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Purpose of review

Diaphragmatic function is a major determinant of the ability to successfully wean patients from mechanical ventilation. There is increasing recognition of a condition termed ventilator-induced diaphragmatic dysfunction. The purpose of the present review is to present evidence that mechanical ventilation can itself be a cause of diaphragmatic dysfunction, to outline our current understanding of the cellular mechanisms responsible for this phenomenon, and to discuss the implications of recent research for future therapeutic strategies.

Recent findings

Many critically ill patients demonstrate diaphragmatic weakness. A large body of evidence from animal models, and more limited data from humans, indicates that mechanical ventilation can cause muscle fiber injury and atrophy within the diaphragm. Current data support a complex underlying pathophysiology involving oxidative stress and the activation of several intracellular proteolytic pathways involved in degradation of the contractile apparatus. This includes the calpain, caspase, and ubiquitin–proteasome systems. In addition, there is a simultaneous downregulation of protein synthesis pathways. Studies in animal models suggest that future therapies may be able to specifically target these processes, whereas for the time being current preventive measures in humans are primarily based upon allowing persistent diaphragmatic activation during mechanical ventilation.

Summary

Diaphragmatic dysfunction is common in mechanically ventilated patients and is a likely cause of weaning failure. Recently, there has been a great expansion in our knowledge of how mechanical ventilation can adversely affect diaphragmatic structure and function. Future studies need to better define the evolution and mechanistic basis for ventilator-induced diaphragmatic dysfunction in humans, in order to allow the development of mechanical ventilation strategies and pharmacologic agents that will decrease the incidence of ventilator-induced diaphragmatic dysfunction.

Keywords

diaphragmatic fatigue, diaphragmatic function, mechanical ventilation, respiratory muscles, weaning failure

Introduction

Mechanical ventilation is a two-edged sword. Although it is life-saving in patients with respiratory failure, mechanical ventilation is also associated with numerous potential complications. For example, although many patients in the ICU are placed on mechanical ventilation as supportive therapy for acute lung injury, there is substantial evidence that the institution of mechanical ventilation itself can paradoxically create and sustain damage to the lungs [1]. The recognition of this phenomenon, known as ventilator-induced lung injury (VILI), eventually led clinicians to adopt ventilator strategies designed to mitigate its occurrence, and this practice has now been linked to improved clinical outcomes in patients with acute respiratory distress syndrome [2,3]. In the past several years, a similar paradigm has been emerging with regard to potential adverse effects of mechanical ventilation on the ventilatory muscles. This entity was originally termed ventilator-induced diaphragmatic dysfunction (VIDD) by Vassilakopoulos and Petrof [4], although it may well involve other respiratory muscles as well [5,6].

Diaphragmatic weakness appears to be very common in patients undergoing mechanical ventilation [7–9]. This is of major importance, as diaphragmatic function plays a crucial role in determining the ability of patients to be successfully weaned from the ventilator [10,11]. Furthermore, difficulties in weaning patients from mechanical

ventilation account for a large proportion of time spent in the ICU [12]. Taken together, these facts suggest that VIDD has the potential to have a major impact on clinical practice and the utilization of healthcare resources. In the present review, we will briefly summarize the existing evidence for VIDD in animal and human studies. We will then review the most recent information about the cellular mechanisms responsible for VIDD, and discuss the possible implications of these findings for future therapeutic strategies.

Brief review of evidence for ventilator-induced diaphragmatic dysfunction

In experimental animals, the ability of the intact diaphragm to generate pressure is reduced by 40–50% within a few days of instituting 'controlled' mechanical ventilation, which permits little or no spontaneous diaphragmatic activity [13–15]. Endurance of the diaphragm also appears to be adversely affected [6], with a reduced ability to sustain diaphragmatic force in the face of an inspiratory resistive load [13]. It should be pointed out that for the purposes of the present review, VIDD refers to changes in diaphragmatic function that arise from alterations outside of the central or peripheral nervous systems. Hence, in animal models of VIDD, nervous impulse transmission at the levels of the phrenic nerve and the neuromuscular junction remain normal [15], and the contractility of isolated (i.e., removed from their neural input) diaphragmatic strips is severely reduced along a similar time course and magnitude to that observed in the intact animal [14,16–19]. Taken together, the above findings indicate that the deleterious effects of mechanical ventilation upon diaphragmatic function are primarily the result of changes that occur within the muscle fibers *per se*. This is consistent with the fact that in the majority of patients with weaning difficulties, the level of neural input to the diaphragm is actually increased, but force generation remains decreased nonetheless [20••]. It is also clear that the loss of diaphragmatic force-generating capacity cannot be ascribed to atrophy alone, as many studies have shown that the force loss is persistent even after correcting for any reductions in muscle cross-sectional area [4,14,16–19].

