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SEMINAL STUDY

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Diaphragmatic fatigue during sepsis and septic shock

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The diaphragm is the primary muscle of respiration, and severe dysfunction of the diaphragm, consisting of decreased maximal force production and increased susceptibility to fatigue, has been documented in animal models of sepsis. A large number of studies have examined the effects of endotoxemia and other sepsis models on diaphragm contractility in spontaneously breathing animals.

Introduction

In the United States sepsis annually affects 700,000 people and accounts for about 210,000 deaths. Respiratory failure has long been known to be a frequent occurrence of this pathological condition and to represent a major contributor to the high associated mortality [1]. This contribution discusses of the effects of sepsis and septic shock on respiratory muscle function and focuses on some of the possible mechanisms involved in the genesis of these effects.

For nearly a century sepsis has been defined as the systemic host response to infection. A consensus definition was formulated a decade ago [2], and the list of symptoms has recently been updated [3]. Sepsis is now defined as infection with evidence of systemic inflammation, with at least two of the following: increased or decreased temperature or leukocyte count, tachycardia, and rapid breathing. In this context septic shock is defined as a state of acute circulatory failure characterized by persistent arterial hypotension unexplained by other causes [3]. Interestingly, the spectrum of responsible micro-organisms seems to have shifted from Gram-negative bacteria in the late 1970s to Gram-positive ones at present [4]. This is an important fact to point out since studies evaluating the effects of sepsis and septic shock on respiratory muscle function have been performed in animal models of sepsis, where Gram-negative bacteria have mainly been used as the infectious agent.

Respiratory muscle dysfunction during sepsis

Twenty years ago Hussain and associates [5] first demonstrated in spontaneously breathing dogs that endotoxic shock resulted in respiratory muscle fatigue, which in turn was the main factor responsible for respiratory failure and death in this experimental model of septic shock (*Escherichia coli* administration). Occurrence of respiratory muscle dysfunction in endotoxic shock has also been reported by our laboratory in mechanically ventilated rats [6]. In this study we observed decreased diaphragmatic strength restricted to the transdiaphragmatic pressure (Pdi) generated at high frequencies of phrenic nerve stimulation (50 and 100 Hz) while both twitch and low frequency Pdi and muscle relaxation rate remained unchanged. Endurance capacity of the diaphragm was curtailed in endotoxemic animals. Contractile dysfunction was associated with a decreased diaphragmatic resting membrane potential. This phenomenon, which has been reported in critically ill patients with various diseases [7] and in septic animal models [8], could impair action potential generation resulting in failure of neuromuscular transmission due to a postsynaptic membrane depolarization and an impaired propagation of electrical excitation along diaphragmatic fibers.

A major common point between the two studies cited above is that blood pressure was significantly reduced in

septic animals. It is well known that blood pressure is a major determinant of muscle metabolic substrate delivery and contractile function. The results of Hussain and coworkers [5] are very similar to those reported by Aubier and coworkers [9] in nonseptic hypotensive spontaneously breathing dogs. The similarity in findings raises the question of the role of hypotension in the pathophysiology of the immediate effects of sepsis on respiratory muscle function, which could be of particular importance in the context of septic shock.

Murphy and associates [10] evaluated the role of Gram-positive bacterial products in muscle dysfunction in 4-week-old piglets. These authors investigated in spontaneously breathing animals the effects on diaphragmatic strength of a continuous infusion of group B streptococcus at a level that caused a decrease in cardiac output, but which avoided hypotension. Diaphragmatic strength was evaluated by measuring Pdi generated during bilateral phrenic stimulation. The main result of this study was that Pdi remained unchanged in septic animals over a 4-h period. However, another study from the same laboratory [10] showed that increasing the dose of streptococcus while avoiding significant hypotension resulted in a transitory but significant decrease in diaphragmatic strength. Hurtado and coworkers [11] investigated the role of hypotension in peripheral muscle dysfunction during sepsis. These authors evaluated the effects of a similar level of septic and nonseptic hypotension on peripheral muscle metabolism and strength generation in rabbits. Blood pressure decreased by approx. 22% of baseline values in both groups of animals. This study showed that by the end of the experiment (180 min after the onset of hypotension) hind-limb force was significantly reduced in septic animals for all the frequencies of stimulation. However, a similar reduction was observed in nonseptic animals. Taken together, these studies suggest that both hypotension and bacterial products make individual contributions to the genesis of the immediate deleterious effects of sepsis on respiratory muscle function. It is unclear whether septic hypotension has none, additive, or synergic effects (in terms of diaphragm dysfunction) with respect to nonseptic hypotension. To our knowledge, no data are available in the literature examining at the same time both septic and nonseptic hypotension. One can imagine that an animal model supporting both septic and nonseptic hypotension would be extremely difficult to manage.

