Oxidative stress leads to severe phenotypes in sepsis through activation of NLRP3-pyroptosis

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Abstract

Oxidative stress is an important contributor to sepsis and one of the most important causes of death in intensive care units. Even though sepsis pathogenesis remains obscure due to its heterogenicity and complexity, there is increasing evidence that oxidants and antioxidants play a key role in its onset and progression. Recent evidence suggests that pyroptosis is required for defense against bacterial infection, and it is active in several cell types during sepsis. One of the most relevant mediators of pyroptosis is the NLRP3 inflammasome, and the oxidative stress/NLRP3 signaling pathway is one of the main upstream signals involved in its activation. So, it is of special relevance to clarify how oxidative stress and antioxidants can modulate pyroptosis signals and therefore decrease the deleterious effects that both oxidative stress and pyroptosis-related cytokines can induce in the tissues during sepsis. Recent studies evaluating several antioxidants are promising, but further trials are needed to confirm their potential role as agents to block NLRP3 and mitigate the exacerbated inflammasome-related responses during sepsis.

Introduction

Sepsis is a life-threatening condition caused by an abnormal host response to an infection that produces altered physiological responses damaging patients' own tissues and results in organ dysfunction and death (Singer et al. 2016).

Sepsis is the leading cause of mortality in the intensive care units (ICU) (Angus et al. 2001). Specifically, the latest epidemiologic studies estimate almost 50 million cases of sepsis worldwide and up to 10 million deaths every year (Rudd et al. 2020, Beltrán-García et al. 2022).

Clinically, sepsis is characterized by a simultaneous hyperinflammatory and anti-inflammatory response, which contribute to the deregulation of the patient’s immune system (Nakamori et al. 2020, Lazzaro et al. 2022). Moreover, circulatory changes, cardiovascular problems (Beltrán-García et al. 2021), neurological deficits (Osca-Verdegal et al. 2021), mitochondrial dysfunction and impaired respiration (Mantzaris et al. 2017) occur in septic patients, increasing the risk of death.

Regarding the deregulation of the immune system during sepsis pathophysiology, pyroptosis has been proposed as one of the mechanisms that mediate sepsis immune dysregulation (Nakamori et al. 2020). Specifically, pyroptosis is defined as a mechanism of programmed cell death that mediates inflammation (Nakamori et al. 2020). In recent years, it has been demonstrated that this type of cell death occurs in many human cells, affecting the immune system (Boise & Collins 2001), CNS (Liu et al. 1999) and cardiovascular system (Yue et al. 2021), among others, highlighting the importance of this mechanism in a large number of biological systems. Likewise, pyroptosis has been described as a key mediator of a wide range of...
diseases, including sepsis (Gao et al. 2018). The reason is that pyroptosis plays a fundamental role in regulating the early immune response against numerous infections, including bacterial, viral, protozoan or fungal infections (Man et al. 2017).

Pyroptosis is triggered by various stimuli, such as changes in membrane potential, oxidative stress, hyperglycemia and even inflammation itself (Lamkanfi & Dixit 2011) (Fig. 1). Among them, oxidative stress has been postulated as a central mediator of pyroptosis, playing a critical role in sepsis (Yang et al. 2019). Recent sepsis guidelines (SEPSIS-3) do not consider oxidative stress as an important contributor to sepsis pathophysiology (Singer et al. 2016). Nonetheless, despite the increasing evidence of the role that oxidative stress is playing in sepsis, the implications of oxidative stress in the activation of pyroptosis have been little explored in sepsis.

The aim of this review is to point out the role of oxidative stress in the modulation of pyroptosis and therefore the immune system during sepsis, as well as their role as an effective goal for therapy in sepsis disease.

**Inflammasome activation is mediated by oxidative stress**

The immune system can be activated in response to pattern recognition receptors (PRRs), which are derived from pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) produced from exogenous and endogenous mediators, respectively (Kang et al. 2015), triggering downstream inflammatory pathways to eliminate microbial infection and repair damaged tissues (Christgen et al. 2020).

