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Review Article

Pharmacological utility of melatonin in the treatment of septic shock: experimental and clinical evidence

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Abstract

Sepsis is a major cause of mortality in critically ill patients and develops as a result of the host response to infection. In recent years, important advances have been made in understanding the pathophysiology and treatment of sepsis. Mitochondria play a central role in the intracellular events associated with inflammation and septic shock. One of the current hypotheses for the molecular mechanisms of sepsis is that the enhanced nitric oxide (NO) production by mitochondrial nitric oxide synthase (mtNOS) leads to excessive peroxynitrite ($ONOO^-$) production and protein nitration, impairing mitochondrial function. Despite the advances in understanding of its pathophysiology, therapy for septic shock remains largely symptomatic and supportive. Melatonin has well documented protective effects against the symptoms of severe sepsis/shock in both animals and in humans; its use for this condition significantly improves survival. Melatonin administration counteracts mtNOS induction and respiratory chain failure, restores cellular and mitochondrial redox status, and reduces proinflammatory cytokines. Melatonin clearly prevents multiple organ failure, circulatory failure, and mitochondrial damage in experimental sepsis, and reduces lipid peroxidation, indices of inflammation and mortality in septic human newborns. Considering these effects of melatonin and its virtual absence of toxicity, the use of melatonin (along with conventional therapy) to preserve mitochondrial bioenergetics as well as to limit inflammatory responses and oxidative damage should be seriously considered as a treatment option in both septic newborn and adult patients. This review summarizes the data that provides a rationale for using melatonin in septic shock patients.

Introduction

Sepsis is a common cause of mortality in intensive care units (Baker & Huynh 1995; Sands et al 1997) and develops as a result of the host response to microbial invasion (Peters et al 2003). Sepsis is clinically characterized by severe hypotension and hyperactivity to vasoconstrictor agents and often culminates in multiple organ failure. Significant complications from sepsis include central nervous system dysfunction, adult respiratory distress syndrome, liver failure, acute renal failure and disseminated intravascular coagulation.

Sepsis is defined as infection with evidence of systemic inflammation (Annane et al 2005; Calandra & Cohen 2005) and can be caused by bacterial pathogens, fungi, viruses and parasites. Lipopolysaccharide (LPS), a component of the cell walls of Gram-negative bacteria, is the predominant agent responsible for the initiation of sepsis (Sriskandan & Cohen 1995; Peters et al 2003). LPS initially binds to the acute-phase LPS-binding protein in the plasma (Jack et al 1997). Although multiple mammalian receptors for LPS have been identified, the most important receptor appears to be CD14. It has been suggested that TRL4 plays a key role in the immune response to Gram-negative infections (Laflamme & Rivest 2001). Under these conditions, there is a rapid activation of the innate immune response and the release of a variety of humoral mediators. LPS induces gene activation and, hence, inflammatory mediator expression (Guha & Mackman 2001). LPS activates a number of intracellular signalling pathways, including nuclear factor κB, thereby allowing rapid gene induction and the expression of inflammatory mediators, which include, in addition to cytokines, chemokines, lipid mediators, inducible

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nitric oxide synthase (iNOS), enzyme activities, adhesion molecules, myocardial depressant substances and heat-shock proteins (Muller et al 2002; Victor et al 2004; Tsiotou et al 2005). In addition, LPS directly inhibits both glucose and lipid metabolism and causes hepatotoxicity, renal failure and lipid peroxidation (LPO) via the induction (Crespo et al 1999). LPO, induced by free radicals, is an important cause of destruction and damage to cell membranes, since polyunsaturated fatty acids of cellular membranes are readily degraded by this process with consequent disruption of membrane integrity. Membrane peroxidation leads to changes in membrane fluidity and permeability and also the rates of protein degradation are accelerated, eventually leading to cell lysis (Garcia et al 1997). Besides these reactions, which are induced by invading microorganisms, also included are the activation of neutrophils, monocytes and microvascular cells (Tsiotou et al 2005). Leukocytes release numerous proteases to combat infections. The progression of the inflammatory response leads to multiple organ dysfunction and multiple organ failure (Hack & Zeerleder 2001).

Despite recent progress in critical care therapy, sepsis is still associated with a high mortality rate (40–50%) (Cohen 2002; Bhatia & Moochhala 2004). Under normal conditions, an equilibrium between proinflammatory and anti-inflammatory mediators exists, but this balance is disrupted in sepsis and the disturbance is manifested by profound changes in the relative levels of production of different mediators (Pinsky 2001).

