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The role of mitochondria in inflammatory syndromes

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ABSTRACT: Several authors have addressed the importance of mitochondrial function in inflammatory syndromes, as it may play a role in the genesis of tissue injury. Sepsis and exposition to environmental particles are examples of inflammatory conditions. Sepsis occurs with an exacerbated inflammatory response that damages tissue mitochondria and impairs bioenergetic processes. One of the current hypotheses for the molecular mechanisms underlying the complex condition of sepsis is that enhanced NO production and oxidative stress lead to mitochondrial dysfunction, bioenergetic derangement and organ failure. The mechanism of particulate matter-health effects are believed to involve inflammation and oxidative stress. Components in particles that elicit inflammation have been poorly investigated, although recent research points out to the contribution of compositional elements and particle size. Oxygen metabolism and mitochondrial function appear to be important areas of study in inflammatory conditions for clarifying molecular mechanisms involved.

Inflammation was classically described as a combination of three clinical signs: vasodilation, hyperthermia, and edema; it is considered as either an acute or a chronic process according to its time course. The phenomenon is associated to cellular mobilization and migration of neutrophils and macrophages to the inflammatory focus.

An extensively studied inflammatory condition is endotoxemia (induced by Gram-negative bacteria) whose primary initiator is lipopolisaccharide (LPS), a component of the cell wall (Darveau, 1998). The binding of LPS to the surface of endothelial cells results in endothelial activation, as shown by the expression of proinflammatory cytokines and adhesion molecules. Several authors addressed the importance of mitochondrial function during endotoxemia and experimental sepsis, as it may play a role in the genesis of tissue injury described in inflammatory syndromes (Navarro and Boveris, 2005, Vanasco *et al.,* 2008). There are several mechanisms by which mitochondria may lead to tissue dysfunction: (a) reduction in cellular adenosine triphosphate (ATP) levels due to impairment of mitochondrial metabolic pathways (Brealey and Singer, 2003); (b) generation of active species, that can damage cell (Vanasco *et al.,* 2010); and (c) involvement in the intrinsic pathway of cellular apoptosis (Bernardi *et al.,* 2001).

Another inflammatory condition is observed during the exposition to polluted environments. There is strong evidence from cell culture and animal models that exposure to particulate matter (PM) is associated with inflammation (Ghio and Devlin, 2001). Components in PM that elicit inflammation have been poorly investigated, although recent research points out to the contribution of compositional elements and particle size. Small (ultrafine) particles can cause inflammation by surface-mediated effects, whereas large (coarse) particles can produce an inflammatory response

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through the presence of endotoxins and other soluble mediators present in the particulate material.

There is current awareness about the central role of mitochondrial dysfunction in the development of organ failure in endotoxemia (Cimolai *et al.,* 2015, Vanasco *et al.,* 2012). Mitochondria provide energy to the cell through the synthesis of ATP by F_o-F_1 ATP synthase. Consequently, a deficient ATP production may result in bioenergetic dysfunction and cardiac failure. In this scenario, mitochondrial biogenesis emerges as a compensatory mechanism that requires nuclear and mitochondrial genomic orchestration. Signals that activate mitochondrial biogenesis include increased production of NO and reactive O_2 species (ROS), and energy deprivation. In line with this observation, we have previously found increased NO, $O2^{\bullet-}$ and H_2O_2 production in muscle mitochondria during endotoxemia (Alvarez and Boveris, 2004). It is of note that mitochondrial biogenesis is triggered *in vivo* in different physiopathological conditions, but this observation does not necessarily imply that resultant mitochondria are functional. The mechanisms by which mitochondrial energy metabolism and sepsis-induced myocardial damage are not fully understood.

Our group recently reported that mitochondrial biogenesis occurs in heart during endotoxemia (Vanasco *et al.,* 2014). Female Sprague-Dawley rats (45 days old) were i.p. injected with LPS (10 mg/kg). Measurements were performed at 0-24h after LPS administration. PGC-1α and mtTFA expression for biogenesis, and p62 and LC3 expression for autophagy, were analyzed by western blot; mitochondrial DNA levels by qPCR, and mitochondrial morphology by transmission electron microscopy. Mitochondrial function was evaluated as oxygen consumption and respiratory chain complexes activity. PGC-1α and mtTFA expression was significantly increased in every time-point analyzed, and mitochondrial mass was increased by 20% ($p<0.05$) at 24h. The expression of p62 was found significantly decreased in a time-dependent manner. LC3-II expression was significantly increased at all-time points analyzed. Ultrastructurally, mitochondria displayed several abnormalities (internal vesicles, cristae disruption, and swelling) at 6 and 18h. Structures compatible with fusion/fission processes were observed at 24 h. Significant decrease in active (state 3) respiration was observed in every time-point analyzed (LPS 6h: 20%, $p<0.05$). Mitochondrial complex I activity was found decreased by 30% in LPS-treated animals at 6 and 24h. Complex II and complex IV showed decreased activity only at 24h. Our study shows that partial restoration of cardiac mitochondrial architecture is not accompanied by improvement of mitochondrial function in acute endotoxemia. The key implication of our study is that cardiac failure due to bioenergetic dysfunction will be overcome by therapeutic interventions aimed to restore cardiac mitochondrial function (Vanasco *et al.,* 2014).

Particulate matter consists of a mixture of particle components, including traffic and combustion-derived carbon-centered particles, secondary particles (nitrates and sulphates), wind-blown dust of geological origin potentially containing endotoxins, and biological particles (spores, pollen) with their associated allergens. Fine (aerodynamic diameter between 0.1-2.5 microns) and ultrafine (aerodynamic diameter less than 0.1 micron) particles can penetrate into the lower airways and alveoli and are closely related with adverse health effects of PM (Schins *et al.,* 2004). A number of hypotheses have been postulated to explain the adverse effects of PM, involving PM size and adsorbed transition metals (Dellinger *et al.,* 2001) or organic compounds (Stenberg *et al.,* 1983).

