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Effect of Ventilator-induced Lung Injury on Skeletal Muscle Oxidative Balance

IN this issue of ANESTHESIOLOGY, Marin-Corral *et al.*[1] report a reduction of several markers of oxidative and nitroxidative stress in the diaphragm and limb muscles of rats exposed to ventilator-induced lung injury (VILI). This finding initially seems counterintuitive because VILI is thought to induce a systemic inflammatory state,[2] which should lead to increased, rather than decreased, oxidative stress also in tissues other than the lung. In this editorial, we will briefly review the basic biochemistry of the oxidative markers measured by Marin-Corral *et al.* to provide some context to these observations, highlight the main results of this study, and define a framework for future investigations of the skeletal muscle effects associated with VILI.

Oxidative Markers

Reactive oxygen species (ROS) are produced physiologically during cellular respiration. Although 95% of oxygen is reduced to H_2O and CO_2 during oxidative phosphorylation, 5% is reduced to superoxide anion radical ($O_2^{\cdot-}$) by capturing a single electron from the mitochondrial transport chain (fig. 1). $O_2^{\cdot-}$ is reduced to hydrogen peroxide (H_2O_2) both chemically and by enzymes such as superoxide dismutase (SOD), and H_2O_2 is converted to hydroxyl radicals ($^{\cdot}OH$). ROS radicals, and in particular $^{\cdot}OH$, oxidize proteins, lipids, and nucleic acids, altering the structure and function of these entities.

ROS production increases significantly above physiologic levels during inflammatory states. In their study, Marin-Corral *et al.* measured two end products of ROS-mediated oxidation: protein carbonyls[3,4] and malondialdehyde (MDA)-protein adducts.[5] Protein carbonyls are formed by several mechanisms including oxidation of primary (serine) or secondary (treonine) alcohol amino acid residues. MDA, instead, is a product of peroxidation of polyunsaturated fatty acids by ROS. It is highly reactive and binds covalently to proteins by alkylating several amino acid residues. Studies *in vitro* have shown that the levels of MDA correlate with those of MDA-protein adducts.[5]

Reactive nitrogen species (RNS) include nitric oxide ($^{\cdot}NO$) and its oxidation products with ROS. RNS can lead to both nitrosation (R-N=O) and nitration (R-NO_2) of amino acid residues (R). Tyrosine nitration has been recognized as a major posttranslational protein modification in cardiovascular[6] and respiratory[7] diseases and is often used as a biomarker of "nitroxidative stress."[8] The two main pathways for tyrosine nitration are as follows[6,8] (fig. 1): (1) reaction of $^{\cdot}NO$ with $O_2^{\cdot-}$ to generate peroxynitrite anion ($ONOO^-$), a highly oxidizing and nitrating compound that reacts with CO_2 to yield nitrogen dioxide ($NO_2^{\cdot}$) and carbonate radical ($CO_3^{\cdot-}$), which oxidizes tyrosine to tyrosyl radical. Tyrosyl radical is then nitrated by $NO_2^{\cdot}$ to yield 3-nitrotyrosine; (2) reaction of nitrite (NO_2^-, generated by oxidation of $^{\cdot}NO$ with molecular oxygen) with hemeperoxidases (*e.g.*, myeloperoxidase) and H_2O_2 to yield tyrosyl radical and $NO_2^{\cdot}$. As in the first pathway, $NO_2^{\cdot}$ then adds to the tyrosyl radical to generate 3-nitrotyrosine. This second pathway seems to be the main venue for tyrosine nitration *in vivo*, especially in heme-rich tissues such as skeletal muscle.[9]

ROS and RNS pathways are intimately interwoven as $O_2^{\cdot-}$ and H_2O_2 play a crucial role in the protein oxidation that forms the basis for tyrosine nitration.

Protection from ROS

The two main mechanisms of protection from ROS are SOD and catalase. SOD converts $O_2^{\cdot-}$ to H_2O_2, and catalase converts H_2O_2 to water. These metalloproteins act as antioxidants. In this study,[1] skeletal muscle and lung levels of catalase and the mitochondrial form of SOD (Mn-SOD) were measured to determine the level of protection from ROS.

VILI and Oxidative Stress

Because VILI has been postulated to initiate and propagate a systemic inflammatory response,[2] and ROS and RNS are critically important mediators of inflammatory states, one might expect that oxidative stress is increased during VILI. In the investigation by Marin-Corral *et al.*, inflammation occurred in the lungs of rats exposed to

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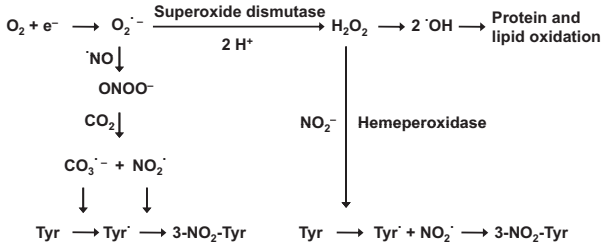

Fig. 1. Schematic representation of reactive oxygen and nitrogen species pathways leading to oxidation of organic substrates and tyrosine (Tyr) nitration.