Endothelial dysfunction plays a central role in the pathogenesis of sepsis (Hack & Zeerleder 2001). The endothelium is a major target of sepsis-induced substance generation and endothelial cell damage accounts for much of the pathology of septic shock (Morrison & Ulevitch 1978; Brackett et al 1990). In addition, endothelial cells themselves may generate proinflammatory mediators in response to bacterial pathogens (Hack & Zeerleder 2001; Henneke & Golenbock 2002). Cyclooxygenase (COX)-2 is induced by proinflammatory stimuli in migratory cells and inflamed tissues (Vane et al 1998). Prostaglandins formed from arachidonic acid by the enzymatic action of COX-2 also play important roles in inflammation (Keifer & Dannhard 2002). The result is an increase in microvascular permeability, oedema formation and hypotension, resulting in a hyperdynamic shock characterized by increased cardiac output and loss of peripheral vascular resistance (Peters et al 2003; Tsiotou et al 2005).

There is considerable evidence supporting a link between free radicals and endothelial injury. Experimental and clinical data reveal that endothelium-derived substances such as endothelin and nitric oxide (NO) are two important mediators in the pathogenesis of septic shock, leading to fatal multiple organ dysfunction (Iskit & Guc 2003). The potent vasoconstrictor, endothelin, appears to produce mesenteric ischaemia, which is also considered an important factor related to the high mortality seen in sepsis-related syndromes (Baykal et al 2000a).

Cytokines in sepsis

An improper activation of the inflammatory processes with increased proinflammatory cytokines characterizes sepsis and septic shock. Cytokines are immunoregulatory peptides with potent inflammatory actions. Tumour necrosis factor α (TNF-α) and interleukin 1(IL-1) are generated by activated macrophages and they act via specific cell membrane receptors (Putensen & Wrigge 2000; Aldridge 2002). TNF-α is an important soluble mediator of inflammation and its release is elevated early during sepsis. Binding of TNF-α to its receptors leads to cell activation through nuclear factor κB translation. In turn, IL-1 stimulates the production of proteases and generation of reactive oxygen species (ROS) (Tsiotou et al 2005), resulting in the initiation of inflammatory cell migration into tissues (Cohen 2002). Moreover, IL-1 and TNF-α act synergistically leading to the expression of factors including other proinflammatory cytokines such as IL-8 and IL-12 (Kumar et al 1996), which are involved in tissue inflammation (Baggionili et al 1994). IL-8 causes neutrophil chemotaxis, directional migration, and expression of surface adhesion molecules (Baggionili et al 1994; Baggionili 1995); circulating levels of IL-8 predict morbidity and mortality (Hack et al 1992). A number of other cytokines, including IL-4, IL-10, IL-13 and interferon γ may also be involved in the pathogenesis of sepsis; they amplify the effects of the above-mentioned inflammatory mediators.

Cytokines released in the course of sepsis are also involved in another series of events. They stimulate a procoagulant state, leading to capillary obstruction, ischaemia and organ dysfunction (Levi 2001). Complement activation, mainly C5a, which is a potent anaphylatoxin and chemoattractant, plays an important role in host defence, resulting in increased vascular permeability, leukocyte attraction, immobilization, phagocytosis and cell lysis. Phospholipase A2, inducible cyclooxygenase and 5-lipoxygenase, which produce proinflammatory prostaglandins and leukotrienes, are likewise induced by cytokines (Annane et al 2005).

Reactive oxygen and nitrogen species

ROS are partially reduced derivatives of molecular oxygen (O_2); included in this group of reactants is the superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\bullet}$) and hypochlorous acid (Fink 2002). Under physiological conditions, a homeostatic balance exists between the formation of ROS and their removal by endogenous scavenging antioxidants (Gutteridge & Mitchell 1999). Oxidative stress occurs when this balance is disrupted by excessive production of ROS and/or inadequate antioxidant defence. In mammalian cells, the majority of ROS are formed during cellular respiration and by leukocytes as part of the normal host defence against infecting microorganisms (Webster & Nunn 1988). ROS act also as messenger molecules in cellular signalling and gene activation, and they enhance nuclear factor κB activation (Horton 2003).

In recent years, increasing evidence suggests the involvement of reactive nitrogen species (RNS), mainly NO, in cellular damage resulting from sepsis. The main source of NO during endotoxaemia is iNOS, which is induced by the cooperative effects of inflammatory cytokines (Lanone et al 2000; Carreras et al 2004). Excessive NO production is clearly implicated in the pathogenesis of septic shock (Thiemermann & Vane 1990) and, as a result, pharmacological inhibition of iNOS would presumably be beneficial in sepsis (Hobbs et al 1999). Unfortunately, clinical trials using iNOS inhibitors

failed to show a beneficial effect in septic shock patients (Ruetten & Thiemermann 2000).

NO is believed to be a key component in vasopressor-resistant and myocardial depression of septic shock (Tsiotou et al 2005). Normally, NO participates in a variety of homeostatic activities, including control of vascular tone, modulation of nerve transmission and regulation of the activity of a variety of proteins. Bacterial products such as LPS, and certain inflammatory mediators, particularly interferon γ, TNF-α, IL-1β and platelet activator factor, are related to iNOS induction (Victor et al 2004). Once expressed, iNOS is continuously active during inflammation, producing elevated amounts of NO in the cell. Although large quantities of NO may kill bacteria, fungi and tumour cells, they may also induce excessive vasodilatation and nitrosative stress to host tissues (Weitzberg 2005).

