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Editorial

Melatonin and mitochondrial dysfunction are key players in the pathophysiology of sepsis

La melatonina y la disfunción mitocondrial son elementos claves en la patofisiología de la sepsis

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Sepsis was first defined as a syndrome in 1991, but it has been described in medicine from a lot longer, at least since 1000 BC by Avicenna as a process characterized by a fever associated with tissue putrefaction. The first consensus on sepsis, defined it as a systemic inflammatory response syndrome (SIRS) to a microbial infection. SIRS is identified if at least two of the following are met: tachypnea, tachycardia, pyrexia or hypothermia, and leukocytosis, leucopenia or neutrophilia.[1]

Recently, the concept of SIRS has been abandoned, given that it has little specificity and that it appears almost universally in all critically ill patients. On the other hand, a better understanding of the pathophysiology of sepsis leads to the last definition in 2016.[2] Now sepsis is defined as a dysregulated host response to infection with involvement of organs remotely from the site of infection. Septic shock continues to be characterized by tissue hypoperfusion with hyperlactacidemia and the need for vasoactive drugs, with a mortality of up to 40–50%. With the appearance of the new definition, the term of severe sepsis has been abandoned, as it ended up being redundant.

Lately research in the field of sepsis makes more emphasis in the study of the response of the host to infection and less to the pathogenicity of the microorganism itself. This has been reflected by the emergence of the 'danger hypothesis',[3] which states that the immune system recognizes microbial patterns and cellular products such as 'danger signals' of infection or tissue damage. This excessive host response ends up producing molecular changes that cause organic damage, leading to multiorgan failure, and the contributing factors being not only the causative pathogen, but also the initial site of infection, comorbidities and iatrogenic interventions. Of note, although infection is the triggering event in this definition of sepsis, the aberrant immune response often remains after successful treatment of the infection.

Organ dysfunction, that is required for sepsis diagnosis, is defined as an acute change in sequential organ failure assessment (SOFA) score ≥2 points due to infection and is associated with a ≥10% increase in mortality. Multiorgan dysfunction syndrome (MODS) most commonly affects the respiratory system (acute respiratory distress syndrome or ARDS), the cardiovascular system (shock, hypoperfusion and myocardial depression), the central nervous system (encephalopathy, altered mentation and delirium) and the kidney (acute kidney injury).

Therefore, sepsis is not a specific disease but rather a syndrome, the definition of which is under continuous review and change, and the diagnosis of which does not have a clear gold standard, but rather is a clinical diagnosis based on a consensus of experts.

Despite the fact that sepsis is a syndrome with a marked impact on health worldwide, we do not have reliable data about the disease burden of sepsis. This is mainly due to the lack of information about the epidemiology in low and middle-income countries. The available data we have are from high-income countries that show a high incidence, from 194 per 100,000 inhabitants in Australia[4] to 580 per 100 000 inhabitants in the United States in 2006.[5]

In terms of mortality, it remains very high, between 15% and 25% in sepsis, and up to 40–50% in septic shock, being the leading cause of death in critically ill patients in the United States. There is evidence, especially from Australia and New Zeeland[6] that shows that mortality from sepsis is decreasing, although other authors[7] have shown that the total number of deaths is increasing given that the incidence of sepsis is increasing. Therefore, as mentioned previously, both the incidence and mortality of sepsis worldwide remain high but with an important variability.[8]

In terms of pathophysiology, sepsis is a dysregulated host response to infection. The immune response that is initiated by an

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invading pathogen fails to return to homeostasis, thus culminating in a pathological syndrome that is characterized by sustained excessive inflammation and immune suppression.

After infection, the invading pathogen encounters the host innate immune system, and innate cells 'sense' pathogens by recognizing pathogen-associated molecular patterns (PAMPs) and host-derived danger-associated molecular patterns (DAMPs) released during this process in the circulation through an assortment of pattern recognition receptors (PRRs) like the toll-like receptors (TLR). TLR recognize these PAMPs and DAMPs inducing an up or downregulation in the transcription of genes encoding for inflammation proteins like cytokines and other proteins involved in multiple pathways including cardiovascular, immune, hormonal, coagulation, metabolic and energetic between others.[9] Some of the products of inflammation are the reactive species such as nitric oxide and superoxide in different quantities, which finally are related to outcome.[10]

There is an important hormonal activation during the early phase of sepsis, to prepare the body for stress with the secretion of acute phase hormones like adrenaline and cortisol, inducing a redistribution of blood flow to vital organs and increasing cardiac output, modifying hepatic proteins production toward acute phase proteins and modulating metabolic activity. Catecholamines also induce an increase in lactate synthesis by the muscle, which will serve as energy for the brain, the liver and the heart.[11]

The cardiovascular system activation also contributes to the increase in the overall cardiac output and redistribution of the blood flow toward the organs that need more oxygen delivery for their greater metabolic requirements during the stress. To all the above, a change in vascular tone and alterations in the endothelium is added to improve the reach of inflammation products and white blood cells into the infection sites.