Once the first reports on the immediate effects of sepsis on respiratory muscle function were published, investigators began to be interested also by the consequences of septic processes lasting several days. Using an in vivo rat model we evaluated the modifications in diaphragmatic function 3 days after *Streptococcus pneumoniae* injection [12] and 2 days after inoculation of *E. coli* endotoxin [13]. Both inoculations were performed subcutaneously, and both models of sepsis were nonlethal, with no change in blood pressure, serum electrolytes and acid-base status. The results of these studies were similar: 2 or 3 days of experimental sepsis in rats impaired diaphragmatic function without affecting muscle mass or histology. Contractile force in response to phrenic stimulation was reduced without a concomitant decrease in the electrical activity of the muscle. Muscle relaxation rate was prolonged, and the diaphragms of septic animals fatigued rapidly in response to a stimulation regimen that was without effect on the diaphragms of control animals.

Similar results were reported by Shindoh and coworkers [14] in *E. coli* endotoxin-inoculated hamsters. More recently Krause and coworkers [15] and Matzcuzak and collaborators [16] showed a decreased diaphragmatic force in experimental models of pancreatitis, suggesting that patients suffering from such disease may be susceptible to respiratory muscle failure. Finally, Drew and associates [17] examined the effects of a chronic infection lasting several weeks, visceral leishmaniasis, on the function of the diaphragm and the peripheral muscles soleus and plantaris. Muscular function was assessed in vitro. Infected animals (intracardiac inoculation of *Leishmania donovani* amastigotes) were maintained for 7–12 weeks until advanced disease characterized by anorexia, weight loss, and weakness was evident. Body weight and the mass of the diaphragm, soleus, and plantaris were reduced in septic animals. Absolute contractile force of the diaphragm and soleus muscles was moderately reduced, and only to the extent that muscle mass was decreased. Force normalized to muscle mass or cross-sectional area was not impaired. In contrast, the force of the plantaris, a fast twitch muscle, was severely reduced even after correcting for loss of muscle mass. The effects of leishmaniasis on the diaphragm and soleus muscles did not differ from those of semistarvation with equivalent weight loss, but these models of sepsis produced much greater loss in plantaris force than occurred with semistarvation.

To summarize, the last 20 years have brought multiple evidence and some explanation regarding the occurrence of severe dysfunction of the diaphragm in animal models of sepsis, dysfunction consisting in decreased maximal force production, and an increased susceptibility to fatigue.

Mechanisms of respiratory muscle dysfunction during sepsis

The underlying mechanisms of respiratory muscle dysfunction occurring during the early phase and after several days of sepsis are certainly different. They encompass energetic and metabolic components as well as the implication of mediators such as prostaglandins, cytokines, reactive oxygen species (ROS), and nitric oxide [18].

Energetics

From a general point of view respiratory muscle dysfunction is thought to occur when blood supply of energetic subtracts to the muscle is not sufficient to meet the muscle's metabolic needs [19]. The efficiency of energy minus uptake by these muscles depends mainly upon the total blood flow that reaches them, the conditions of perfusion of the microvascular network, and the ability of muscle cells to utilize metabolic substrates. All of these processes can be altered by the septic condition.

