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## Inactivity-induced diaphragm dysfunction and mitochondria-targeted antioxidants: New concepts in critical care medicine

Sanford Levine, MD,

Murat T. Budak, MD, PhD,

Jamil Dierov, MD, PhD, ScD,

Sunil Singhal, MD

University of Pennsylvania and Gift of Life Donor Program Philadelphia, PA

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Over the past decade, the Powers lab has contributed a remarkable series of publications (1-4) indicating that controlled mechanical ventilation (MV) in the rat—according to a standardized protocol carried out for 12–18 hrs—predictably elicited diaphragm inactivity that was accompanied by the triad of oxidative stress, myofiber atrophy, and contractile dysfunction. The clinical relevance of these studies became apparent in 2008, when we (5) reported that 18–72 hrs of MV and human diaphragm inactivity—*in brain dead organ donors*—elicited some evidence of oxidative stress and dramatic (i.e., 50%) atrophy of both slow and fast diaphragm myofibers. Furthermore, we noted increased activity of caspase-3, a cytoplasmic serine protease, and increased expression of messenger ribonucleic acid coding for atrogen-1 and MuRF-1, key components of the ubiquitin–proteasome proteolytic pathway (UPPP), and we suggested that increased proteolysis played a major role in producing the myofiber atrophy.

Additionally, on the basis of the data in our article, we calculated that maximum transdiaphragmatic pressure of these diaphragms would be dramatically decreased. We (6) later demonstrated that the concentration of myosin heavy chain proteins (the molecular motor of contraction) was decreased to <50% of control concentrations, and this provides one possible mechanism for the postulated contractile dysfunction. More recently, Jaber et al (7) used cervical magnetic phrenic nerve stimulation to directly demonstrate progressive decreases in diaphragm peak twitch tension (assessed by airway occlusion pressures) in patients on prolonged MV; additionally, their biopsy data indicated myofiber atrophy, increased UPPP activity, and increased protein expression of the calpains (i.e., calpains I, II, and III), another family of serine cytoplasmic proteases. We presume that these increases in calpain expression represented an increase in calpain activity; this inductive leap is important since the UPPP cannot degrade intact actinomyosin complexes that are attached

to the myofiber lattice. Indeed, recent work by Whidden et al (4) and others (8) indicates that increased activity of both the calpains and caspase-3 in conjunction with oxidative stress cleaves structural proteins such as titin (9) and thereby effects the release of actin, myosin, and other myofibrillar proteins from the lattice for further degradation by the UPPP.

Other recent work by Hussain and colleagues (10) demonstrated that the diaphragms of brain dead organ donors—exposed to prolonged MV and inactivity—also exhibited autophagy, and this mechanism can account for the degradation of cytoplasmic organelles (e.g., mitochondria) that occur during the myofiber atrophy process. Equally important, Hussain et al (10) were the first to directly demonstrate that, in human diaphragms exposed to prolonged MV and inactivity, increased protein carbonylation and increased 4-hydroxynonenal–protein adducts are manifestations of oxidative stress. In summary, both human and rat studies indicate that the combination of prolonged MV and diaphragm inactivity elicits a pathologic triad characterized by oxidant stress, myofiber atrophy, and contractile dysfunction.

How do we prevent this syndrome from developing in patients on prolonged MV? Prior work from the Powers lab demonstrated that antioxidant therapy with Trolox (an analogue of vitamin E that is not approved for clinical use) attenuated the oxidant stress, myofiber atrophy, and contractile dysfunction that predictably occur in their rat MV model (2, 4, 11). After exploring several possible oxidant generating pathways, these workers postulated that mitochondria were the major source of oxidants in the MV rat model, and the experiments of Kavazis et al (12) demonstrated that diaphragm mitochondria from their MV rats—in comparison to control rats—generate increased reactive oxidative species (ROS) during both states 3 and 4 respiration. The seminal paper by Powers et al (13) in this issue of *Critical Care Medicine* suggests that the systemic administration of mitochondria-targeted antioxidants may represent future preventive treatment for patient diaphragms exposed to prolonged MV and inactivity. Specifically, these workers administered the “mitochondria-targeted antioxidant”—Szeto-Schiller peptide 31—to one group of rats that were undergoing MV according to their standard protocol; they noted that, despite presumed inactivity, these rat diaphragms did not exhibit the expected increase in mitochondrial generation of ROS, as well as any myofiber atrophy, or contractile dysfunction. In contrast, control rats that were pretreated with saline—and underwent the same MV protocol—exhibited increased mitochondrial ROS production, myofiber atrophy, and contractile dysfunction. Therefore, we believe that the data in the paper by Powers et al strongly support the concept that mitochondria are the major source of ROS in diaphragm inactivity and this increase in diaphragm ROS appears to be necessary for development of the pathologic triad of oxidative stress, myofiber atrophy, and contractile dysfunction.

Conceptually, we view the type of oxidative stress—manifest in the diaphragms of both the MV rat model and human studies in the literature—as an increase in the generation of ROS that is initiated in close proximity to the inner mitochondrial membrane (where the respiratory complexes are located); this increase in ROS then spreads throughout the mitochondria and diffuses to all areas of the myofiber, producing oxidant damage to proteins, lipids, and deoxyribonucleic acid. This model suggests that the increase in ROS will be greatest in the inner mitochondrial membrane, resulting in progressive

damage to the respiratory complexes that in turn will cause progressive increases in ROS production. A major problem in treating this pathology is that the only currently Food and Drug Administration approved antioxidant (i.e., N-acetylcysteine) is thought to lack the ability to effect adequate concentrations in the mitochondria. Therefore, the theoretical advantage of this new class of mitochondria-targeted antioxidants is that, following systemic administration, the ratios between mitochondrial matrix concentrations and those in plasma range from 500:1 to 1000:1 (14, 15). (We recognize that a recent paper in this journal by Agten et al (16) casts some doubt on this concept; however, a detailed discussion of this paper is beyond the scope of this editorial.) The members of this new class of drugs are Szeto-Schiller peptide 31 (15), which has completed phase I studies, and the extensively studied drug mito-Q (14), which has completed phase I as well as some phase II studies.

In 2004, in a prophetic perspective article, Vassikopoulos and Petrof (17) introduced the term “ventilator-induced diaphragm dysfunction” to describe the pathologic changes (noted above) in human and experimental animal diaphragms exposed to prolonged MV. On the basis of this concept, they suggested that partial ventilator support modes would be less likely to be associated with this syndrome. Indeed, this has now become accepted clinical practice in patients who are suitable for this ventilator modality. However, at the present time, we believe that the term “inactivity-induced diaphragm dysfunction” may be a more useful concept because regardless of the settings on the ventilator, early observations by Powers et al (3) indicate that the diaphragms of MV rats were inactive (as assessed by electromyographic observation) and the human diaphragms of brain dead organ donors reported by our group (5, 6) as well as Hussain et al (10) were also inactive. Furthermore, the two signaling pathways (i.e., IGF1-PI3K-AKT, NF- $\kappa$ B) that have been demonstrated to play a role in eliciting the increase in UPPP activity in these diaphragms are known to be activated by disuse (6, 7).

In summary, we hope that the inactivity-induced diaphragm dysfunction concept will stimulate human studies on therapeutic modalities that either eliminate prolonged diaphragm inactivity (i.e., periodic electrical stimulation for short periods of time [18]) or drugs that will prevent the diaphragm mitochondrial pathology associated with inactivity (i.e., mitochondria-targeted antioxidant drugs).

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