In the areas of infection control, where the inflammation is maximum, there is an increase in coagulation and the thrombus formation to exclude these areas from the rest of the circulation and to avoid the spread of bacteria, DAMPs, toxins to the organism. In sepsis, the excessive host-response will affect organs initially unaffected by the infection. The initial increase in the cardiac output will be counterbalanced by capillary leak and the loss of great amounts of interstitial fluid. Thus, the final result will be a decrease in the overall cardiac output due to a decrease in the preload on the one hand, and on the other due to a decrease in myocardial contractility due to high levels of mediators of inflammation such as NO and a significant loss in vascular tone that jeopardizes organ perfusion.[12] The consequences of these processes are MODS and high mortality.

The mitochondria have several important physiological roles, but to state the most important: ATP production, reactive oxygen species (ROS) production, heat generation[13] and it also triggers cell death pathways (necrosis when ATP falls below minimum levels or necrosis by releasing cytochrome c into the cytoplasm). Except erythrocytes, all cell types possess mitochondria.

During sepsis, microbial antigens, similar as in trauma or other injuries, trigger a systemic inflammatory response. DAMPs are released into the circulation in response to stress or tissue injury. These molecules include histones, DNA and biomolecules of mitochondrial origin.[14]

The hypoperfusion seen in sepsis due to endogenous intravascular fluid loss and exogenous losses, besides divert blood flow, decreased vascular tone and myocardial depression all lead to tissue hypoxia and insufficient oxygen for the mitochondria to create ATP. The function of mitochondrial complex IV (CIV) enzymes is compromised in these circumstances and might trigger cell death pathways. There is evidence of the decrease of the complex I mitochondrial activity in patients with septic shock[15] and an association between complex I activity with NO levels. The excessive levels of ROS including NO induce direct damage to the mitochondria respiratory function and to the lipid membrane. The DAMPs, in the early phase of sepsis, induce a downregulation of the genes transcribing mitochondrial proteins that also affects the efficiency of the mitochondrial ATP production and respiratory chain activity. To all the previously described as mitochondrial damage caused by stress, the toxicity induced by polymedication in critical patients is added.

The mitochondrial affectation would end up activating death cell pathways, but as previously stated, this does not seem to be the final route in sepsis. As a hypothesis to explain this, an increase in the production of non-mitochondrial ATP (increased glycolytic activity) and the appearance of a cellular hibernation state[16] have been described, decreasing the global energy requirements so that the ATP levels do not decrease enough to trigger cell death. So instead of the organ failure induced by sepsis, there are authors that talk about energetic and apoptotic shutdown, a reversible situation that could explain the minimal cell death.[17]

Melatonin is an endogenous indolamine synthesized every night by the pineal gland mostly, although it is also found in many other organs and tissues as in lymphocytes, bone marrow, gastrointestinal tract, skin, etc. It is a molecule that interferes in several physiological processes, such as in the circadian cycle, but it has also been shown to act as a modulator in the immune response and antioxidant activity,[18] protecting against free radicals in case of bacterial, viral or parasitic infection.

The importance of the release of the mediators of inflammation and free oxygen radicals and the mechanisms of death in sepsis has already been discussed. Melatonin has an indirect effect in all these processes, promoting the expression of antioxidant enzyme genes on the one hand and on the other it has a significant anti-inflammatory role, reducing the synthesis of cytokines and suppressing nitric oxide synthetase expression genes.

Melatonin, as a scavenger of free radicals, is able to act, through donation of electrons to directly detoxify ROS as the hydroxyl radical and other oxidizing agents such as peroxynitrite anion, singlet oxygen, nitric oxide, and peroxynitrous acid.[19] It also stimulates several antioxidant enzymes including superoxide dismutase, glutathione peroxidase, glutathione reductase, and gamma-glutamyl-cysteine synthase.[19]

Lorente and his group have compared the mitochondrial function between severe sepsis patients and healthy control subjects according to mitochondrial (mtDNA) haplogroup and platelet respiratory CIV activity.[20] It was a prospective, multicentric observational study conducted in 6 ICUs, with a total of 198 septic patients and 96 healthy subjects, of whom samples have been collected on days 1, 4 and 8 from the diagnosis of severe sepsis. Determinations of mtDNA haplogroup and mitochondrial respiratory CIV activity in circulating platelets have been made, the endpoint being 30-day mortality.

Of the 198 septic patients, in 38 the haplogroup JT of mtDNA was detected and the remaining 160 had a mtDNA haplogroup non-JT. While of the 96 healthy subjects, 16 had JT haplogroup and 80 patients had mtDNA haplogroup non-JT.