The septic state is characterized by generalized blood flow misdistribution among the different organs including the respiratory muscles. However, this phenomenon is modulated by the degree of contractile activity. Either immediately or lately after the beginning of the septic process, blood flow decreases if the diaphragm is at rest [5] and increases if it contracts [20]. The increase in respiratory muscles blood flow during septic shock can reach dramatic levels, resulting in reduced blood flow to the brain, gastrointestinal tract, and other skeletal muscles [5]. It is predictable that in this state the function of the vital organs other than the respiratory muscles is compromised. However, in spite of this finding the values for diaphragmatic blood flow observed during septic shock are much lower than the maximum reported in normotensive conditions [5]. Therefore, although diaphragmatic blood flow is significantly increased during sepsis, a septic-induced limitation to the maximal blood flow is operational. This limitation can occur at the microcirculatory level. Using an in vivo experimental model in rats we have shown that the number of perfused-diaphragmatic capillaries decreases significantly after *E. coli* endotoxin inoculation [21]. In addition to the microcirculatory limitation in metabolic substrates delivery to the respiratory muscles, the ability of muscle cells to utilize metabolic substrates is compromised in sepsis. *E. coli* endotoxin inoculation in rats induces an impairment in diaphragmatic mitochondrial respiration associated with an increased production of hydrogen peroxide [22, 23], secondary to induction of the inducible isoform of nitric oxide synthase (NOS II) in the muscle (see below).

For many years it has been recognized that the septic process is the result of extensive triggering of the body defense mechanisms by the invading micro-organisms and their products. Studies performed in the past 15 years have shown that respiratory muscle dysfunction during sepsis can be attributed to the actions of endogenously produced mediators, such as prostaglandins, cytokines, ROS, and NO.

Mediators

Several studies indicate that prostaglandins play a role in the development of peripheral skeletal muscle dysfunction during sepsis [24]. Elevated prostaglandin E_2 levels have been found in peripheral muscles of septic animals [25, 26], and pharmacological inhibition of prostaglandins synthesis has been shown to protect septic animals from peripheral skeletal muscle impairment [24]. In a similar line, we have found that the cyclooxygenase inhibitor indomethacin prevents the reduction in diaphragmatic strength found in *E. coli* endotoxemic animals [13]. In addition, this agent prevents peripheral muscles atrophy. Similar results have been reported by Murphy and coworkers [27] in septic piglets. The latter study found that systemic administration of thromboxane A_2 mimics the reduction in diaphragmatic strength observed in septic animals.

Among cytokines tumor necrosis factor (TNF) α has received substantial attention in the context of the septic process. In vitro studies show a dose-dependent decrease in diaphragmatic strength elicited by incubation of muscular fibers with murine or human TNF-α [28], with a synergistic effect of interleukin-1β on diaphragmatic contractility [29]. Furthermore, in vivo TNF-α induced a significant decrease in diaphragmatic force in dogs beginning 4 h after administration [30]. Inoculation of rats with *E. coli* endotoxin induced TNF-α mRNA expression in the diaphragm along with a decreased force [31], and pretreatment of the animals with an anti-murine TNF-α antibody prevented the deterioration in diaphragmatic contractile properties [31]. Together these findings suggest that TNF-α induces a decrease in diaphragmatic force generation. Different mechanisms may explain the effects of TNF-α on diaphragmatic contractility. Wilcox and coworkers [32] showed a role of prostaglandins and Reid and associated [33] demonstrated that TNF-α decreases force by blunting the response of muscle myofilaments to calcium activation. Whether these effects are mediated directly by TNF-α or indirectly by the induction of molecules such as ROS or NO (see below) warrants further investigation.

Reactive oxygen species

ROS are produced by all aerobic organisms as a consequence of oxygen consumption and cell respiration. They play a role of intracellular mediators at physiological concentrations, but in stress situations increasing production of ROS can lead to cellular injury. During sepsis the rate of ROS produced by respiratory muscles increases, releasing a large amount of superoxide anion, hydroxyl radical, and hydrogen peroxide [34]. This enhanced ROS production derives from different cellular compartments: one part of these ROS depends on mitochondrial chain respiration impairment following hemodynamic failure [35] while another part comes from sepsis-activated constitutive skeletal muscle NAD(P)H oxidase [36]. The participation of ROS in septic dia-

