Minireview

Ventilator-induced diaphragm dysfunction in critical illness

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Impact statement

Mechanical ventilation (MV) is life-saving for patients with acute respiratory failure but also causes difficult liberation of patients from ventilator due to rapid decrease of diaphragm muscle endurance and strength, which is termed ventilator-induced diaphragmatic damage (VIDD). Numerous studies have revealed that VIDD could increase extubation failure, ICU stay, ICU mortality, and healthcare expenditures. However, the mechanisms of VIDD, potentially involving a multistep process including muscle atrophy, oxidative loads, structural damage, and muscle fiber remodeling, are not fully elucidated. Further research is necessary to unravel mechanistic framework for understanding the molecular mechanisms underlying VIDD, especially mitochondrial dysfunction and increased mitochondrial oxidative stress, and develop better MV strategies, rehabilitative programs, and pharmacologic agents to translate this knowledge into clinical benefits.

Abstract

Mechanical ventilation is an essential intervention for intensive care unit patients with acute lung injury. However, the use of controlled mechanical ventilation in both animal and human models causes ventilator-induced diaphragm dysfunction, wherein a substantial reduction in diaphragmatic force-generating capacity occurs, along with structural injury and atrophy of diaphragm muscle fibers. Although diaphragm dysfunction, noted in most mechanically ventilated patients, is correlated with poor clinical outcome, the specific pathophysiology underlying ventilator-induced diaphragm dysfunction requires further elucidation. Numerous factors may underlie this condition in humans as well as animals, such as increased oxidative stress, calcium-activated calpain and caspase-3, the ubiquitin–proteasome system, autophagy–lysosomal pathway, and proapoptotic proteins. All these alter protein synthesis and degradation, thus resulting in muscle atrophy and impaired contractility and compromising oxidative phosphorylation and upregulating glycolysis associated with impaired mitochondrial function. Furthermore, infection combined with mechanical stretch may induce multisystem organ failure and render the diaphragm more sensitive to ventilator-induced diaphragm dysfunction. Herein, several major cellular mechanisms associated with autophagy, apoptosis, and mitochondrial biogenesis—including toll-like receptor 4, nuclear factor-κB, Src, class O of forkhead box, signal transducer and activator of transcription 3, and Janus kinase—are reviewed. In addition, we discuss the potential therapeutic strategies used to ameliorate ventilator-induced diaphragm dysfunction and thus prevent delay in the management of patients under prolonged duration of mechanical ventilation.

Keywords: Acute lung injury, mitochondria, nuclear factor-κB, endotoxemia, toll-like receptor 4, ventilator-induced diaphragm dysfunction

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Clinical prevalence of ventilator-induced diaphragm dysfunction

Although mechanical ventilation (MV) is life saving for patients with acute lung injury (ALI), it causes weaning failures in approximately 20% of patients due to rapid deterioration of diaphragm muscle endurance and strength; this condition is called ventilator-induced diaphragm dysfunction (VIDD).[1–4] In most of the intensive care unit (ICU) patients (80%), diaphragmatic dysfunction can occur on admission or during subsequent stay.[4–6] Accumulating clinical evidence has revealed that VIDD aggravates ventilator-associated pneumonia, extubation failure, in-hospital mortality, ventilator dependence, and health costs.[4–6] VIDD has a pathophysiology similar to ventilator-induced lung injury (VILI), which is characterized by diffuse

inflammation and increased oxidative stress, ultimately leading to impaired gas exchange.[7] However, the mechanism underlying VIDD—potentially involving a multistep process including oxidative stress, muscle weakness (arising from caspase-3, calpain, ubiquitin–proteasome system (UPS) activation, and autophagy-lysosomal pathway (ALP)), structural damage, and myofiber remodeling[8–10]—requires further elucidation. Therefore, a detailed knowledge of the molecular mechanisms underlying VIDD is crucial for designing potential strategies and reducing prolonged MV use, ICU stay, and thus ICU mortality.