No significant differences were found in the activity of the CIV complex between healthy individuals and surviving septic patients with JT mtDNA haplogroup on days 1, 4 and 8 after the diagnosis. However, non-surviving septic patients of this cohort, had lower platelet IV complex activity than healthy patients with the same haplogroup on days 1, 4, and 8. Moreover, survivors and nonsurvivors severe sepsis patients with mtDNA haplogroup non-JT have also lower CIV activity than healthy individuals.

In human genetics, the haplogroups are determined by the variations found in human mitochondrial DNA (mtDNA). These haplogroups trace the matrilineal descent to the origins of the human species in Africa and from there, to its subsequent

dispersion over the entire surface of the planet. Haplogroup JT is a human mitochondrial haplogroup typical of Western Eurasia conformed in turn by haplogroups J and T. Descended from the pre-JT haplogroup, its genetic markers are 11251, 15452A and 16126 and have a probable origin 50,000 years ago.

Mitochondria are cytoplasmic organelles responsible for producing energy in the form of ATP, through a system called oxidative phosphorylation system (OXPHOS), formed by the electron transport chain (CTE) and ATP synthase. The two genomes (JT) encode polypeptides that are part of OXPHOS, the final pathway of mitochondrial energy metabolism that leads to the synthesis of ATP.

Previously it had been reported that the OXPHOS function is reduced in septic patients[22] and that there is an association between the haplogroup mtDNA and survival and an association between the haplogroup mtDNA and the CIV specific activity platelet.

This study is interesting and novel because for the first time it is found that the surviving patients with mtDNA haplogroup JT do not present differences at the level of the CIV activity with respect to healthy volunteers and on the contrary, the patients of the same non-surviving haplogroup show a more reduced CIV platelet activity than healthy volunteers and that patients with mtDNA haplogroup non-JT, both survivors and non-survivors, have a more impaired mitochondrial function than healthy volunteers of the same haplogroup. This finding has important clinical implications since it allows the identification of patients with mitochondrial dysfunction that could have worse clinical evolution and could be tributaries of therapeutic measures that aim to treat mitochondrial dysfunction. In addition to the clinical implications already mentioned, this finding also has implications at the research level, since by allowing the identification of patients with mitochondrial dysfunction, opens the door to biomedical research on potential therapeutic agents that modulate the system of OXPHOS.

The same group of Lorente have also assessed the role of melatonin in severe sepsis.[21] The objective of the study was to determine if there is any relationship between the serum levels of melatonin in the first week of the diagnosis of sepsis and the severity and mortality of sepsis.

Therefore, a multicentric, observational study from 2008 to 2009 has been designed, collecting a total of 308 patients with severe sepsis. The levels of melatonin, malondialdehyde and tumor necrosis factor-alpha (TNF-alpha) have been determined at days 1, 4 and 8 from the sepsis diagnosis. The endpoint was 30 days mortality.

At 30 days, 103 patients died and when comparing the levels of melatonin with the 205 surviving patients, higher levels were observed on days 1, 4 and 8 in the non-survivors ($P < 0.001$). When performing a logistic regression, it was observed that the levels of melatonin on days 1, 4 and 8 are associated with mortality adjusted by age, lactate, SOFA and diabetes mellitus.

Lorente et al. found significantly higher levels of melatonin in non-survivor septic patients and this relationship is maintained for the first seven days. They also found a correlation, albeit weakly positive, with organ dysfunction, inflammatory response and lactate levels. Previously, his group had already described that in the initial period of sepsis, melatonin levels are higher in non-survivor septic patients, there being an association between melatonin levels and the mortality of septic patients.[23] In addition, in this study they also found a positive association between melatonin levels and SOFA score, IL-6 and lactate levels. The novelty of the current study is that these findings are maintained during the first week of sepsis evolution. They argue that the association between melatonin and mortality may be due to the fact that non-survivor patients have a higher oxidative state than survivor patients, demonstrated by an increase in malondialdehyde levels and that the increase in melatonin levels in the non-survivors could reflect a compensatory response to try to control, although without success, the oxidative state. This is an attractive theory and would support the hypothesis that the exogenous administration of melatonin could have a beneficial effect in patients with sepsis. However, it would have been desirable that Lorente et al. show in the current study a more detailed analysis of cytokines (especially anti-inflammatory ones) and different components of the oxidative system in order to evaluate their inter-relationships. Although, it seems that melatonin may have utility as a biomarker of poor prognosis, more studies are needed to help elude the mechanisms of action through which melatonin may have a beneficial role in sepsis.

We anticipate a future in which personalized medicine is available for patients with sepsis and is guided by repeated measurements of biomarker sets that reflect targetable malfunction in several pathways, like the ones discussed in this manuscript and others.

Conflict of interest

None

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