NO overproduction interferes with mitochondrial respiration and induces apoptosis and necrosis. NO can also bind to a component of catalase and reversibly inhibit H_2O_2 breakdown by this enzyme (Brown 1995, 2001). NO reacts with $O_2^{\bullet-}$, leading to $ONOO^-$ formation (Lipton et al 1993), a cytotoxic pro-oxidant, which oxidizes sulfhydryl groups that in turn generate $HO^\bullet$ (Beckman et al 1990), causing tissue injury during sepsis. Another RNS, namely nitrogen dioxide, can also produce critical cellular injury. Additionally, RNS cause DNA damage, an important means of tissue injury associated with oxidative stress (Liaudet et al 2002).

ROS and RNS disrupt tissue integrity and function, which leads to cell necrosis. During oxidative stress, these reactive species are responsible for cellular LPO, DNA and protein oxidation, and mitochondrial impairment, which alter membrane fluidity and permeability (Garcia et al 1997; Yerer et al 2003), thereby disrupting cell function (Zimmerman 1995). Oxidative damage to mitochondria results in altered respiration and uncoupling; mitochondria may be changed to a degree such that cytochrome c release, caspase activation and apoptosis follow. Thus, pharmacological intervention with agents that either block the production of ROS and/or RNS, or scavenge these reactive molecules once they have been formed, may ameliorate the severity of sepsis (Bhatia & Moochhala 2004).

NO and mitochondria

At low concentrations, NO competes with O_2 at the level of complex IV (cytochrome c oxidase) and reversibly inhibits this enzyme in the mitochondrial electron transport chain (ETC) (Brown 2001). It is now recognized that the rate of cellular energy supply depends on the mitochondrial O_2/NO ratio (Boveris et al 2001). This indicates that NO is a physiological regulator acting directly on the ETC.

In a variety of studies in experimental animal and human tissues, mitochondrial dysfunction during sepsis was observed (Mela et al 1970; Crespo et al 1999; Boveris et al 2002; Escames et al 2003). High levels of NO produced by iNOS are a major cause of the cytotoxic and genotoxic effects of sepsis, and they are related to the irreversible inhibition of mitochondrial respiration and cell death (Titheradge 1999). High concentrations of NO reduce electron transfer along the mitochondrial ETC. The impairment of the ETC increases electron leakage and the production of $O_2^{\bullet-}$, which, in turn, reacts with NO to yield $ONOO^-$. Both NO and $ONOO^-$ are capable of irreversibly inhibiting the four respiratory complexes and ATP synthase, thereby decreasing ATP production (Lizasoain et al 1996; Cadenas et al 2000; Escames et al 2003, 2006). $ONOO^-$ also inhibits other enzymes, including aconitase, NADH- and succinate-dehydrogenases and superoxide dismutase (SOD); changes in the activities of these enzymes further perturb mitochondrial dysfunction (Brown 1999, 2001).

Recently, a specific mitochondrial isoform of NOS (mtNOS) was reported (Ghafourifar & Richter 1997); this enzyme is responsible for the intramitochondrial production of NO (Giulivi et al 1998). This constitutively expressed mtNOS isoform presumably derives from a nNOS isoform (Tatoyan & Giulivi 1998; Kanai et al 2001; Elfering et al 2002). We recently demonstrated that endotoxaemia induces the expression and activity of a mtNOS isoform, which is associated with mitochondrial impairment (Escames et al 2003). Further experiments confirmed the existence of an inducible mtNOS isoform derived from iNOS (Acuña-Castroviejo et al 2005; López et al 2005). The terms mtcNOS and mtiNOS have been proposed for the constitutive, nNOS-derived and inducible, iNOS-derived mtNOS, respectively (Acuña-Castroviejo et al 2005). Although constitutively expressed in mitochondria, mtiNOS mainly influences mitochondrial function after its induction in pathophysiological states such as sepsis (Escames et al 2003, 2006; Lopez et al 2005). Thus, inhibition of mitochondrial respiration and the resulting energy failure observed in sepsis may reflect the overproduction of NO due to mtiNOS induction rather than NO produced by cytosolic iNOS (Acuña-Castroviejo et al 2005; Escames et al 2006). While high levels of NO are responsible for a significant portion of the pathophysiological manifestations resulting from mitochondrial damage during sepsis, the situation is further aggravated by the coupling of NO with $O_2^{\bullet-}$ to form $ONOO^-$. $ONOO^-$ inhibits the ETC leading to a further increase in $O_2^{\bullet-}$ production and oxidative damage, eventually culminating in apoptosis (Escames et al 2003). Thus, mtiNOS seems to be a significant contributor to the mitochondrial impairment observed in sepsis (Acuña-Castroviejo et al 2005; Escames et al 2006). Mitochondrial impairment during sepsis is consistent with the reduced body temperature and the decreased whole body oxygen uptake that are characteristic of septic shock (Boveris et al 2002).