Pathophysiology of VIDD

Diaphragmatic atrophy

A study on VIDD in humans demonstrated a substantial reduction (~53%–57%) in the diameters of both slow-twitch and fast-twitch muscle fibers in 14 adults who met the brain-dead criteria for 18–69 h.[11] Notably, in these patients, the pectoralis muscle was unaffected during the same period of dysfunction, suggesting that the rapid weakness was related to the diaphragm. Recent studies on ICU patients revealed that diaphragm thickness measured by ultrasound is associated with a lower daily probability of successful weaning, prolonged ICU stay, and high risk of ventilator-associated complications.[4,6,12] In previous studies, the clinical impact of diaphragm atrophy was demonstrated, revealing that MV caused rapid onset of sarcomeric disarray, disuse atrophy, and impaired contractility in the diaphragm.[11–14] Studies have indicated that diaphragm dysfunction causes diaphragm atrophy, whereas excess inspiratory efforts aggravate VILI and damage the diaphragm.[13–15]

Contractile dysfunction

Diaphragm strength is crucial in weaning patients from MV and transferring them to long-term care facilities; it also determines ICU mortality.[16,17] A recent study indicated that during their ICU stay, approximately 80% of mechanically ventilated ICU patients demonstrated various patterns of ICU-acquired diaphragm weakness after the initial use of MV.[4] In addition, diaphragm inactivity is found two times as frequent as limb inactivity in critically ill patients.[16] Multimodal evaluation of the diaphragm (magnetic stimulation of the phrenic nerve, ultrasound measurement of the diaphragm excursion and thickening fraction, and maximal inspiratory pressure) in ICU patients revealed that diaphragm dysfunction is often in patients with ICU-acquired inactivity and is associated with a higher rate of weaning failure and ICU mortality.[6]

Oxidative stress

MV-induced oxidative stress in the diaphragm may impair diaphragm contractility and is a crucial signaling event leading to proteolytic pathway activation.[13,18–21] During ALI, reactive oxygen species (ROS) are the primary oxidants in the diaphragm; they occur in mitochondria, sarcolemma, sarcoplasmic reticula, transverse tubes, and cytosol within 6 h of MV.[10,18,19,22] Oxidative loads inactivate skeletal muscles through the interaction of different oxidant production pathways: (1) superoxide radical generation in mitochondria, (2) generation of hydroxyl radicals because of elevated cellular reactive iron levels, (3) NO production by nitric oxide synthase (NOS), and (4) ROS generation by xanthine oxidase.[23,24] ROS disassembles proteins from the muscle fibers by activating calcium-activated proteases, such as caspase-3 and calpain, and degrades muscle proteins through the UPS.[23,24] Elevation in intracellular calcium is a prerequisite for calpain activation. Oxidative stress can augment calpain cleavage of Z line-related proteins, such as titin and nebulin in diaphragmatic myofibers by dampening the activity of plasma membrane Ca^{2+}-ATPase.[25,26] These modifications may desensitize muscle fiber to calcium and increase intracellular calcium accumulation. Caspase-3, a cysteine protease upregulated by oxidative loads, can increase calpain activity and upregulate proapoptotic proteins to elicit intrinsic apoptosis correlated with mitochondrial abnormalities.[27,28] Furthermore, the UPS deteriorates monomeric muscle fibers, which are liberated from actomyosin complexes after caspase-3 and calpain breakdown.[29] However, there are contradictory findings regarding the mechanisms of developing diaphragm dysfunction in a clinical study of ventilated critically ill patients undergoing surgery.[30] van den Berg *et al.*[30] found that diaphragm muscle biopsies from these patients exhibited substantial atrophy and reduced contractility triggered by a redox imbalance, but lacked upregulated oxidative markers and impaired mitochondrial biogenetics. The authors explained the inconsistencies between their results and those from ventilated animals and brain-dead organ donors were attributed to different clinical features. Further clinical investigations are warranted to clarify the causative role of oxidative stress involved in the pathogenesis of VIDD.