phragmatic failure has been clearly demonstrated in experimental models by the protective effect of antioxidant treatments, such as *N*-acetylcysteine [13], catalase, and superoxide dismutase [14]. Among the different ROS superoxide anion and hydroxyl radical are the two species that play the central role in reducing fibers calcium sensitivity and altering contractile protein capacity [37]. ROS reduce skeletal muscle force-generating capacity by inhibiting mitochondrial oxygen consumption, especially during ADP-stimulated (state 3) diaphragm mitochondrial oxygen utilization [23]. In septic patients an association has been found between antioxidant depletion, mitochondrial dysfunction and organ failure and outcome [38], underlying the importance of oxidative stress in generating energetic failure. Oxidants can structurally alter other, different components of excitation-contraction coupling system: T-tubules, sarcoplasmic reticulum calcium ATPase, and head of myosin oxidation (leading to inhibition of actin-myosin binding). Protein oxidation in skeletal muscle comes early during sepsis and is significantly correlated to the decline in mitochondrial respiration. Moreover, oxidized proteins are more sensitive to degradation. Proteolysis takes part to the development of muscular weakness observed in sepsis. Finally, myoglobin oxidation decreases oxygen storage capacity of the muscle.

Nitric oxide and its metabolites

NO is a secondary messenger molecule which participates in numerous biological processes, including vasodilatation, neurotransmission, and bronchodilatation. NO is synthesized by a group of enzymes referred to as NOS which are responsible of the conversion of L-arginine to L-citrulline and NO in presence of oxygen. Three NOS isoforms (I–III) have been identified so far, and they all are expressed in respiratory muscles, particularly in diaphragm [39, 40, 41]. In animal models of sepsis it has been extensively demonstrated that NOS II expression is induced in the diaphragm, both at mRNA and protein levels, with a resultant increase in NO production [41, 42, 43]. Several lines of evidence suggest that impaired diaphragmatic contractility is a result of NO overproduction during sepsis. Boczkowski et al. [41] were the first to propose a link between in vivo induction of diaphragmatic NOS II and its involvement in the genesis of diaphragmatic contractile dysfunction after *E. coli* endotoxin inoculation in rats. First, this study showed that the time course of NOS II induction in diaphragmatic myocytes and that of the decrease in diaphragmatic force are similar, and, second, that inhibition of NO synthesis by either N^{ω}-monomethyl-L-arginine (L-NMMA), an inhibitor of NOS activity, or dexamethasone, an inhibitor of NOS II induction, significantly improves the decrease in diaphragmatic force observed in endotoxemic animals. Similar results have been reported by El-Dwairi et al. [44] using *S*-methylisothiourea as NOS activity inhibitor. In an attempt to define the exact role of the different NOS isoforms in lipopolysaccharide (LPS) induced diaphragmatic contractile injury, two studies by Comtois and collaborators [45, 46] investigated their role in genetically engineered mice, knockouts (KO) for either NOS II or NOS I. Taken together, the findings of these studies suggest that both NOS I and NOS II isoforms play protective roles in attenuating LPS-induced reduction in diaphragmatic contractile function, despite leading, respectively, to a decreased and an increased NO synthesis. Interestingly, another study in NOS II KO mice [47], showed that LPS injection induces less tyrosine nitration than in wild-type mice, although deficiency for NOS I or NOS III does not affect this protein modification. This points out the great importance of the environment in which NO is synthesized. However, the mechanism(s) by which NO participates in the alteration of diaphragmatic contractile function remain(s) to be determined.

NO by itself has a deleterious effect on mitochondrial respiration, with the inhibition of several enzymes such as aconitase and cytochrome oxidase [48, 49]. This effect of NO may contribute to poor oxygen extraction observed in sepsis and thus participate in altered muscular function. Moreover, NO can produce its deleterious effects by its reaction with superoxide anion to form peroxynitrite anion ($ONOO^-$), a very strong oxidizing agent [50], which targets various molecules such as thiols, lipids, and proteins containing aromatic amino acids, and irreversibly inhibits several mitochondrial enzymes such as aconitase, NADH and succinate dehydrogenases, and superoxide dismutase [51, 52, 53]. Several authors have described peroxynitrite formation in the diaphragm of endotoxemic animals [41, 44, 47], mainly in the mitochondrial and membrane fractions of LPS-treated rats diaphragm [47], a treatment with L-NMMA leading to a diminished nitration of diaphragmatic mitochondrial proteins [22]. Finally, studies on the role of peroxynitrite on diaphragmatic contractile function show that in vitro exposure of muscular samples to peroxynitrite itself or peroxynitrite-generating agents leads to a decreased force generation [54]. It must be pointed out, however, that exogenously generated peroxynitrite, considering its short half-life at physiological pH [55], may not react in the same way as endogenously produced peroxynitrite. The exact relationship between peroxynitrite generation and contractile function impairment is not entirely clear, but one possible explanation lies in the oxidating and nitrating properties of peroxynitrite which can lead to the alteration of proteins involved in the contractile process, such as actin [56] and the sarcoplasmic reticulum Ca^{2+}-ATPase [57].