Antioxidants

For counteracting free radical damage, cells are equipped with a variety of antioxidants and free radical scavengers. Antioxidants prevent oxygen radical formation by enzymatically removing their precursors or by converting radical products to less reactive molecules; these enzymes include SOD, glutathione peroxidase (GPx) and catalase. SOD rapidly promotes the conversion of $O_2^{\bullet-}$ to H_2O_2. Catalase and GPx, the latter requiring reduced glutathione (GSH) for its action,

catalyse the conversion of H_2O_2 to H_2O. GSH itself also has direct antioxidant activity, via donation of hydrogen ions, to repair damaged DNA (Victor et al 2004). GSH is an essential intracellular antioxidant in protecting normal cells from oxidative injury. Free radical scavengers react directly with ROS already formed; the best known of these scavengers are vitamin C and E (Victor et al 2004). Vitamin E, β-carotene, lycopene, and coenzyme Q, due to their lipid solubility, especially protect cellular membranes from free radical damage, while vitamin C, because of its aqueous solubility, reduces oxidative damage in the cytosol. Mitochondria contain their own system of antioxidants, which includes an isoform of SOD and the glutathione redox cycle.

The total antioxidant potential of patients with sepsis is significantly reduced resulting in massive free radical damage and eventually death (Cowley et al 1996; Victor et al 2004). Besides alterations in GSH metabolism in sepsis, LPS produces oxidative stress, which reduces plasma antioxidant capacity and potentiates depletion of GSH (Carbonell et al 2000). Transgenic mice overexpressing human GPx exhibit a decreased sensitivity to endotoxic shock and an increased rate of survival even after the administration of a large LPS dose (Mirochnitchenko et al 2000). Thus, the GSH-related antioxidant system is an important protective mechanism of the cell against oxidative damage and, additionally, it is a critical factor in the development of the immune response.

Therapeutic approaches in septic shock

The economic cost of severe sepsis is considerable. In the USA, around 700 000 patients per year develop severe sepsis (Angus & Wax 2001; Angus et al 2001; Martin et al 2003), with a financial cost of about $24 000 per patient (Angus et al 2001). However, in contrast to the major therapeutic achievements in other fields of medicine, there is not yet a specific treatment for septic shock, in part due to our lack of knowledge of the underlying mechanisms (Iskit & Guc 2004). The current treatments for sepsis remain largely symptomatic and supportive. A combination of antibiotic treatment, which reduces infection, and haemodynamic, respiratory and metabolic support are used to restore tissue perfusion and to normalize cellular metabolism. When hypotension results mainly from myocardial depression, inotropic agents are used first (Annane et al 2005). Optimization of the haemodynamic status may require blood transfusion. Patients may be treated with O_2 and, when they have acute lung injury or acute respiratory distress syndrome, with intensive mechanical ventilation (Petrucci & Lacovelli 2004). Daily haemodialysis or continuous veno–venous haemofiltration is used in patients with acute renal failure (Schrier & Wang 2004).

Besides symptomatic treatment, blocking specific steps in the septic cascade have been tested as to their potential benefits. However, attempts to modify endotoxin release failed to improve outcome (Ziegler et al 1991; McCloskey et al 1994). A randomized trial with the platelet-activating factor, acetylhydrolase, for reducing the inflammatory response in septic patients did not yield conclusive results (Opal et al 2004). High doses of corticosteroids and non-steroid anti-inflammatory drugs have been also used to combat the inflammatory response in sepsis, but these drugs failed to improve survival (Bernard et al 1997; Annane et al 2004, 2005). Monoclonal antibodies targeting LPS (Greenberg et al 1991; Zeigler et al 1991) also proved ineffective (Warren et al 1993).

Serum concentrations of anticoagulants often decrease during sepsis, whereas diffuse microembolism thrombus formation is thought to have a central role in the development of organ dysfunction. Natural anticoagulant proteins such as antithrombin III, activated protein C and tissue-factor-pathway inhibitor have been examined in septic patients. Only activated protein C significantly decreased mortality (Bernard et al 2001; Gluck & Opal 2004), but its use is currently restricted to patients with a greater severity of the disease or coagulation disorders (Polderman & Girbes 2004). Recent studies with other anti-inflammatory agents suggest that the severity of sepsis, as reflected by the mortality rate, may be of importance in reducing $O_2^{\bullet-}$ generation (Eichacker et al 2002; Cui et al 2004). Other interventions used to reduce either ROS generation or their actions have exerted minor beneficial effects in a variety of experimental models of shock. These therapeutic approaches include the use of *N*-acetylcysteine (Ozdulger et al 2003), tempol (Thiemermann 2003), and SOD mimetics (Salvemini & Cuzzocrea 2003). However, the mortality rates of patients with severe sepsis remain high (Angus & Wax 2001). Since the elevated mortality rate in patients with septic shock still remains, other approaches to address this serious condition require exploration (Parrillo 1993). We discuss here the rationale for the use of melatonin in the pharmacological treatment of sepsis.