Changes in proteolytic protein synthesis

The major proteases in the skeletal muscle include (1) lysosomal enzymes, (2) calcium-related proteases, and (3) the UPS.[3,31] The lysosomal pathway is specific for the degradation of cytosolic proteins and organelles, including mitochondria and peroxisomes, whereas the last two are responsible for myofilament protein degradation.[31] The connection of ubiquitin to protein substrates necessitates E1 enzymes, E2 carrier protein, and (in many cases) specific E3 enzymes.[9,32] The 26S proteasome complex, consisting of a core 20S proteasome combined with a pair of 19S regulators, is activated after ubiquitin binding to protein substrates and labels them for degeneration. However, if the protein substrate is monoubiquitinated or diubiquitinated, then it is degraded through internalization and lysosomal transport. The activation of muscle-specific ubiquitin E3 ligases F-box protein atrogin-1 and muscle RING-finger proteins-1 (MuRF-1) is pivotal for the degradation of monomeric myofibrillar proteins in the diaphragms of animals and patients with MV; both proteins are modulated by transcription factor nuclear factor (NF)-κB or class O of forkhead box (FoxO).[33] Moreover, according to a murine

study, MuRF-1 plays a crucial role in regulating ALI-associated muscle atrophy.[34]

Intracellular signaling pathways

Relationships among VIDD, sepsis, autophagy, and apoptosis

Animal studies have indicated that infection is a primary cause of impaired diaphragm dysfunction.[18,35,36] Sepsis was demonstrated to be a crucial risk factor for diaphragm dysfunction in ICU patients.[37] A study on diaphragm contractility in mechanically ventilated ICU patients indicated that the union of infection and ventilator-mediated diaphragm weakness might induce sufficient diaphragm abnormalities to adversely affect patient outcomes, including higher mortality and longer weaning periods.[17] Sepsis-enhanced diaphragmatic weakness and VIDD seem to have several molecular mechanisms, including elevated oxidative loads and mitochondrial dysregulation (mitochondrial biogenesis inhibition and increased mitochondrial permeability) within the diaphragm myofibrils, indicating that sepsis may be an accessory contributor for VIDD.[17,18,38–40] Moreover, sepsis and MV-mediated oxidative stress may increase generation of inflammatory cytokines, including high mobility group box (HMGB) 1, interleukin (IL)-6, macrophage inflammatory protein (MIP)-2, and tumor necrosis factor (TNF)-α.[18,20,27,41–43] These inflammatory mediators can suppress diaphragmatic contractility and exacerbate sepsis-induced systemic translocation via mechanisms including reduced protein synthesis, atrogin-1 and MuRF-1 induction, and signal transducer and activator of transcription (STAT) 3-myostatin pathway activation.[41,44]

Autophagy is a catabolic process marked by the expulsion of intracellular components, such as mitochondria, in the muscle fibers, and it is designated to regulate cell proliferation and death (or survival), innate and adaptive immune responses, and mitochondrial turnover.[26,45,46] Mitochondria are a major source of diaphragmatic free radicals, a vital upstream mediator that starts the signaling pathways leading to diaphragm muscle atrophy during endotoxemia or mechanical stretch.[47,48] The activity of electron transport chain isoform complexes II, III, and IV was reduced in mitochondria separated from the diaphragms of rats with 12 h of MV.[49] Autophagy may prevent or promote the progress of pulmonary diseases by exerting its diverse functions. Although basal autophagy is crucial for regulating cell survival, uncontrolled autophagy enhances abnormalities, such as intrinsic apoptosis, muscle weakness, and mitochondrial morphological damage in the diaphragm of patients with sepsis.[32,46] Animal investigations on VIDD have shown that MV augmented diaphragmatic weakness through excessive ROS generation by enhancing proteolysis and microtubule-related protein light chain (LC) 3.[47,50,51] LC3-I becomes autophagosome-bound LC3-II by conjugating to phosphatidylethanolamine and upregulation of LC3-II expression is a biomarker of increased autophagosome formation.[45,47] LC3-II accumulation in the diaphragm after MV could be majorly because of pathological abnormalities of autophagosome breakdown, rather than activation of the ALP.[52] In particular, recent studies have demonstrated that increased autophagy is boosted by oxidative stress, resulting in selective degradation of the endogenous antioxidant catalase by eliminating peroxisomes and mitochondria, thus further increasing both ROS generation and autophagy.[50,51]