Beyond decreased diaphragmatic strength, a number of histological and biochemical changes have been described in the diaphragms of animals with VIDD. These include the muscle fiber atrophy [5,13,16,17,19,21], which appears to be the result of decreased protein synthesis [22,23•] as well as increased protein breakdown [21,24–27]; muscle fiber remodeling, as indicated by changes in the expression of multiple structural [28] and muscle-specific proteins such as myosin heavy chain, MyoD, and myogenin [17,29]; and signs of muscle fiber injury, including disrupted myofibrils, increased numbers of vacuolar structures, and abnormal mitochondria [5,14,24,30].

In human individuals, there is more limited but nonetheless compelling evidence for the development of VIDD. In a postmortem analysis of neonates, diffuse diaphragmatic muscle fiber atrophy was found in patients who received ventilatory assistance for 12 days or more immediately before death, whereas such changes were not present in extradiaphragmatic muscles from the same patients or diaphragms of infants ventilated for 7 days or less [31]. More recently, in a landmark study, Levine *et al.* [32••] evaluated diaphragm biopsy specimens from adult brain-dead organ donors who had undergone mechanical ventilation for variable periods of time (18–69 h) prior to organ harvest, and compared them with specimens obtained from control patients who were undergoing thoracic surgery for benign lesions or localized lung cancer (mechanical ventilation for 2–3 h). In comparison with the controls, biopsy specimens from the organ donor group showed a decreased cross-sectional area of slow-twitch and fast-twitch fibers (atrophy), decreased glutathione levels (suggesting increased oxidative stress), and greater expression of active caspase-3 and the E3 ubiquitin ligases atrogin-1, and muscle RING-finger protein-1 (MuRF-1; implicated in muscle proteolysis). Several of these results have recently been confirmed by another similarly designed study, which additionally reported rapidly progressive contractile dysfunction and diaphragmatic injury in mechanically ventilated humans [33]. Taken together, the above findings are remarkably similar to those that have been documented in animal models of VIDD.

It should be noted that mechanical ventilation constitutes a rather unique form of muscle 'disuse', in the sense that the diaphragm is at the same time mechanically unloaded, electrically quiescent, and subjected to changes in myofiber length by cyclical lung inflation or positive end-expiratory pressure (PEEP). The diaphragm itself is also unique. It is normally exposed to a negative pressure environment along its pleural surface that can potentially serve as a stretch-like hypertrophic stimulus [34], which is removed by the application of positive-pressure ventilation. In addition, the diaphragm is more active than most other skeletal muscles (30–40% of the time, 24 h/day). All of these factors may help to explain the very rapid diaphragmatic atrophy and force loss observed during mechanical ventilation. It has been estimated that 12 h of mechanical ventilation in rats is approximately equivalent to 96 h of locomotor muscle unloading in terms of the muscle wasting responses that are induced [35].

Major cellular mechanisms of ventilator-induced diaphragmatic dysfunction

Our current understanding of the cellular mechanisms causing VIDD is derived primarily from animal models. Numerous studies have shown that mechanical

ventilation is associated with an increase in markers of oxidative stress in the diaphragm [21,32••,36–38]. The onset of oxidative modifications is rapid, occurring within 6 h of instituting mechanical ventilation in rats [36]. Expression levels of antioxidant enzymes or free radical scavengers such as glutathione, superoxide dismutase, catalase, heme oxygenase-1, and others are variably decreased [28,37,38] or increased [28,39•], with the latter presumably being an attempt to limit oxidant-mediated injury. Interestingly, the cellular targets of diaphragmatic protein oxidation may involve elements of the contractile machinery such as myosin and actin [36]. Moreover, treatment with an antioxidant (the vitamin E analogue Trolox; Hoffman-La Roche, Nutley, New Jersey, USA) during mechanical ventilation mitigated diaphragmatic proteolysis and also prevented the loss of diaphragmatic force [40]. This same antioxidant also attenuated diaphragmatic fiber atrophy in a manner that was independent of alterations in insulin-like growth factor-1 (IGF1)-phosphoinositide-3 kinase (PI3K)-Akt signaling or ubiquitin ligase expression [41], despite the association of these pathways with diaphragmatic atrophy [19,25]. Collectively, the findings strongly suggest that mechanical ventilation-induced oxidative stress plays a major role in atrophy as well as the intrinsic contractile impairment of the diaphragm found in VIDD.