Properties of melatonin

Besides its ubiquitous presence in animals, melatonin is also present in plants (Reiter & Tan 2002; Kolar & Machackova 2005). In the animal kingdom, melatonin is a highly conserved molecule found in organisms from unicells to humans (Hardeland & Fuhrberg 1996; Hardeland & Poeggeler 2003) In vertebrates, melatonin is the main secretory product of the pineal gland, from where it is released into the blood (Reiter 1991) and perhaps into the cerebrospinal fluid (Skinner & Malpaux 1999; Reiter & Tan 2003); in both these fluids its concentration exhibits a circadian rhythm since melatonin secretion occurs during darkness. Melatonin is also found in high concentrations, much higher than in the blood, in other body fluids and tissues, and it is differentially distributed in organs and in subcellular organelles (Menendez-Pelaez & Reiter 1993; Tan et al 1994; Skinner & Malpaux 1999; Acuña-Castroviejo et al 2003; Reiter & Tan 2003). Besides its synthesis in the pineal gland, melatonin is also produced in other organs, including the retina (Ivanova & Iuvone 2002), gastrointestinal tract (Motilva et al 2001), skin (Slominski et al 2005) and bone marrow (Tan et al 1999). Its broad extracellular and intracellular distribution in addition to its synthesis in a variety of organs may explain the role of melatonin in modulating a number of physiological processes through a variety of mechanisms.

Melatonin is a powerful antioxidant and directly scavenges both ROS and RNS (Tan et al 1993; Reiter 1995; Acuña-

Castroviejo et al 2001; Reiter et al 2001; Allegra et al 2003; Reiter & Tan 2003). Melatonin prevents LPO (Escames et al 1997) and preserves membrane permeability by increasing its fluidity (Garcia et al 1997). Melatonin reduces mitochondrial hydroperoxide levels and restores GSH homeostasis and mitochondrial function in organelles under oxidative stress (Martín et al 2000a, b, 2002; Acuña-Castroviejo et al 2001, 2003). Melatonin also stimulates γ-glutamylcysteine synthase, thereby increasing intracellular GSH synthesis (Urata et al 1999). Moreover, the indoleamine has indirect antioxidant effects, since it stimulates the activities of the two enzymes involved in the GSH cycling, namely GPx and glutathione reductase (Martín et al 2000a; Reiter et al 2000; Rodriguez et al 2004; Escames et al 2006; López et al 2005). In addition to melatonin's multiple direct and indirect antioxidant and anti-inflammatory actions, several metabolites that are formed when melatonin functions as a free radical scavenger, that is *N*1-acetyl-*N*2-formyl-5-methoxykynuramine (AFMK) and *N*1-acetyl-5-methoxykynuramine (AMK), also possess significant antioxidative and anti-inflammatory activity (Ressmeyer et al 2003; Mayo et al 2005). COX-2 inhibition by melatonin and AFMK or AMK may account for many protective effects, including those observed in neurodegenerative diseases. Drugs that have high potency for inhibiting COX-2 and a lesser effect on COX-1 would provide potent anti-inflammatory activity with fewer side-effects. Melatonin prevents specifically the activation of the proinflammatory enzymes COX-2 without simultaneous inhibition of the COX-1 enzyme (Mayo et al 2005). Thus, not only the parent molecule, melatonin, but also its metabolites are protective against oxidative stress (Tan et al 2003).

More recently, melatonin was found to counteract the inhibition of respiratory complexes and restore normal function of the respiratory chain by increasing the activities of mitochondrial complex I and IV (Martín et al 2000a, b). These protective actions of melatonin may involve an effect on the expression of mtDNA. Melatonin increases the expression of mtDNA encoded polypeptide subunits I, II and III of complex IV in mitochondria from rat liver in a time-dependent manner, which correlates with the increase in complex IV activity (Acuña-Castroviejo et al 2002, 2003). One consequence of melatonin's action in mitochondria is an increase in ATP production (Martín et al 2002). Thus, melatonin restores the functional mitochondrial activity that is depressed in some pathological situations (Acuña-Castroviejo et al 2002; León et al 2004).

Melatonin reduces O_2 consumption by liver mitochondria, an effect that may protect this organelle from excessive oxidative damage (Sewerynek et al 1999; Karbownik et al 2000; Reyes Toso et al 2003). Furthermore, the genomic effects of melatonin include the inhibition of the expression of iNOS and mtiNOS (Crespo et al 1999; Escames et al 2003), two pro-oxidant enzymes involved in the septic response and cellular impairment. Melatonin also reduces apoptosis due to its antioxidant and free radical scavenging properties (Jou et al 2004), although a direct interaction between melatonin and mitochondrial permeability transition pore has been also proposed (Andrabi et al 2004; Rivas et al 2005).