One mediator of interest could be cGMP, as it is widely known that NO activates the soluble guanylyl cyclase, leading to cyclic GMP synthesis [58]. Kobzik et al. [40] demonstrated that agents able to increase intra-

cellular cGMP content, such as 8-bromo cGMP, reverse the protective effect of NOS inhibitors on muscular force. However, in another study [41], activation of guanylyl cyclase observed in diaphragmatic muscle after LPS inoculation showed a biphasic time course; early activation appeared to be due to NO synthesized by NOS II, while late activation was independent of NO. Thus the exact role of cGMP in mediating the effects of NO in sepsis-induced diaphragmatic contractile dysfunction still remains to be elucidated. Finally, the pharmacological approach used by several authors brings some insights to better understand the exact role that NO could play in the alteration of respiratory muscle function. Inhibition of NOS activity, by administration of the NOS inhibitor N^{ϕ}-nitro-L-arginine methylester leads to a protection against the reduction in myofiber calcium sensitivity observed in endotoxemic rats [37]. Moreover, administration of L-NMMA, another NOS inhibitor, significantly reduces LPS-induced diaphragm sarcolemmal injury and alters resting membrane potential in rats [43]. This could have a direct effect on muscular function. It is important to mention in this context a study by Ebihara and coworkers [59] which determined the impact of mechanical ventilation on rat diaphragm sarcolemnal injury, NOS II expression, and oxidative stress during endotoxemia. These authors demonstrated that starting ventilation at the time of infusing endotoxin into rats partially prevents the impaired diaphragmatic contractility due to sepsis. Mechanical ventilation also prevented the injury to the sarcolemma of diaphragmatic cells [59], but surprisingly did not reduce the increase in expression of NOS II. This should not lead us to the conclusion that nitric oxide and oxidative stress are less important than in the context of muscle injury caused by sepsis. Indeed, in the same study [59], using an in vitro system to independently modulate oxidative and mechanical stresses, the authors demonstrated that these two factors act in a synergistic fashion to favor the occurrence of sarcolemmal injury.

Conclusion

There is no doubt that sepsis impairs the function of respiratory muscles. This impairment is observed soon after the onset of the septic process and may be still present after several weeks, depending on the duration of the infectious aggression. The precocious dysfunction is strongly related to the hypotension that can be present in this condition. In contrast, the later effects (days to weeks) appear to be independent of hemodynamic alterations and are connected to pathophysiological processes that need some time (days to weeks) to develop. From an integrated point of view it is possible to postulate that sepsis impairs respiratory muscle function by acting at two levels. The first is by disturbances at different steps of the chain of muscular energy supply: blood flow and metabolic substrate extraction and utilization. The second is a direct impairment of the contractile process. These effects of sepsis are probably the result of the action of septic mediators. The result is a complex series of effects on the respiratory muscles that have the potential for profound clinical consequences. Despite the recent advances in the field much remains to be learned, and several questions are still unresolved. Answers to these questions will allow the clinicians to better manage respiratory failure in septic patients and particularly the mechanical ventilation procedure. Recommendations for the use of mechanical ventilation during sepsis will depend substantially on the clinical status of the patient. Putting the respiratory muscles at rest when they are fatigued has been shown to be beneficial at least during the weaning process of mechanical ventilation. However, resting the respiratory muscles by mechanical ventilation could also be deleterious. The decision as to mechanical ventilation during sepsis should therefore be based on the respiratory as well as circulating parameters, both leading to respiratory failure.

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