Recent studies have begun to search for the main sources of the reactive oxygen species (ROS) generated in the diaphragm during mechanical ventilation. There is no evidence for increased diaphragmatic inflammatory cell infiltration [32••,42] or a major upregulation of proinflammatory cytokine gene expression in the diaphragm [28]. Nitric oxide synthases also do not appear to be involved [43]. NADPH oxidase, which can generate superoxide within muscle fibers [44], was only found to be minimally increased in the mechanically ventilated diaphragm [45]. Xanthine oxidase, another potential source of superoxide, is also upregulated in the diaphragm during mechanical ventilation, and its inhibition modestly improved diaphragmatic contractility but failed to have an impact upon atrophy [46]. Given the above, mitochondria could be the main source of excessive ROS production in the diaphragm during mechanical ventilation. In this regard, mitochondria isolated from mechanically ventilated rats release significantly more ROS and also exhibit biochemical evidence of oxidative damage [39•]. Furthermore, abnormalities of diaphragmatic mitochondrial respiration have been found in several animal species during mechanical ventilation [5,39•,47]. Interestingly, these findings are also consistent with morphological evidence of damaged mitochondria in the diaphragm after mechanical ventilation [5].

There is good evidence that the calpain, caspase, and ubiquitin–proteasome proteolysis pathways all play significant roles in the development of mechanical ventilation-induced atrophy. Calpains are calcium-dependent proteases that have long been known to be capable of degrading cytoskeletal proteins in muscle [48], whereas recognition of the involvement of caspases in muscle atrophy is more recent [49]. Myofilament proteins, which constitute approximately two-thirds of bulk muscle protein, must first be partially cleaved and disassembled in order to be processed and degraded by the ubiquitin–proteasome system [50]. This initial step of proteolytic release of myofilaments from their native state can be accomplished by either calpains or caspases, both of which are upregulated in the diaphragm during mechanical ventilation [21,26,27]. Interestingly, the calpain and caspase systems demonstrate considerable crosstalk and can be mutually reinforcing [51]. The role of lysosomal proteases is less clear, but cathepsin activity is also increased [27]. Intriguingly, vacuolar structures in the mechanically ventilated diaphragm [5,30] also suggest the possibility of autophagy, another lysosomally mediated process recently shown to be involved in skeletal muscle atrophy associated with increased oxidative stress [52•].

Several studies have shown that myofiber atrophy and impaired contractility in the mechanically ventilated diaphragm can be uncoupled from one another [14,46,53]. For example, administration of oxypurinol (xanthine oxidase inhibitor) improved diaphragmatic contractility but not atrophy [46]. Conversely, allowing intermittent trials of spontaneous breathing between periods of mechanical ventilation was able to prevent mechanical ventilation-induced atrophy, but reductions in diaphragmatic contractility were persistent [53]. These observations suggest that the mechanisms responsible for atrophy and intrinsic contractile dysfunction are not identical, although they are likely to be linked. For instance, one possibility is that the disassembly of actomyosin complexes by calpains or caspases, which is a prerequisite for myofiber atrophy via the proteasome, also plays a critical role in causing intrinsic contractile impairment of the diaphragm. Moreover, calpains and caspases are activated by ROS and also have the ability to mediate activation of the proapoptotic protein Bid [54–57]. This could potentially lead to mitochondrial damage and drive further ROS generation, thereby sustaining a vicious cycle. The histological evidence of mitochondrial damage and particularly the presence of myofibrillar disarray, which has been reported in several studies and significantly correlated with abnormal contractile function of the diaphragm [5,14,24,30], are consistent with the above hypothesis.