The action of melatonin on mitochondria may be mediated by the antioxidant and direct free radical scavenging properties of the indolamine, which protect this organelle from oxidative damage. Also, its actions on mtDNA, which increase the expression of the respiratory complex subunits encoded by mtDNA, as well as a direct interaction of melatonin with the mitochondrial permeability transition pore, may contribute to melatonin's protective effects at the mitochondrial level (León et al 2005).

In a number of pathologies, including models of neurodegenerative diseases, in which high production of free radicals is a primary cause of the condition, melatonin has documented protective actions (Reiter et al 2004). Indeed, melatonin has potent neuroprotective properties. Melatonin reduces the damaging effect of kainic acid and *N*-methyl-D-aspartate in-vivo (Dabbeni-Sala et al 2001; Escames et al 2004). In a model of Parkinson's disease, melatonin ameliorated the reduction of tyrosine hydroxylase positive fibres and the lipid peroxidation in the striatum (Acuña-Castroviejo et al 1997), reduced mitochondrial DNA damage (Tan et al 1994; Chen et al 2005), prevented the inhibition of mitochondrial complex I activity induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (Khaldy et al 2003) and inhibited dopamine autoxidation (Khaldy et al 2000). In models of Alzheimer's disease, melatonin prevented neurodegenerative changes (Matsubara et al 2003; Feng et al 2004; Lahiri et al 2004; Wang et al 2004; Esparza et al 2005) and, in humans, it significantly slowed the progression of the disease (Cardinali et al 2002; Wu & Swaab 2005). Many reports have shown a protective effect of melatonin on epilepsy in humans (Molina-Carballo et al 1997).

These data indicate that melatonin efficiently counteracts oxygen radical pathology and suggests that mitochondria are an important target for the action of melatonin in the cell. Melatonin improves the bioenergetics of the cell, promotes more efficient nuclear and mitochondrial genomic repair mechanisms, increases GSH levels and elevates ATP production (Acuña-Castroviejo et al 2001).

Melatonin in sepsis

Considering its multiple actions, and the fact that NO seems to be a key factor in sepsis and iNOS is one of the main targets of melatonin action, it is not surprising that melatonin administration is beneficial in restoring homeostasis in sepsis. Sewerynek et al (1995a, b) first documented a reduction in LPS-induced oxidative damage after melatonin administration. In these studies, melatonin reduced LPO of membranes and attenuated hepatic leukocytosis. More recently, also using the LPS model of sepsis in rats, it was shown that melatonin counteracted a variety of metabolic alterations (Crespo et al 1999). In these experiments, we demonstrated that melatonin significantly reduced LPO and counteracted the LPS-induced NO production in lungs and liver. These results document a dose-dependent inhibition of iNOS activity by melatonin. Melatonin also reduced the expression of iNOS mRNA. Thus, melatonin may reduce NO production during sepsis, mainly by inhibiting iNOS expression.

In other studies, melatonin counteracted LPS-induced multiple organ dysfunction syndrome and protected animals against endotoxaemia and death (Crespo et al 1999; Reynolds 2003). Wu et al (2001) reported that melatonin inhibits both

TNF-α release into the plasma and $O_2^{\bullet-}$ production in the aorta of septic rats. Thus, the beneficial haemodynamic actions of melatonin were likely associated with its anti-inflammatory effects on cytokines such as TNF-α, its suppression of iNOS expression and its antioxidant properties. Additional studies have demonstrated that melatonin significantly reduces $ONOO^-$ formation and poly(ADP-ribose) synthase activation (Cuzzocrea et al 1998; Cuzzocrea & Caputi 1999), limits LPO and replenishes the GSH content of several organs (El-Sokkary et al 1999; Paskalolu et al 2004; Sener et al 2005) in experimental sepsis. Melatonin also inhibits the elevated myeloperoxidase activity and scavenges hypochlorous acid (Zavodnik et al 2004), suggesting that the protective effects of melatonin are in part related to the inhibition of neutrophil infiltration, scavenging of the reactant that neutrophils generate, and restores the contractility of muscle tissues (Paskalolu et al 2004; Sener et al 2005).

We have previously demonstrated that melatonin prevents mitochondrial oxidative damage and inhibits mtiNOS expression and activity induced by LPS in rat lung and liver mitochondria (Figure 1) (Escames et al 2003). In these experiments, melatonin also restored ETC activity which was inhibited by sepsis (Figure 2). Moreover, melatonin is a scavenger of both NO and $ONOO^-$ or radicals generated therefrom (Blanchard et al 2000). The inhibition of mtiNOS by melatonin, in addition to its scavenging of both ROS and RNS may account for many of the protective effects of the indolamine against endotoxaemia, including the recovery of ETC activity and ATP production. Since melatonin increases the efficiency and activities of the respiratory complexes, reduces electron leakage, and thereby curtails $O_2^{\bullet-}$ and H_2O_2 generation, it limits damage due to LPS toxicity.