Inflammasomes are a group of intracellular multimeric protein complexes that activate inflammatory caspase-1 by the cleavage of pro-caspase-1 (Franchi et al. 2009). Inflammasome's activation is a major inflammatory pathway and as being part of innate immunity, it can be induced by infectious agents and also by sepsis (Franchi et al. 2009, Christgen et al. 2020). Some inflammasomes are activated by AMPs and DAMPs, while others are activated by cell homeostatic environment modifications caused by those patterns (Christgen et al. 2020). The sensor protein (PRR) characterizes the inflammasome. Five different PRR have been described to form inflammasomes: the nucleotide-binding oligomerization domain (NOD), absent-in-melanoma 2 (AIM2), leucine-rich repeat (LRR)-containing proteins, nucleotide-binding oligomerization domain-like receptor (NLR) family members NLRP1, NLRP3 and NLRC4, in addition to pyrin (Lamkanfi & Dixit 2014, Sharma & Kanneganti 2016).

Among the different PRRs, NLR family members and particularly NLRP3 stands out over others since it is the most extensively studied inflammasome. NLRP3 is a crucial protein participating in the innate immune response to infection (Sutterwala et al. 2014). The most probable reason for the widespread interest in NLRP3 above other NLRs is because it is activated by several stimuli such as extracellular ATP, biological particulate matter and pore-forming toxins and pathogens, including bacteria, viruses and fungi (Mariathasan et al. 2006,
Martinson et al. 2006, Li et al. 2008, Gross et al. 2009, Yazdi et al. 2010, Wang et al. 2014). Importantly, NLRP3 was the only one activated by oxidative stress (Sharma et al. 2018). In this regard, Lucena Lage et al. demonstrated recently a direct relationship between lipid peroxidation and NLRP3 activation, broadening the several activators of NLRP3 as a consequence of increased oxidative stress (Lage et al. 2021).

Specifically, NLRP3 inflammasome is formed by different subunits: the NLR family, pyrin domain (PYD)-containing 3 (NLRP3) and an adapter molecule, apoptosis-associated speck-like protein containing a CARD (ASC), which is an adapter that connects the PRR with the downstream effector, caspase-1 (CASP1) (Christgen et al. 2020). After the activation of CASP1, it processes the proinflammatory cytokines interleukin-1β (IL-1β) and IL-18 into their mature forms (Thornberry et al. 1992). Moreover, CASP1 is also responsible for cleaving gasdermin D (GSDMD) protein, which oligomerizes to form pores in the cell membrane, allowing the proinflammatory cytokines to leak out of the cell, thus completing pyroptotic cell death (Yaron et al. 2015, Liu et al. 2016, Sborgi et al. 2016, Ding et al. 2016) (Fig. 1). Activation of NLRP3 inflammasome enables several immune cells such as monocytes and macrophages to release high levels of interleukin 1-α (IL-1α) and IL-18 into the cell, thus completing pyroptotic cell death (Yaron et al. 2015, Liu et al. 2016, Sborgi et al. 2016, Ding et al. 2016) (Fig. 1). Activation of NLRP3 inflammasome enables several immune cells such as monocytes and macrophages to release high levels of interleukin 1-α (IL-1α) and IL-18 into the cell, thus completing pyroptotic cell death (Yaron et al. 2015, Liu et al. 2016, Sborgi et al. 2016, Ding et al. 2016) (Fig. 1).

NLRP3 upstream regulatory molecules

The upstream regulatory molecules participating in NLRP3 inflammasome activation have not been yet well characterized. It is unknown if NLRP3 is activated directly by a specific ligand through indirect signals or even through both mechanisms. Many direct stimuli have been described as NLRP3 inflammasome activators, such as ATP, K+ and Ca2+ dysregulation, lysosomal disruption by crystalline particulates, mitochondrial DNA (mtDNA) and ROS (Mariathasan et al. 2006, Dostert et al. 2008, Hornung et al. 2008, Yaron et al. 2015, Zhong et al. 2018). Owing to the high number of molecules able to activate the NLRP3 inflammasome, and the chemical and structural differences existing between some of the molecules inducing inflammasome activation, it is expected that there is not a single ligand activating this inflammasome. A new study has described a protein necessary for NLRP3 activation, so it is feasible to think that NLRP3 inflammasome is activated by indirect pathways (Samir et al. 2019). Specifically, the authors found that DDX3X, which is sequestered by stress granules found in cytoplasmic compartments, helps cells survive different stressors (Protter & Parker 2016). Since DDX3X drives inflammasome activation, NLRP3 inflammasome and stress granules compete for DDX3X molecules to orchestrate the innate responses activation and subsequent cell-fate decisions under stress conditions (Samir et al. 2019).