Potential therapeutic strategies and future directions

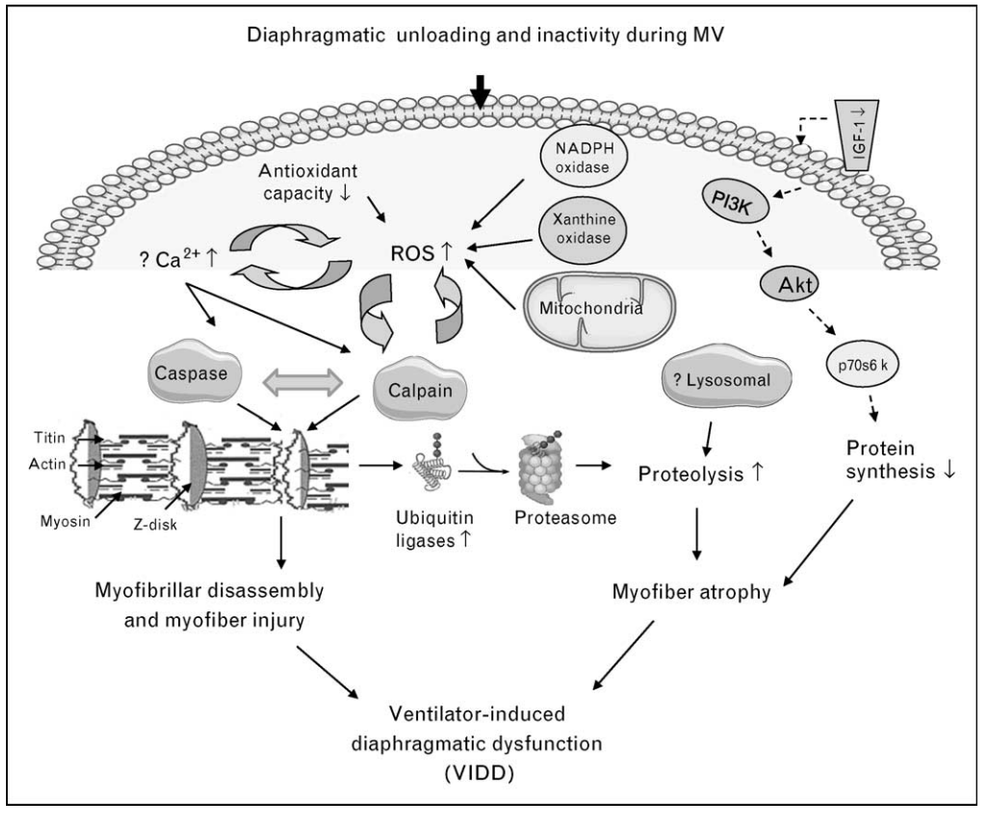
Animal studies indicate that VIDD is alleviated (but not completely prevented) when using partial support modes

of mechanical ventilation, in which a significant degree of diaphragmatic effort is permitted [53,58,59•]. From a purely practical standpoint, it appears logical to permit as much diaphragmatic activity as possible, as long as this allows for adequate patient comfort and gas exchange. However, further study is needed to ascertain the optimal level of diaphragmatic effort and to determine whether the specific method of promoting diaphragmatic effort during mechanical ventilation (e.g., spontaneous breathing trials, assist-control, pressure-support, newer modes such as NAVA, etc.) has any impact upon the risk of developing VIDD. In addition, it is important to recognize that even when using partial support modes of mechanical ventilation or allowing for intermittent periods of spontaneous breathing, studies have found evidence of persistent oxidative stress [59•] as well as a substantial residual deficit of diaphragmatic force production, even in the absence of atrophy [53,58]. Taken together, the above findings suggest that other measures designed to target the specific cellular pathways involved in muscle injury, may be required in order to completely prevent or reverse VIDD.

With regard to pharmacological interventions, treatment with the vitamin E analogue Trolox during mechanical ventilation prevents the loss of diaphragmatic contractility and attenuates atrophy in rats over the short-term [40]. It is also interesting to note that in a study of critically ill surgical patients, an antioxidant supplement containing vitamins E and C was reported to reduce the duration of mechanical ventilation in comparison with nonsupplemented patients [60]. Although these findings are encouraging and support the use of antioxidants to prevent VIDD, these agents can also have deleterious as well as nonspecific effects. For example, it appears that the beneficial effects of apocynin on VIDD in rats may be more a function of its ability to upregulate a calpain inhibitor (calpastatin) rather than any specific antioxidant action [45].