In a search for the source of mtiNOS, we recently performed a series of experiments in a model of sepsis induced by caecal ligation and puncture in both normal and iNOS knockout mice (Escames et al 2006; López et al 2005). The activity of mtNOS and nitrite levels significantly increased after sepsis in $iNOS^{+/+}$ mice. These animals also exhibited a significant inhibition of the respiratory chain activity and an increase in mitochondrial oxidative stress. Interestingly, mtNOS activity remained unchanged in $iNOS^{-/-}$ septic mice (Figure 3), and the mitochondria of these animals were unaffected by sepsis. Our results further confirm that melatonin

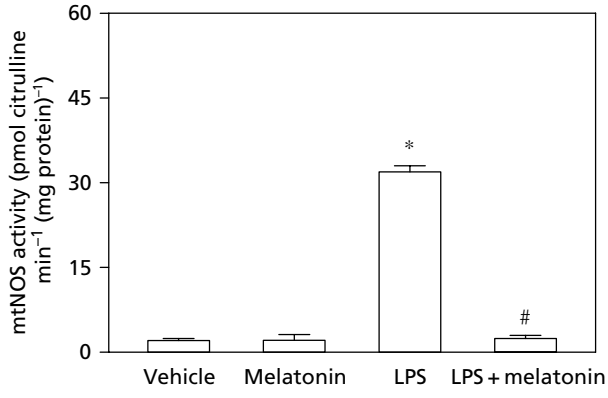

Figure 1 Effects of melatonin on lipopolysaccharide (LPS) induced mitochondrial nitric oxide synthase (mtNOS) activity in lung mitochondria of 3-month-old rats. Mean ± s.e. of six to eight experiments per group, each assayed in duplicate. *$P < 0.001$ versus vehicle; #$P < 0.001$ versus LPS (data from Escames et al 2003).

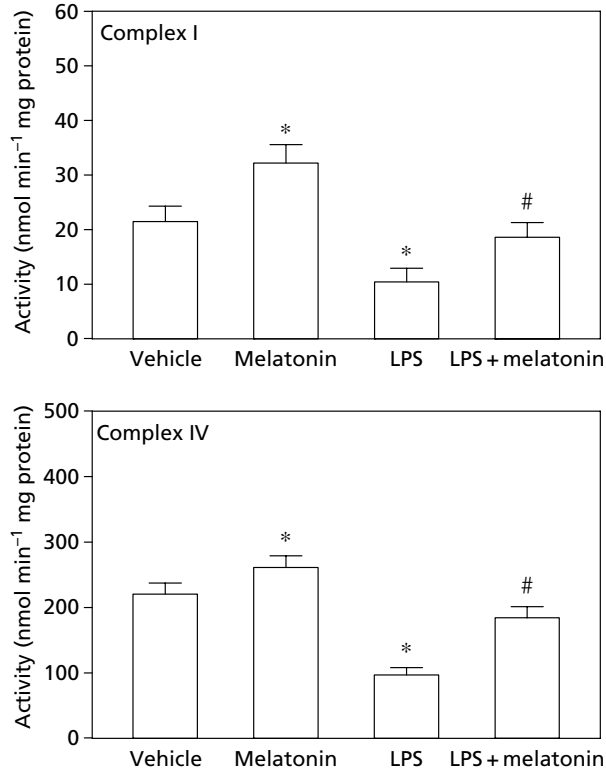

Figure 2 Effects of melatonin on the activities of complex I and IV in lung mitochondria of lipopolysaccharide (LPS) treated 3-month-old rats. Mean ± s.e. of six to eight experiments per group, each assayed in duplicate. *$P < 0.001$ versus vehicle; #$P < 0.001$ versus LPS (data from Escames et al 2003).

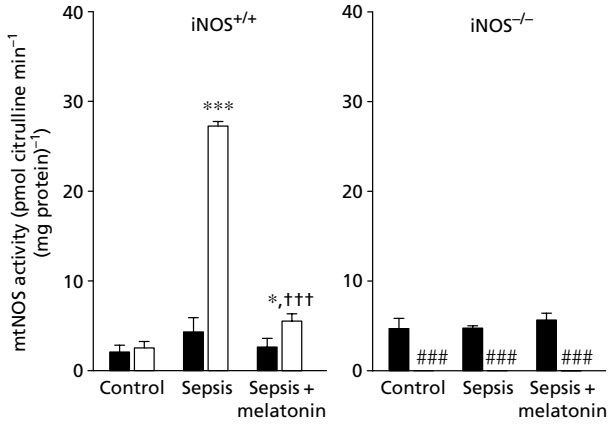

Figure 3 Mitochondrial nitric oxide synthase (mtNOS) activity measured in skeletal muscle mitochondria. Closed bars represent constitutive mtNOS and open bars represent inducible mtNOS activity. *$P < 0.05$ and ***$P < 0.001$ versus control; †††$P < 0.001$ versus sepsis; ###$P < 0.001$ versus $iNOS^{+/+}$.