MV-induced elevation in mitochondrial ROS level is associated with oxidation of lipid and protein, inducing breakdown of mitochondrial structures.[19,28,53] Liberation of cytochrome *c* from mitochondria to cytosol subsequently mediates apoptotic cell death.[28,53] Sepsis may affect mitochondria by (1) generating free radicals and reactive nitrogen species, thus increasing lipid peroxidation and protein oxidation within mitochondria; (2) impairing perfusion of mitochondria, leading to tissue hypoxia, and triggering the cell death pathway; and (3) altering hormones and downregulating genes transcribing mitochondrial proteins.[54] Moreover, myonuclear apoptosis can be induced by (1) mitochondrial ROS and elevated cellular calcium levels, (2) Fas ligand- and TNF-α receptor-mediated pathways, and (3) sarcoplasmic (endoplasmic) reticulum (SR) stress-induced activation of caspase-3 and calpain.[21,28,47] Mitochondrial biogenesis is modulated principally at the transcriptional level and needs coordinated expression of both mitochondrial- and nuclear-encoded proteins, involving mitochondrial transcription factor A, nuclear respiratory factors 1 and 2, peroxisome proliferator-activated receptor coactivator (PGC)-1α, and 5′-adenosine monophosphate-activated protein kinase.[19,26,54,55] The downregulation of oxidative phosphorylation but upregulation of glycolysis, reduction in mitochondrial membrane potential, cytochrome *c* leak into the cytosol, and constitutive opening of mitochondrial pores have all been associated with apoptosis pathways.[54,55]

The NF-κB signaling pathway, a primary transcription factor for inflammatory cytokines, may increase diaphragm atrophy through transcriptional regulation of the manifestations of atrogin-1 and MuRF-1.[13,21,27] In a murine endotoxemia study, transgenic (muscle-specific inhibitor IκBα super-repressor) mice induced with endotoxemia demonstrated that skeletal muscle fiber-specific inhibition of canonical NF-κB signaling prevents lipopolysaccharide (LPS)-induced diaphragmatic injury.[27] In addition, NF-κB is pivotal in modulation of autophagy and the apoptotic pathway correlated with mitochondrial abnormalities in the diaphragm.[21,28,47] Toll-like receptor (TLR)4 is the most thoroughly investigated receptors of the TLR family and is important for the recognition of damage-associated molecular patterns, involving HMGB1, extracellular matrix components, and LPS.[43,56,57] Recent murine studies on inflammatory myopathies and Duchenne muscular dystrophy in the diaphragm have shown that HMGB1 could be an early inducer of skeletal muscle dysfunction by activating the TLR4 signaling pathway.[57–59] Studies have revealed that stimulation of TLR4 pathway by LPS enhances the expression of cytokines, such as TNF-α, MIP-2, and IL-6 in skeletal muscles.[37,44] Increases in free intracellular calcium levels in the calcium-dependent calpain and caspase-3 system have been identified in animal models of endotoxemia to

amplify proinflammatory cytokines associated with diaphragm contractility.[29,60]

Murine studies on endotoxemia have exhibited that TLR4 regulates diaphragm inflammation and autophagy by activating the p38 mitogen-activated protein kinase (MAPK) or NF-κB pathways.[57,59,61] In a myogenic cell line and murine study of endotoxemia, TLR4 activation augmented autophagosome generation in a p38 MAPK-dependent pathway.[61] Sepsis-induced systemic inflammation may sensitize diaphragm stretch-related injuries by increasing sarcolemma membrane fragility. It may also increase disturbances in different steps of the muscular energy supply chain, including hypoxic ischemia and cytopathic ischemia, and it may directly impair contractile proteins through inflammatory cytokines.[6,16,18] In a murine study of sepsis, TLR4 homozygous knockout could inhibit the ALP and attenuate mitochondrial ultrastructural changes by suppressing TLR4/NF-κB signaling.[62]

