (b) translocation of lipids from intracellular organelles to the plasma membrane. In effect, cells increase their surface area through active transport and thereby accommodate externally imposed changes in their surface to volume ratio. Since the lipid bilayer of the plasma membrane is relatively stiff and its lytic tension low [13], elastic distension of the membrane, that is an increase in the intermolecular distance of bilayer phospholipids, is not an effective means of cell surface area regulation. In light of the above considerations, interventions which either increase the amount of excess plasma membrane or which enhance deformation-induced lipid trafficking ought to be cytoprotective

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against ventilator-induced lung injury. As detailed below, exposing the lung to hyperosmolar solutions may have such an effect.

Osmotic stress induces a rapid cellular volume change in lower organisms [14]. This property is preserved in mammalian cells even when they live in a very tightly regulated iso-osmotic environment. Volume regulation after shrinkage and swelling is physiologically relevant since extracellular and particularly intracellular osmotic challenges may jeopardize critical cell functions. Even under iso-osmotic conditions with a relatively constant extracellular osmolality volume constancy is challenged by transport of osmotically active substances across the membrane and formation of intracellular products of metabolism that may alter the intracellular osmolality [15]. Numerous transporters and membrane channels are known to be involved in cell volume regulation; however, the sensing and signaling mechanisms by which cells regulate their volume is complex and still poorly understood. Extensive reviews have summarized the growing knowledge in this field of cell biology [14–17].

Even though plasma membrane tension and cell surface area regulation are sensitive to cell volume and shape, their interdependence is by no means straightforward. The tension hypothesis of surface area regulation proposes that when plasma membrane tension increases, the plasma membrane is added from endomembranes. Alternatively, when tension decreases, excess plasma membrane is retrieved through endocytosis [18]. In some systems such as molluscan neurons it is possible to osmotically manipulate cell volume independent of plasma membrane surface area [19]. If similar responses can be achieved in the lung without impairing deformation-induced lipid trafficking, then cells might tolerate larger surface to volume ratios (that is, higher tidal stretch) without developing lytic plasma membrane tensions.

Plasma membrane tension and surface area may be inferred from measurements of lipid tether mechanics. The use of optical (laser) tweezers to measure the elastic recoil of plasma membrane lipid tethers was pioneered by Sheetz and colleagues [20–22] and has laid the foundation for current views on the biophysical determinants of endocytosis and plasma membrane remodeling [23]. Lipid tethers are formed by attaching 1- μm beads to plasma membrane proteins that do not readily associate with the cytoskeleton. Figure 1 shows how tether force-length tracings may be interpreted as readout of excess plasma membrane, that is the plasma membrane reservoir. Once the tether is formed, the force required to lengthen it remains nearly constant as long as excess plasma membrane pro-

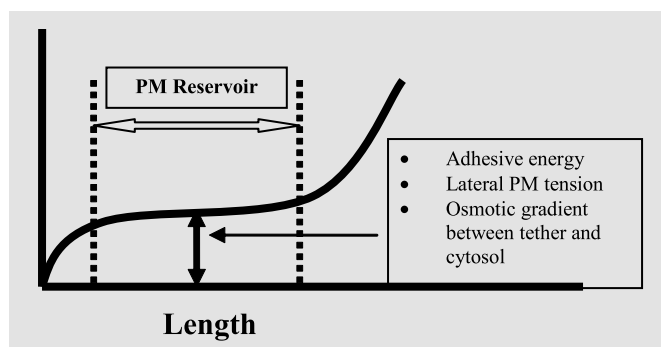


Fig. 1. Determinants of lipid tether force/length relationship. PM = Plasma membrane. Adapted from Raucher et al. [24].

vides a source of lipid for the elongating tether. To the extent to which tether elongation also promotes deformation-induced lipid trafficking, both rate and amplitude of applied force become additional determinants of the size of the apparent plasma membrane reservoir. This technique is therefore ideally suited to test cell volume and surface area responses of lung cells to osmotic challenges.

The hypothesis that anisomotic conditioning of the lungs protects lung cells from physical injury is also being tested in whole organ systems. We have developed and validated an isolated perfused rat lung model of ventilator-induced lung injury which focuses on plasma membrane injury and repair as surrogate endpoints [9]. Validation studies fashioned after experiments originally conducted by Slutsky's group [25] produced the expected physiologic and histological injury responses. The novel aspect of this model is the use of confocal microscopy to assess plasma membrane integrity and repair in subpleural cells. Mechanically ventilated lungs are perfused with the membrane impermeable label propidium iodide (Molecular Probe, Eugene, Oreg., USA). When propidium iodide enters a cell through a membrane defect, it intercalates with DNA and emits a red fluorescence upon excitation with blue light. We have conducted a series of validation experiments and have shown that propidium iodide at the concentrations used does not enter cells with intact membranes in sufficient amounts to label DNA or RNA. This is true even after stimulation with phorbol ester which triggers fluid phase endocytosis.

In summary, plasma membrane excess and cellular lipid trafficking are important determinants of deformation-related increases in plasma membrane tension. Given the high incidence of plasma membrane stress failure in ventilator-injured lungs, both determinants deserve a better definition in lung cells including the alveolar epithelium.

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