Although upstream pathways are not well elucidated (Yuk et al. 2020), it was shown that K+ efflux activates NLRP3 in response to a high amount of stimuli, including membrane pores formed by toxins, and has been proposed to be a common mechanism of NLRP3 activation (Pétrilli et al. 2007, Muñoz-Planillo et al. 2013). In fact, trans-golgi network (TGN) disturbance has been related to the activation of NLRP3 through K+-dependent and K+-independent NLRP3 activators (Chen & Chen 2018). Nevertheless, NLRP3 inflammasome can be activated also by molecules that are K+-efflux independent, such as those small molecules that target the mitochondria (Groß et al. 2016), which release ROS and mtDNA. Importantly, both ROS and mtDNA have been involved in NLRP3 inflammasome activation, emphasizing the mitochondrial relevance for NLRP3 activation (Dostert et al. 2008, Misawa et al. 2013, Liu et al. 2018, Zhong et al. 2018).

Occasionally, NLRP3 levels within the cell are not enough to generate a strong inflammasome activation. Therefore, the current NLRP3 canonical activation model proposes a two-step process. The first signal guides the upregulation of NLRP3 inflammasome components, at transcriptional and post-translational levels, facilitating its activation (Sutterwala et al. 2014, Christgen et al. 2020). After priming, a later unchain signal is required. In this scenario, NLRP3 oligomerizes and binds to ASC, and inflammasome activation continues (Sutterwala et al. 2014, Christgen et al. 2020) (Fig. 1).

NLRP3 activation mediated by ionic imbalance

There are many DAMPs and PAMPs that cause an efflux of K+ activating NLRP3 inflammasome. These molecules can cause a K+ efflux by direct pathways, such as nigericin, that forms pores on plasma membranes, or by indirect pathways, such as ATP-induced NLRP3 inflammasome activation, that is activated by the passage of ATP through P2X7, a cation channel that works as a receptor of extracellular ATP (Gustin et al. 2015, Di et al. 2018, Gong et al. 2018, Hafner-Bratković & Pelegrín 2018). However, potassium efflux has also been found to be mediated by GSDMD (Shi et al. 2015, Orning et al. 2018, Sarhan et al. 2018, Chen et al. 2019).

One of the major events that modulate the activation of NLRP3 is changes in the membrane potential. In
fact, several authors demonstrated that damaging the plasmatic membrane could increase the levels of NLRP3 (Beckwith et al. 2020, Paik et al. 2021), and this activation is usually mediated by changes in the membrane potential led by different ion concentrations (Fig. 2).

In this regard, potassium has a prominent role in modulating the activation of NLRP3 (Muñoz-Planillo et al. 2013, Gong et al. 2018), since other inflammasomes’ activation is not affected by the concentration of K⁺. In fact, lower concentration of cytosolic K⁺ has been related to induction of NLRP3 activation and high concentration of K⁺ has been associated with an inhibition of NLRP3 inflammasome activation (Franchi et al. 2007, Pétrilli et al. 2007, Muñoz-Planillo 2013).