Relationships among VIDD, hyperoxia, and mitochondrial dysfunction

The management of ALI often requires the support of MV with high levels of oxygen to maintain adequate oxygenation of the brain and other vital organs. However, concurrent MV and hyperoxia may interact to worsen ALI and result in generation of inflammatory cytokines, including MIP-2, TNF-α, and plasminogen activator inhibitor (PAI)-1.[63,64] Src, a critical nonreceptor protein tyrosine kinase serving in intracellular signal transduction, mediates leukocytes influx and acute inflammatory reactions triggered by oxidative stress.[33,65] Mechanical stretch of C2C12 myoblasts upregulates p38 MAPK activity by activating Src-induced TNF-α-converting enzyme.[66] In a murine model of VILI, Src plays a principal role in the activation of ROS formation and lung inflammation.[67] Moreover, in a murine Duchenne muscular dystrophy model, persistent Src activation, which enhanced autophagy through phosphoinositide 3-OH kinase/serine/threonine kinase/protein kinase B (Akt) phosphorylation, was noted.[68]

In skeletal muscle in mdx mice, Src and Rac1 were shown to play important roles in eliciting ROS production via NADPH oxidase 2.[68,69] The increased ROS in skeletal muscle unloading may activate the NF-κB and FoxO signaling pathways.[13,70] FoxO1 is a mammalian FoxO transcription factor responsible for the regulation of cellular proliferation, apoptosis, and cell-cycle arrest.[26,71–73] FoxOs activate atrophic proteins (i.e. atrogin-1 and MuRF-1) and autophagy-related proteins (B cell lymphoma (Bcl)-2 19-kilodalton interacting protein 3, cathepsin L, and LC3).[20,26,50,51] In the unstimulated state, FoxO is phosphorylated by Akt, which inhibits FoxO transcriptional activity.[22,73] However, MV suppresses FoxO1 phosphorylation mediated by Akt and translocates FoxO1 to the nucleus to trigger autophagy-related gene transcription in animal and human diaphragms.[33,61] Although the activation of atrogin-1 and MuRF-1 is substantially upregulated by MV with hyperoxia, their expression is reduced by suppression of Src-dependent FoxO1 signaling.[33] Moreover, FoxOs augment apoptotic signaling by upregulating the activity of Fas ligand and stimulating the members of the Bcl-2 family (e.g. the apoptosis facilitator gene, Bcl2-interacting mediator (Bim), which controls mitochondrial membrane permeability), as evidenced by loss of cytochrome c release and membrane potential in an isolated mitochondrial study.[26,28,72,74] In particular, accumulated lipid level in human diaphragm during MV implicates accelerated glycolysis, which generate fatty acids converted from intermediate substances but suppresses fatty acid breakdown due to impaired mitochondrial function.[19] In addition, p62, a biomarker of protein turnover, binds with polyubiquitinated proteins and LC3, functioning as a cargo receptor for the autolysosome degradation process.[45–47] The role of mitochondrial dynamics and biogenesis in ALI is complex. In some studies, reduced mitochondrial membrane potential, mitochondrial fragmentation, and DNA damage are demonstrated in the diaphragms of both animals and patients subjected to MV.[24,44,47–49,55] However, no alteration of mitochondrial bioenergetics and morphology is reported in a recent study of ICU patients.[30] Further clinical trials are required to delineate this discrepancies.

Other signaling pathways

1. A recent work suggested that infection elicits cytokine production, resulting in cell-surface neutral sphingomyelinase receptors upregulation. The muscle ceramide metabolism is altered by the activation of these receptors, increasing mitochondrial ROS formation and triggering the generation of oxidative stress.[60]
2. Murine studies on endotoxemia have revealed that myostatin may induce muscle atrophy mediated by Smad3, atrogin-1, and FoxO3 signaling, and blocking the Akt/mammalian target of the rapamycin (mTOR) pathway. Furthermore, the muscle fibrosis-related PAI-1 and the mitochondrial biogenesis-related PGC-1α were inhibited by Smad3 activtion.[55,75–77]
3. Intracellular calcium overload in the diaphragm can trigger proinflammatory cytokine production and proteolysis in sepsis. Diaphragm contractility was recovered after curbing the release of HMGB1 by calcium antagonists in the septic diaphragm of mice.[29]
4. A murine model of cecal ligation puncture-induced sepsis demonstrated that skeletal muscle calcium-dependent phospholipase (cPL) A2 is upregulated by cytokines and connected with mitochondrial superoxide formation, and that cPLA2-induced ROS production induces calpain activation in skeletal muscle fibers.[60]
5. STAT3 and Janus kinase (JAK), constituting a signaling cascade activated by hormones, growth factors, and inflammatory cytokines via ligand–receptor interaction, can be rapidly phosphorylated or upregulated in a ventilated, inactive diaphragm.[44] STAT3, an upstream inducer of mitochondria-derived ROS generation in the nucleus, can activate the expression of proteins, including Bim, uncoupling protein, and Cox5A, which decrease efficiency of ATP formation and mitochondrial membrane potential. Suppressing