The proteolytic systems activated during VIDD are also logical targets for therapeutic intervention. In this regard, a single administration of leupeptin (inhibitor of calpain/cathepsin) at the onset of mechanical ventilation, not only blocked atrophy, but also prevented intrinsic contractile

Figure 1 Cellular mechanisms implicated in ventilator-induced diaphragmatic dysfunction



Several of the mechanistic pathways implicated in the development of VIDD are illustrated. Protein synthesis pathways are downregulated (indicated by dashed lines). In addition, ROS generated from several possible sources can activate downstream proteolytic pathways involved in myofiber injury and atrophy, including the calpain and caspase systems; these have the potential to be mutually reinforcing and to drive further ROS production. The role of lysosomally-mediated proteolysis, and particularly autophagy, remains to be explored. IGF-1, insulin-like growth factor-1; MV, mechanical ventilation; PI3K, phosphoinositide-3 kinase; ROS, reactive oxygen species.

impairment in the rat diaphragm [27]. Somewhat surprizingly, acute high-dose corticosteroid administration was also found to prevent calpain upregulation and mitigate VIDD in rats [61•], although corticosteroids are unlikely to be a viable treatment for VIDD given their association with acute thick filament loss and other forms of myopathy [62]. Less surprizing is the fact that neuromuscular blocking agents can synergize with mechanical ventilation to exacerbate VIDD, with attendant increases in activation of the calpain and ubiquitin–proteasome systems [63,64]. Interestingly, recent lessons from the sepsis literature suggest that inhibition of the proteasome pathway may not be an effective way to prevent the loss of contractile force associated with enhanced proteolysis in the diaphragm [65•]. These findings are consistent with the notion that events upstream of the proteasome (e.g., caspase-mediated or calpain-mediated myofilament dissociation [23•] and other forms of injury) are more likely to be responsible for the early reductions in diaphragmatic force-generating capacity in VIDD, but this hypothesis needs to be confirmed.

Finally, there are many questions of both clinical and scientific importance that need to be addressed by future studies. For example, what are the effects of mechanical ventilation on calcium homeostasis? Many of the processes implicated in VIDD, including injury, can be triggered by increases in intracellular calcium, but there is no direct information available on this point. Furthermore, although investigations to date have elegantly dissected the relationships of several cellular pathways to muscle fiber atrophy, the mechanisms leading to injury and contractile dysfunction remain obscure. There is also very little information about how VIDD is influenced by underlying conditions commonly found in mechanically ventilated patients, such as hyperglycemia [66] and sepsis [67], or by the preexisting state of the muscle (e.g., if fatigued or injured) prior to initiating mechanical ventilation. Most importantly, data are sorely lacking in human patients regarding most aspects of the VIDD phenomenon, including the time course for development of diaphragmatic weakness during mechanical ventilation and its relationship to injury, atrophy, and the candidate cellular pathways implicated in the animal models discussed in this review (see Fig. 1).

Conclusion

There is now evidence that mechanical ventilation itself may be an important cause of diaphragmatic weakness, associated with a combination of mechanical ventilation-induced diaphragmatic atrophy and injury, which is collectively referred to as VIDD. It is likely that several factors (e.g., underlying disease state, infection, drug therapy, etc.) can converge with VIDD to exacerbate diaphragmatic weakness in critically ill patients. Indeed, due to the presence of multiple confounding factors, it is not currently possible to definitively diagnose any given patient with VIDD, just as no individual patient can be assigned a diagnosis of VILI with certainty. Nevertheless, as in the case of VILI, research into the basic mechanisms underlying the phenomenon of VIDD is allowing us to develop a conceptual framework for understanding the problem and applying this knowledge to clinical practice. At present, the best approach for preventing VIDD is to avoid controlled mechanical ventilation and the use of neuromuscular blocking agents to the greatest extent possible. In the future, the challenge will be to develop strategies or modes of mechanical ventilation which decrease the likelihood of VIDD, as well as to determine the ability of pharmacological interventions such as antioxidant therapy or inhibitors of muscle proteolysis pathways, to preserve diaphragmatic function in mechanically ventilated patients.

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