Similarly, numerous studies indicated that NLRP3 inflammasome requires calcium influx or mobilization for its activation (Compan et al. 2012, Lee et al. 2012, Murakami et al. 2012, Zhong et al. 2013, Yaron et al. 2015). Nigericin, one of the best-characterized pyroptosis NLRP3-specific inducers, works by causing plasma membrane pores that allows the Ca²⁺ in-flow, therefore activating NLRP3 inflammasome (Murakami et al. 2012). Likewise, Brough et al. demonstrated that when Ca²⁺ is chelated, the inflammasome activation is inhibited (Brough et al. 2003). Other authors also demonstrated how the endoplasmic reticulum (intracellular Ca²⁺ reservoir) plays a critical role in modulating the activation of NLRP3 (Lee et al. 2012). Increased levels of extracellular Ca²⁺ induce the NLRP3 activation by stimulating G protein-coupled calcium-sensing receptors and GPRC6A and activation of the phosphatidyl inositol/Ca(2+) pathway (Rossol et al. 2012).

Additionally, other ions like Cl⁻ have been related to NLRP3 inflammasome activation. Verhoef and colleagues reported a decrease in intracellular Cl⁻-induced IL-1β secretion (Verhoef et al. 2005). In this regard, Cl⁻ channel blockers such as flufenamic acid, mefenamic acid and benzoic acid, among others, have been demonstrated to be able to inhibit NLRP3 inflammasome (Channappanavar et al. 2016, Daniels et al. 2016, Tang et al. 2017). Specifically, they found that a reduction in intracellular Cl⁻ concentration leads to an increase in cation flow hence producing the activation of NLRP3. Moreover, other authors also demonstrated that chloride intracellular channels act downstream of the potassium efflux–mitochondrial reactive oxygen species (ROS) axis to promote NLRP3 inflammasome activation (Daniels et al. 2016, Tang et al. 2017). These findings demonstrate that Cl⁻ efflux and influx play an important role in NLRP3 inflammasome activation and could be even more important in neuroinflammation, as a consequence of GABA receptors activation that modulates NLRP3 activation through changes in intracellular Cl⁻ (Lang et al. 2020, Omer et al. 2020).

Comparable to K⁺, Ca²⁺ and Cl⁻, Na⁺ is other molecule that is involved in NLRP3 activation (Muñoz-Planillo et al. 2013). Na⁺ influx activates NLRP3 inflammasome by reducing the decrease in intracellular K⁺ (Schorn et al. 2011). So, Na⁺ influx can modulate the activation of NLRP3 by decreasing intracellular K⁺. Moreover, other ions such as Zn²⁺ have also been proposed to induce the activation of NLRP3 in some immune cells such as macrophages (Summersgill et al. 2014), because zinc depletion damages the integrity of the lysosomal
Mitophagy and mitochondrial oxidative stress

Autophagy is defined as a non-selective degradation process for intracellular cargos (Yuk et al. 2020), but it has been also described as a selective process that is able to target some organelles and substances (Khaminets et al. 2016). Particularly, mitophagy has been described as a selective autophagy process that is able to target and destroy the mitochondria for maintaining its homeostasis, through regulation of mitochondrial ROS generation and elimination of damaged mitochondria (Ashrafi & Schwarz 2013, Wei et al. 2015, Hamacher-Brady & Brady 2016, Gustafsson & Dorn 2019). Mitophagy dysregulation causes a cascade of reactions, including mitochondrial dysfunction, increased production of mitochondrial ROS and translocation of mtDNA into the cytosol, leading to the inflammasome's activation, mitochondrial dysfunction, increased production of mitochondrial ROS and translocation of mtDNA into the cytosol (Zhao et al. 2018, Fan et al. 2019, AA et al. 2020), since mtDNA acts as a crucial DAMP signal to activate NLRP3 inflammasome (Elliot & Sutterwala 2015, Jin et al. 2017, Bae et al. 2019). In this regard, defective mitophagy leads to mitochondrial dysfunction and impairment of mitochondrial membrane integrity, opening mitochondrial permeability transition pore (mPTP) which causes an mtDNA leak from damaged mitochondria, thus forming a vicious cycle that ultimately contributes to inflammation (Harrington et al. 2017, Rottenberg & Hoek 2017, Xu et al. 2020) (Fig. 2). This is of special interest since defective mitophagy causes inflammasome activation, which in turn contributes to the pathogenesis of many human diseases, including sepsis (Harris et al. 2018, Kim et al. 2016, Wu et al. 2019).