the JAK/STAT pathway substantially ameliorates oxidative loads, diaphragm inactivity, and proteolysis in rats.[26,44,78,79]

6. In both human and murine models of VIDD, MV promptly modulates the ryanodine receptor on the SR membrane through oxidation, S-nitrosylation, and Ser-2844 phosphorylation, causing the instability in the receptor complex and leading to calcium leakage.[25] The dysregulated calcium homeostasis can result in impaired muscle contractility and reduction of muscle mass through activation of caspase-3, calpain, and oxidative stress,.[26,35] Persistent elevated level of cytosolic calcium can also upregulate MAPKs, protein kinase C, and histone deacetylase (HDAC) 4. Calcium-dependent upregulation of protein phosphatases, including calcineurin, can also occur.[80]

Potential therapeutic strategies

Prolonged MV use can increase the risk of ventilator-associated pneumonia and lung fibrosis with restrictive ventilatory impairment; this not only causes physical and mental suffering but also affects quality of life and increases financial burden on patients and their families.[40,81] Therefore, identifying effective clinical parameters and molecular mechanisms to facilitate liberating patients from long-term ventilator use is crucial.

Clinical care

Treating electrolyte imbalances and endocrine disorders, including hypoalbuminemia, hypophosphatemia, hypocalcemia, hypomagnesemia, hyperglycemia, severe untreated renal failure, and hypothyroidism, as well as avoiding neuromuscular blocking reagent overuse and sustained corticosteroid administration is of primary importance.[39,40] Several animal and clinical studies have suggested that adjusting sedation and ventilation mode to keep appropriate levels of inspiratory muscle effort and reduce patient-ventilator asynchrony may minimize diaphragm atrophy.[12,82] Furthermore, recent investigations have demonstrated that phrenic nerve stimulation may serve in increasing diaphragmatic activity during MV.[15,83] A randomized clinical trial (ClinicalTrials.gov identifier: NCT03096639) is investigating the use of a temporary diaphragm pacing therapy system to facilitate liberating ventilated patients who have failed at least two weaning attempts.[83]

Antioxidants

In addition to diminishing oxidative loads, antioxidants may modulate the expression of proteolysis-related genes; for example, administration of high-dose vitamin E to animals alleviates the expression of several proteases, such as caspase-3 and calpain.[24,84] Mounting evidence demonstrates that the use of antioxidants, such as N-acetylcysteine and trolox can ameliorate the detrimental effects on respiratory muscle function induced by controlled or prolonged MV.[45,85,86] Treatment of animals with SS-31, a mitochondria-targeting antioxidant selectively functioned on the inner mitochondrial membrane, prevented rat diaphragms from prolonged MV-induced muscle atrophy by countering oxidative stress and protease activation.[24,82]

Theophylline

The molecular mechanisms of theophylline include (1) dilating airway smooth muscles by inhibiting phosphodiesterase-3 activity and antagonizing adenosine A1 and A2 receptors; (2) functioning as an anti-inflammatory agent by augmenting the effect of IL-10 and blocking the translocation of proinflammatory transcription factor NF-κB; and (3) enhancing HDAC2 activity (which is reduced by oxidative stress) to decrease peroxynitrite radical generation.[84] In a rodent study, theophylline alleviated diaphragm atrophy and recovered the decrease of transdiaphragmatic pressure resulting from resistive loaded breathing in newborns.[87] It also facilitated diaphragmatic perfusion by improving cardiac output and providing vasodilation in diaphragmatic arterioles.[88] A retrospective cohort study disclosed that low-dose theophylline substantially improved contractility in the diaphragm without significant adverse drug reactions in patients admitted to medical ICU with VIDD.[89]