VILI, because inflammatory cell infiltration and MDA-protein adducts increased in their lungs compared with nonmechanically ventilated controls. Moreover, protection from ROS seemed to decrease as Mn-SOD and catalase levels were lower than in the lungs of controls. However, instead of seeing evidence of increased oxidative stress in skeletal muscle, protein carbonyls, MDA-protein adducts, and protein tyrosine nitration decreased in skeletal muscle.

How can we explain these observations? One possibility is that, because of its hemodynamic effect, VILI impairs perfusion to peripheral tissues, including skeletal muscle, making them ischemic and limiting the amount of ROS that can form, akin to the ischemic phase of ischemia–reperfusion injury where the bulk of ROS is formed during the reperfusion rather than the ischemic phase. However, markers of oxidative stress as well as nitrotyrosine have been shown to increase within 40 min of renal ischemia even without reperfusion.[10] Furthermore, as Marin-Corral *et al.* point out, protein oxidation and nitration were also reduced in the moderate tidal volume group, which did not experience the hypotension and acidosis of the group receiving the higher tidal volume. Consequently, hypoperfusion is unlikely to be the sole explanation for these findings.

A more intriguing explanation is that the reduction in protein tyrosine nitration reflects an increase, rather than a decrease, in oxidative stress. As Bian *et al.*[9] elegantly showed, the relationship between H_2O_2 concentration and tyrosine nitration (and, to a lesser extent, also carbonyl formation) is biphasic: nitration increases steeply up to 0.5 mM H_2O_2 but decreases for higher concentrations of H_2O_2. This is probably because excess H_2O_2 causes suicide inactivation of peroxidases and degradation of heme in metalloproteins such as myoglobin.[9] Consequently, tyrosine nitration through the second pathway is expected to decrease at high concentrations of H_2O_2. Inactivation of peroxidase by excess H_2O_2 could also account for the apparently contradictory finding of decreased protein nitroxidation despite increased leukocyte infiltration in skeletal muscle, which would be expected to lead to increased tyrosine nitration by activation of the pathway by myeloperoxidase. In fact, all these biochemical assays measure reaction by-products of ROS and RNS, which may not necessarily correlate with the level of the reactive species themselves (see fig. 2B of Reference 9).

Finally, it is possible that oxidative and nitroxidative stress are simply not part of the early VILI-induced molecular changes in skeletal muscle, as the authors suggest.

Clinical Implications

What clinical inferences, if any, can we draw from this experiment? We know that loss of aeration in Acute Respiratory Distress Syndrome (ARDS) is highly heterogeneous[11] and the distribution of tidal volume uneven, such that very high regional stress and strain can develop in the ARDS lung even with clinically acceptable tidal volumes.[12] The value of studying the effect of these high levels of strain in normal lungs is to isolate the contribution of VILI to these biochemical phenomena while eliminating the confounding effect of other sources of lung injury. In this respect, studies in normal lungs provide "proof-of-concept" and are just as valuable as experiments in models of ARDS in which a second insult is imposed on the lung. It is quite possible that levels of strain comparable with those imposed on the whole lung in this study develop on a regional basis in ARDS. Thus, the results of this study suggest that VILI-induced skeletal muscle oxidative imbalance could contribute to muscle weakness and potentially to critical-illness myopathy in ARDS. If the observed decrease in protein oxidation was a marker of decreased ROS production, a reduction in muscle contractility could be expected because certain levels of ROS are required for optimal contractility.[13] If instead the decrease in protein oxidation and nitration was "paradoxically" a marker of increased ROS production, a reduction in contractility might be expected as a result of the inflammatory process associated with excessive ROS.

Future studies should clarify the relationship between levels of the primary noxious stimuli (*e.g.*, ROS or RNS), their reaction by-products (*e.g.*, protein carbonyls or nitrotyrosine), and the ensuing functional impairment (*e.g.*, reduced muscle contractility). How to interpret these associations and whether they have pathogenetic significance, however, require a focused assessment of the causal relation between the biomarker and the functional or structural abnormality. The big question that remains is whether the measured oxidative and nitroxidative protein changes induced by VILI affect skeletal muscle function and how this effect occurs.

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Guido Musch, M.D., Jeanine P. Wiener-Kronish, M.D., Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts. guido.musch@gmail.com

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ANESTHESIOLOGY REFLECTIONS

The Lungmotor for Adults

In 1930 cartoonist and screenwriter Reuben L. "Rube" Goldberg (1883–1970) and his colleagues needed a resuscitating apparatus for a rescue scene in *Soup to Nuts*, the film debut of a trio now known as "The Three Stooges." The apparatus chosen for the comedy was America's adult version of Germany's Draeger Pulmotor—the "Lungmotor" manufactured by the Life Saving Devices Company. No laughing matter, one Lungmotor helped revive a mother and then her son from carbon monoxide poisoning just 35 miles from what is today's American Society of Anesthesiologists headquarters. Careful inspection of the example above (*courtesy of the Wood Library-Museum*) reveals the initial wording of the upside-down metal-punched hallmark of "THE LUNG MOTOR" on the apparatus' base. (Copyright © the American Society of Anesthesiologists, Inc. This image appears in color in the *Anesthesiology Reflections* online collection available at www.anesthesiology.org.)

George S. Bause, M.D., M.P.H., Honorary Curator, ASA's Wood Library-Museum of Anesthesiology, Park Ridge, Illinois, and Clinical Associate Professor, Case Western Reserve University, Cleveland, Ohio. UJYC@aol.com.