As previously described, the inflammasome is activated as a consequence of mitochondrial perturbation and dysfunction. It is noteworthy that the NLRP3 inflammasome is highly active due to an overload of mitochondrial Ca$^{2+}$ that increases the generation of mitochondrial ROS (Tschopp 2011, Abais et al. 2015, Elliot & Sutterwala 2015, Yu & Lee 2016).

Mitochondrial DAMPs (mtDAMPs) have gained relevance among researchers in the last years. They include mtDNA, mitochondrial transcription factor (TFAm), cardiolipin and ATP (Khwaja et al. 2021). These molecules are released during tissue injury and activate a similar immune response to that caused by pathogens (Kang et al. 2015). Among them, probably the most important molecule in this process is mtDNA, whose release causes the activation of NLRP3 inflammasome through TLR9-NF-κB pathway (Shimada et al. 2012, West & Shadel 2017, Khwaja et al. 2021). The mtDNA can be leaked into the cytosol easily when the mitochondrial membrane potential is decreased as a consequence of mitochondrial stress (Harrington et al. 2017, Liu et al. 2018). Recently, the relationship between mtDNA, mitochondrial ROS and NLRP3 has been described (Sok et al. 2021, Xian et al. 2021). Sok et al. observed that by inhibiting the release of oxidized mitochondrial DNA, the mitochondrial ROS generation was decreased and subsequently, NLRP3 inflamasomes were inhibited (Sok et al. 2021). Wu et al. (2020) performed several experiments inducing delayed resuscitation in rats and showed that delayed resuscitation caused liver damage and oxidative stress, developing a liver injury through mitochondrial destruction and mtDNA/NLRP3 axis activation. Moreover, they also observed that the structure and function of mitochondria were protected by antioxidants directed to inhibit the release of mtDNA (Wu et al. 2020). These results denoted that mtDNA has a direct role in the development of systemic inflammation and organ dysfunction, via the NLRP3 inflammasome. Interestingly, some studies have correlated the plasmatic levels of mtDNA with the septic shock clinical outcome (Jiménez-Sousa et al. 2015, Hu et al. 2017, Schneck et al. 2020) and have proposed mtDNA as a biomarker to predict mortality in patients with sepsis better than lactate concentration or sequential organ failure assessment score (SOFA) (Kung et al. 2012). Since elevated circulating levels of mtDNA have been directly linked to the activation of NLRP3 inflammasome, it is plausible to use the circulating mtDNA levels as a biomarker for sepsis severity and prognosis.

Evidence supporting the relevance of oxidative stress in sepsis therapies

NLRP3 has been postulated as an important mediator of sepsis (Cassel & Sutterwala 2010, Kalbitz et al. 2016, Zhang et al. 2019), and some therapeutic strategies under research are directed to block NLRP3 overactivation, demonstrating their important role in sepsis development and pathogenesis (Danielski et al. 2020, Zhang & Ning 2021).

In recent years, several studies have postulated that sepsis pathogenesis is affected by oxygen metabolism accelerating the disease progression and causing multiple organ failure (Kozlov & Grillari 2022). Similarly, the cGMP-dependent protein kinase 1 alpha (PRKG1) activation...
by oxidative stress induces a blood vessel dilatation, increasing permeability and therefore decreasing cardiac output (Banday & Lokhandwala 2019) and organ perfusion as well as oxygen delivery, inducing ischemia to organs and finally organ failure (Rudyk et al. 2013). In addition, excessive oxidative stress is a common feature of sepsis, mainly the disruption of the redox homeostasis (Lopes-Pires et al. 2021). For example, some reactive species found in sepsis pathogenesis include superoxide (O$_2^-$), hydroxyl radical (·OH), hydrogen peroxide (H$_2$O$_2$), peroxynitrite (ONOO$^-$) and hypochlorous acid (HOCl) (Parihar et al. 2008). Conversely, levels of some antioxidants such as α-tocopherol (Takeda et al. 1984, Ogilvie et al. 1991), selenium (Mishra et al. 2007, Sakr et al. 2007), ascorbic acid (Borrelli et al. 1996), reduced glutathione (GSH) (Brealey et al. 2002), vitamin A, β-carotene and lycopene (Rossol et al. 2012) have been found to be decreased in septic patients. Consequently, therapies targeting redox abnormalities have been postulated as promising strategy for improving the management and treatment of septic patients.