Other pharmacological agents

1. The calcium sensitizer levosimendan, a positive inotropic agent, has been applied to chronic obstructive pulmonary disease patients. It strengthens the muscle contractility of the diaphragm by increasing the calcium sensitivity of the contractile proteins.[90,91] The agent may exert and energetically promote diaphragm contractility and mean that less calcium is required to maintain force generation.[90,91] Furthermore, levosimendan can augment contractile function of the diaphragm in healthy humans performing inspiratory loading tasks.[92] Further research using levosimendan to regain respiratory muscle function in mechanically ventilated patients is currently underway (ClinicalTrials.gov identifier NCT01727434).
2. The mTOR pathway is essential for the regulation of adipogenesis and muscle protein synthesis.[93,94] Controlled MV increases lipid accumulation and deteriorates diaphragm contractility. These detrimental effects are partially blocked by the mTOR inhibitor rapamycin.[95]

Novel therapy

Considerable research on the use of stem cell therapy for ALI treatment is underway.[64,96] Induced pluripotent stem cells (iPSCs) derived from human fibroblasts by delivering four reprogramming factors can differentiate into patient-specific progenitor cells and tissues for any of the three germ layers and facilitate personalized therapy in future clinical application.[97] Li *et al*.[33] demonstrated that hyperoxia-augmented VIDD can be attenuated by iPSC therapy through suppressing the Src-FoxO1 signaling pathway. The authors conducted a preclinical investigation by using iPSCs and iPSC-conditioned media to study the