It has been suggested that mitochondrial function plays a central role in cardiovascular diseases associated with PM (as residual oil fly ashes, ROFA) inhalation. The aim of our study was to evaluate this hypothesis, with focus on cardiac O_2 and energetic metabolism, and its impact on cardiac contractility (Marchini *et al.,* 2013). Heart O_2 consumption was

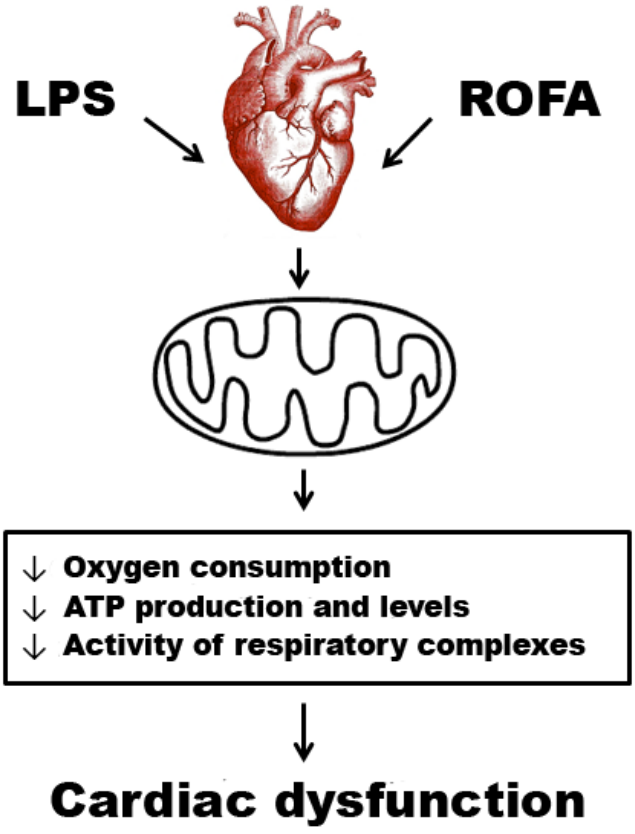

FIGURE 1. Scheme showing the main effects of the inflammatory condition (caused by LPS or ROFA) in cardiac mitochondria. Mitochondrial bioenergetics derangements would be one of the main causes of cardiac dysfunction in inflammatory syndromes.

assessed in tissue cubes. In these conditions, heart O_2 consumption was found to be significantly decreased by 23% as early as 1 h after the ROFA instillation, with a minimum at 3 h after the treatment, indicating deficient tissue O_2 uptake. The observed decrease seemed to be not only time-dependent but also reversible, since O_2 consumption rates of ROFA-exposed mice were not significantly different from control animals at 5 h after the treatment. Under physiological conditions, 85-90% of tissue O_2 uptake is consumed by mitochondria in the oxidative phosphorylation process (Hill *et al.,* 2009). Therefore, the evaluation of mitochondrial respiration was carried out in order to clarify the observed alteration in tissue O_2 consumption. Mitochondrial resting (state 4) and active (state 3) respiration were found to be decreased by 30 and 24%, respectively (controls: state 4: 88±5 ng-at O/min mg protein; state 3: 240±20 ng-at O/min mg protein, $p<0.05$). These findings were associated with decreased complex II activity, mitochondrial depolarization and deficient ATP production. These results suggest that the altered mitochondrial function accounts for the decrease in tissue O_2 consumption, and indicates a decreased mitochondrial function due to an inhibition of the electron transport chain with maintenance of mitochondrial membrane integrity. When cardiac function was evaluated, even though basal contractility was not modified (control: 75± 5 mm Hg), isolated perfused hearts failed to properly respond to isoproterenol in ROFA-exposed mice. Tissue O_2 consumption rates positively correlated with cardiac contractile state in controls ($r^2=0.8271$), but not in treated mice ($r^2=0.1396$). The study shows an impaired mitochondrial function associated with deficient cardiac contractility, which could represent an early cardiovascular alteration after the exposure to environmental PM (Marchini *et al.,* 2013). Interestingly, in a recent study we have shown that impaired cardiac oxygen metabolism and contractile function induced by an acute exposure to air pollution particulate matter can be attenuated by blocking TNF-α-dependent systemic inflammation with Infliximab, which emphasizes the importance of environmental factors and inflammation in cardiovascular disease (Marchini *et al.,* 2015).

To the extent that mitochondrial dysfunction contributes to impaired organ function (as shown in Fig. 1), new therapeutic opportunities should be considered. Additional investigations detailing the mechanisms and timing of mitochondrial injury during inflammatory conditions are needed before it were possible to develop highly effective mitochondrial protection strategies for application in the clinical setting. The overall mechanisms by which sepsis leads to organ dysfunction remains to be established. Although microvascular flow abnormalities occur, findings of decreased mitochondrial oxygen consumption despite elevated tissue oxygen tensions (cytopathic hypoxia) suggest that the problem lies in cellular oxygen utilization by mitochondria. Mitochondria utilize 90% of total body oxygen consumption to generate ATP, so organ dysfunction could be a consequence of impaired bioenergetics. Thus, mitochondria play a central role in the intracellular events associated with inflammation.

Mitochondrial bioenergetics and architecture in inflammatory syndromes appear to be important areas of study necessary to understand free radical mechanisms behind inflammatory syndromes.

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