Several antioxidants have been studied in animal models as well as in clinical trials to develop effective therapies against sepsis. In this regard, Escames et al. tested melatonin in a mice model of sepsis (cecal ligation puncture (CLP) sepsis model) (Escames et al. 2007). They observed that sepsis increased the expression levels of iNOS and mitochondrial NOS, accompanied by an increase in oxidative stress and reduced ATP production and respiratory chain impairment, despite ATPase levels did not change. In addition, mitochondrial NOS induction was associated with heart mitochondrial dysfunction. The treatment with the antioxidant and circadian regulator melatonin was able to reduce iNOS and mitochondrial NOS expression levels avoiding the mitochondrial homeostasis impairment in sepsis mice and reestablishing mitochondrial ATP production (Escames et al. 2007).

Similarly, in a CLP sepsis study in rats, sepsis induced a decrease in GSH concentrations while producing an increase in malondialdehyde (MDA) levels and myeloperoxidase (MPO) activity (neutrophil aggregation) in the evaluated tissues (liver, lung, heart, kidney and brain). Likewise, sepsis also led to higher plasma aspartate aminotransferase (AST) and alanine transaminase (ALT) activity, as well as creatinine (Cre) levels and blood urea nitrogen. Treatment with melatonin (10 mg/kg i.p.) before and after the surgery restored the MDA levels in the evaluated tissues and restored GSH levels in kidney, diaphragm and brain. In cardiac and pulmonary tissues, no improvement was reported. Moreover, melatonin treatment reduced MPO activity in all tissues and returned to normal levels of AST, ALT, blood urea nitrogen (BUN) and Cre levels (Zhang et al. 2017). These findings highlight the potential of melatonin as therapy or co-therapy (supporting therapy) for sepsis disease.

Another promising antioxidant in sepsis is N-acetylcysteine (NAC). Some studies demonstrated that lipopolysaccharide (LPS) injections into mice (LPS sepsis model) increase BUN, LDH, Cre and CPK levels, as well as other pro- and anti-inflammatory cytokines such as IL-1β, TNF-α, IL-6 and IL-10 levels. The mean arterial blood pressure (MAP) was found to be decreased after exposition to LPS mice treatments, which represents a serious risk of death and end-organ failure. Conversely, treatment with NAC moderately avoids the decrease in MAP and markers of organ injury (BUN, LDH, Cre and CPK) and pro- and anti-inflammatory biomarkers (IL-1β, TNF-a, IL-6, and IL-10) caused by LPS injection (sepsis) (Hsu et al. 2006).

The work by Blackwell and colleagues supports the idea of the use of NAC as potential treatment against sepsis (Blackwell et al. 1996). In this work, authors administered NAC before injecting endotoxin to rats (sepsis animal model) demonstrating that NAC reduced NF-kB activation in a dose-dependent manner, reducing the cytokine-induced neutrophil chemoattractant expression in lung tissue (Blackwell et al. 1996). Since NF-kB is a master regulator of NLRP3 and pyroptosis (Cornut et al. 2020), NAC treatment, through downregulating NF-kB activation, demonstrated to be able to diminish the damage and the inflammatory response, hence alleviating several symptoms of sepsis typically associated to respiratory distress (Blackwell et al. 1996).

Other well-known antioxidants are vitamin C and vitamin E. Both vitamins have demonstrated their therapeutic potential against sepsis in in vivo studies. For example, recently, in a study carried on by Marik et al., vitamin C reduced the elevation of aminotransferase and hepatic lipid peroxide and MDA levels in a CLP sepsis rat model. Moreover, hepatic GSH concentration was higher in the sepsis group, after vitamin C treatment. In addition, vitamin C also prevented the expression of the vasodilatory gene ET1 and its receptor ETB in the liver. Finally, the increased levels of iNOS and heme oxygenase-1 found in septic rats were also attenuated by vitamin C (Kim & Lee 2004).