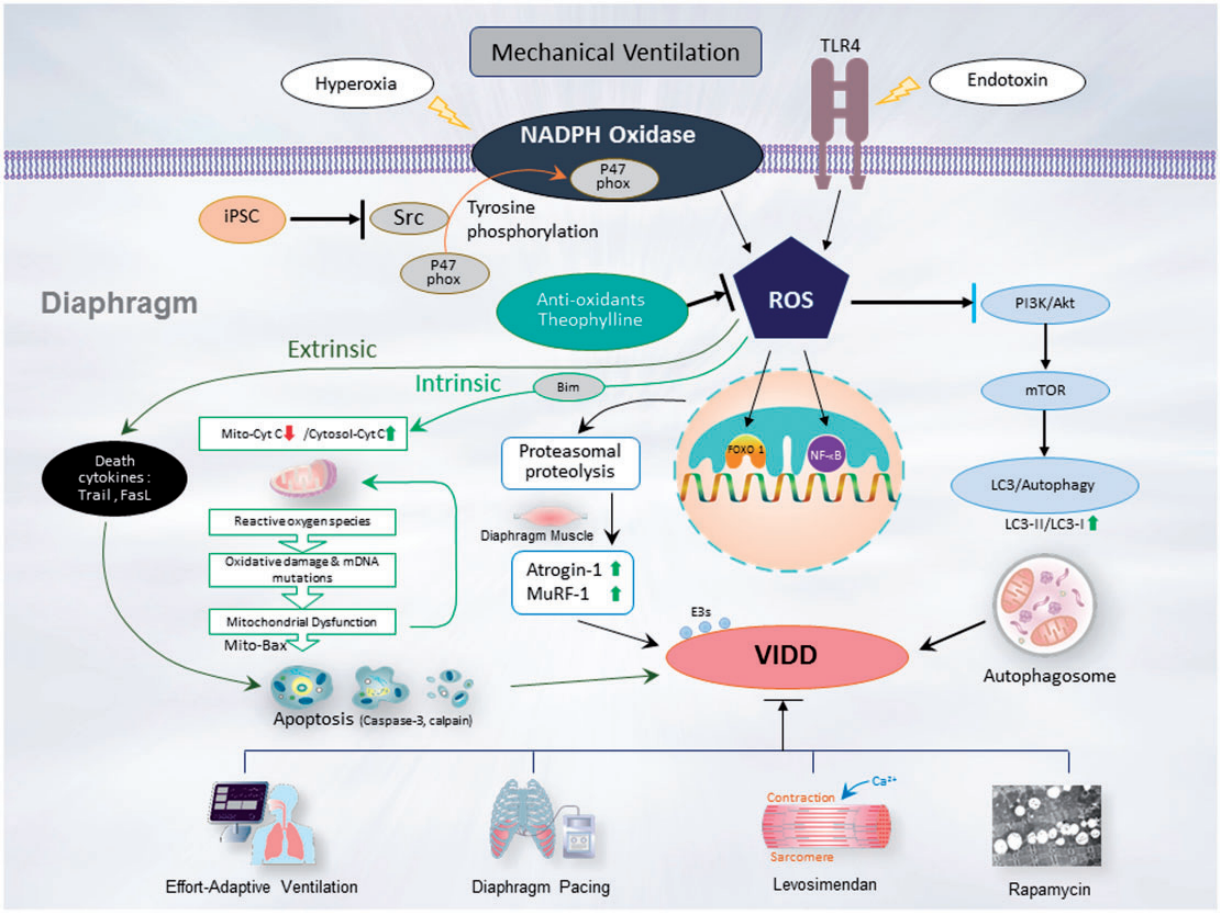

Figure 1. Schematic of the signaling pathway implicated in VIDD development. Endotoxin- or hyperoxia-induced augmentation of mechanical stretch-mediated ROS generation and diaphragm injury are associated with diaphragm proteolysis, apoptosis, mitochondrial dysfunction, autophagy, as well as activation of the caspase-3, calpain, and ubiquitin–proteasome pathways. Diaphragm weakness can be attenuated by administering iPSCs, antioxidants, theophylline, levosimendan, or rapamycin, or through partial support MV or diaphragm pacing through PI3K/Akt, Src, and TLR4 pathway inhibition. Akt: serine/threonine kinase/protein kinase B; Bax: Bcl2-associated X; Bim: Bcl2-interacting mediator; Bnip3: Bcl-2 nineteen-kilodalton interacting protein 3; FoxO1: Class O of forkhead box1; iPSCs: Induced pluripotent stem cells; LC3: light chain 3; mTOR: mammalian target of rapamycin; MuRF-1: muscle ring finger-1; NADPH: nicotinamine adenine dinucleotide phosphate; NF-κB: nuclear factor kappa B; PI3-K: phosphoinositide 3-OH kinase; ROS: reactive oxygen species; TLR4: toll-like receptor 4; VIDD: ventilator-induced diaphragm dysfunction. (A color version of this figure is available in the online journal.)

mechanisms and beneficial effects of stem cell therapy on combinatorial MV and hyperoxia-induced oxidative stress, proteolysis, apoptosis, autophagy, and functional impairment simulating the clinical scenario. Our results indicate that stem cell therapy may provide a novel therapeutic option for VIDD.[33]

Conclusion

Without adequate preventive and therapeutic strategies, a resting and inactive diaphragm muscle after prolonged MV may experience fast morphological and functional alterations, including accelerated protein degradation, muscle atrophy, and impaired contractile force. Numerous pathogenic mechanisms underlying the destructive effects of MV on diaphragmatic structure and contractility have been demonstrated in animal and human models of VIDD. However, these studies, performed in healthy animals, did not consider the effects of risk factors, such as sepsis and multisystem organ failure, in ICU patients. Because of the presence of multiple confounding factors, reaching a definitive diagnosis of VIDD in ICU patients is not easy; nevertheless, physicians should be careful of the occurrence of VIDD when a ventilated patient demonstrates poor progress during weaning trials in despite of clinical improvements in underlying diseases.[39,40,98,99] Furthermore, the effort adaptive ventilation should be used as soon as possible to attenuate the deleterious effects of MV on the diaphragm. Further research on the mechanistic framework of this condition is required to understand the molecular mechanisms underlying VIDD (Figure 1), particularly mitochondrial dysfunction and increased mitochondrial ROS emission, and for developing further improved MV strategies, rehabilitative programs, and pharmacological agents to translate this knowledge into clinical benefits.

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All authors have read the journal's policy on disclosure of potential conflicts of interest and declared that no competing interests exist.

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