Similarly, in another study, it was demonstrated that not only vitamin C but also vitamin E treatment reduced the increase of aminotransferase and MDA during sepsis. Similar to the previous study, the decreased expression levels of hepatic CYP1A1 and CYP2E1 found in rat sepsis model were prevented by both vitamins, vitamins C and E.
Since elevated levels of aminotransferase and MDA have been related to elevated levels of NLRP3 (Wu et al. 2017, Lin et al. 2018, Chen et al. 2020), it is plausible that antioxidant combinatory therapy could modulate inflammasome activation and therefore prevent the hyperinflammatory response in septic patients.

Regarding therapies already being applied in sepsis, Manzanares and colleagues performed a meta-analysis study on the effects of antioxidant co-therapy in septic patients based on 21 clinical trials (Manzanares et al. 2012). They found that the antioxidant combinatory therapy induced a reduction of the mechanical ventilation need and a decrease in infections, although there was no effect related to ICU or hospital length of stay. Furthermore, the use of combined antioxidants was correlated with a mortality reduction in septic patients with elevated risk of death (Manzanares et al. 2012).

From different clinical studies performed so far, selenium, used as co-adjuvant antioxidant therapy, demonstrated to be one of the most feasible candidates to be used as a therapy, since its use showed decreased mortality in critically ill patients (Hardy et al. 2012, Huang et al. 2013). Specifically, in sepsis, clinical studies performed on septic patients demonstrated that selenium supplementation increased glutathione peroxidase activity, as well as a significant negative correlation between plasmatic selenium and SOFA score (Mishra et al. 2007). Similarly, another clinical study performed on critically ill patients in ICUs reported that selenium supplementation reduced renal failure and improved patient outcomes (Angstwurm et al. 1999).

Several clinical studies, applying antioxidant molecules as therapy or co-adjuvant therapy against sepsis, have been developed, with the aim of ameliorating the effects of oxidative stress in septic patients. So far, 47 clinical trials have been registered on clinicaltrials.gov, focused to mitigate the damage mediated by oxidative stress in sepsis. The strategies developed to date are diverse, from the use of cellular antioxidants such as selenium (NCT00207844) or glutamine (NCT00133978, NCT01367223) to vitamin C and vitamin D (NCT04216459) or vitamin B1 (NCT03380507), some molecules with antioxidant activity as melatonin (NCT03295162, NCT01724424) and other strategies focused on decreasing oxidative damage as probiotic-based dietary supplement (NCT01474629), specific molecules as acetaminophen (NCT01739361), dopexamine, epinephrine and norepinephrine (NCT00134212), deferasirox (NCT01349699) or atazanavir (NCT00916448), or even some medical strategies such as polymyxin B hemoperfusion (NCT01756755). Nevertheless, despite several of these therapies having already demonstrated improved clinical outcomes in septic patients, many of these proposed therapies have yet demonstrated enough efficacy to be used as a sepsis treatment alone. Further studies are needed in order to demonstrate its potential as co-adjuvant agent to be used in a combinatory therapy against sepsis (see Table 1).

Conclusions

It is broadly accepted that oxidative stress plays an important role in the pathogenesis of sepsis (Prauchner et al. 2017). In physiological conditions, an equilibrium between oxidant and antioxidant systems inside the cell usually occurs. Oxidative stress is an important contributor to sepsis physiopathology (Mantarlis et al. 2017). Continuous oxidative stress leads to an increase in the inflammatory response, inducing mitochondria alterations, which in turn can lead to mitochondrial dysfunction and finally cell death, mediating sepsis-induced organ failure (Exline & Crouser 2008).

It is noteworthy, that during an inflammatory process, the immune system produces a huge amount of oxidant species in a process called respiratory burst, which contributes to the immune defense against invading pathogens. However, ROS produced during the respiratory burst can also damage cellular components and interestingly can act as signals to modulate the initiation of pyroptosis, through the activation of NLRP3, since it is the only type of inflammasome activated by oxidative stress. Furthermore, in recent years, the notion that sepsis pathogenesis also involves an inability of the cell to consume oxygen has increased. Because consumption of molecular oxygen by mitochondria accounts for 98% of the body’s O₂ usage (Singer 2014), it is expected that problems in O₂ consumption contribute to mitochondrial dysfunction and play an important role in the pathogenesis of sepsis (Singer 2014, Rahmel et al. 2020). Despite sepsis being a heterogeneous and complex condition, oxidative stress is a common feature of sepsis, so therapies focused on reducing the redox changes may be useful to improve the management of septic patients, specifically therapies directed to modulate the NLRP3 activation, which subsequently attenuates the inflammatory response during sepsis (Zhang et al. 2018). Unfortunately, a whole understanding of the mechanisms leading to oxidative stress in sepsis progression is not currently available; however, it is well-established that an energy deficit in cells by mitochondrial dysfunction and impaired vascular tone are central events that lead to multi-organ dysfunction and
Table 1  Sepsis therapies have been tested currently in clinical trials modulating oxidative stress levels.

| Drug/therapies       | NCT          | Recruitment status | Type of study                  | Status | Sponsor                                                      |
|----------------------|--------------|--------------------|--------------------------------|--------|--------------------------------------------------------------|
| Selenium             | NCT00207844  | Completed          | Interventional                 | Ph. 2  | Centre Hospitalier de Meaux, Ministry of Health, France    |
| Glutamine            | NCT00133978  | Completed          | Interventional                 | N.A.   | Clinical Evaluation Research Unit at Kingston General Hospital, Canada (Fundació Sant Joan de Déu, Spanish National Health System, Spain) |
|                      | NCT01367223  | Completed          | Interventional                 | Ph. 4  | Mansoura University, Egypt                                  |
| Vitamin C and vitamin D | NCT04216459  | Completed          | Interventional                 | Ph.2-Ph.3 | Hamad Medical Corporation, Qatar                          |
| Vitamin B1           | NCT03380507  | Completed          | Interventional                 | Ph.1-Ph.2 | Ain Shams University, Egypt                               |
| Melatonin            | NCT03295162  | Completed          | Interventional                 | Ph.1   | University of Aberdeen, Scotland, UK                       |
| Acetaminophen        | NCT01739361  | Completed          | Interventional non-randomized  | Ph. 2  | Vanderbilt University, Tennessee, USA                      |
| Dopexamine, epinephrine and norepinephrine | NCT00134212 | Completed          | Interventional randomized      | Ph. 2  | Rennes University Hospital, France                         |
| Deferasirox          | NCT01349699  | Completed          | Interventional randomized      | N.A.   | Radboud University Medical Center, Netherlands             |
| Atazanavir           | NCT00916448  | Completed          | Interventional randomized      | N.A.   | Radboud University Medical Center, Netherlands             |
| Polymyxin B hemoperfusion | NCT01756755  | Completed          | Interventional randomized      | N.A.   | National Taiwan University Hospital, Taiwan                |

N.A., not applicable; Ph, clinical phase.

therefore poorer clinical outcomes and increased mortality in septic patients (Mantzaris et al. 2017).

In this regard, numerous trials have been performed to identify potential treatments against sepsis over the years. Particularly, antioxidants targeting mitochondria stand out among them, since they can accumulate inside the inner membrane of the mitochondria, and therefore, they can modulate different molecular mechanisms such as pyroptosis, inflammation and cellular respiration, among other molecular events that occur during sepsis. Nevertheless, the results obtained in clinical trials are not sufficient for their implementation in sepsis management, and further research is still needed, especially to understand the molecular mechanisms that underlie oxidative stress during sepsis physiopathology and its contribution to NLRP3 inflammasome activity.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Author contribution statement
J B-G and R O-V: manuscript drafting. F V P and J L G-G: critical revision. All the authors read and approved the final manuscript.

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