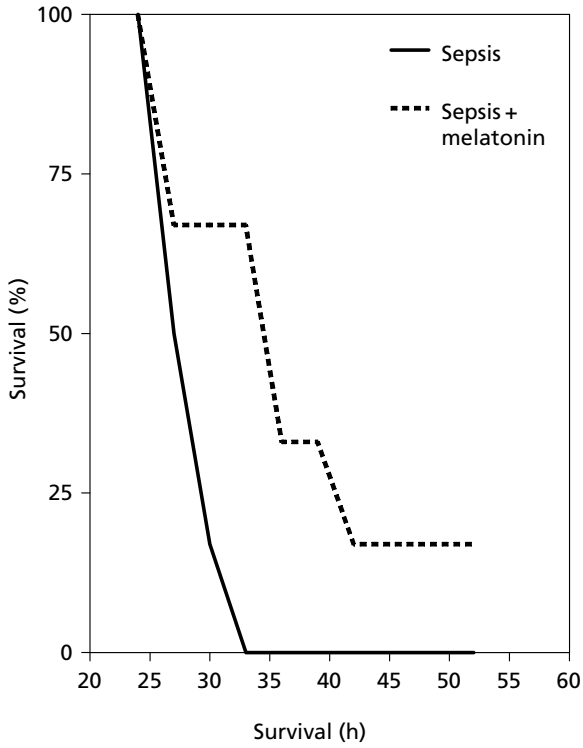

Figure 4 Percent and duration of survival of septic mice without or with melatonin treatment. Sepsis was induced by caecal ligation and puncture. Each group contained 20 mice at the onset.

administration to $iNOS^{+/+}$ mice counteracts mtNOS induction and respiratory chain failure and improves the activity of complexes I, II, III and IV. Melatonin normalizes the GSH pool and increases the glutathione reductase activity. Melatonin also restores normal membrane dynamics and limits LPO in septic animals. The results suggest that sepsis-induced mtNOS is responsible for the increase of mitochondrial impairment due to oxidative stress, while melatonin treatment counteracts mitochondrial failure to the same extend as the lack of the iNOS gene. Melatonin treatment is also a protective factor against vascular injury during endotoxic shock since it limits vascular smooth muscle contractile dysfunction (d'Emmanuele di Villa Bianca et al 2004).

It is known that $O_2^{\bullet-}$ oxidizes catecholamines, including dopamine, noradrenaline (norepinephrine) and adrenaline (epinephrine), to products that cause vasoconstriction (Macarthur et al 2000). Thus, vascular hyporeactivity to catecholamines impairs successful treatment of hypotension in septic shock (Heikkila 1985). d'Emmanuele di Villa Bianca et al (2004) demonstrated that the vasoconstrictive actions of catecholamines were increased by melatonin. They also investigated the mechanism of the loss of vascular contractility in aorta rings collected from rats treated with LPS and they found that melatonin inhibits PARS expression in the aorta. PARS is a nuclear enzyme that is activated following DNA damage; this leads to a cascade of events that cause necrosis (Ha & Snider 1999). Erythrocyte deformability, which is critical for a microcirculatory system to function effectively, is also prevented by melatonin (Yerer et al 2004). Furthermore, melatonin inhibits endothelin converting enzyme-1, which is required for endothelin synthesis (Kilic et al 2004). The reduction in endothelin would also assist melatonin in protecting against septic shock. In high doses, melatonin also directly reduces mesenteric blood flow and LPS-induced TNF-α generation (Baykal et al 2000b). These beneficial effects of melatonin are likely related to its antioxidant properties and inhibition of iNOS protein expression (Crespo et al 1999; Escames et al 2006; López et al 2005).

It is well established that circadian melatonin secretion is impaired in severe septic patients (Mundigler et al 2002). Cytokines such as IL-1β and TNF-α were found to drastically reduce pineal serotonin content in pineal explants or cell cultures from neonate animals (Tay et al 2001). Since serotonin is a precursor in the synthesis of melatonin, a reduction in this amine in the pineal gland would likely limit melatonin generation and the reduction in melatonin may in part contribute to the pathophysiology of septic shock (Jiang-Shieh et al 2005).

Melatonin clearly prevents multiple organ failure, circulatory failure and mitochondrial damage in experimental sepsis (Crespo et al 1999; Wakatsuki et al 2001; Wu et al 2001; Escames et al 2003, 2006; López et al 2005). The evidence is compelling that melatonin improves survival in animals with septic shock (Figures 4 and 5) (Maestroni 1996; Wichman et al 1996; Wu et al 2001; Reynolds et al 2003). Based on these findings, Gitto et al (2001) utilized melatonin to treat septic human newborns. This study demonstrated the efficacy of melatonin as a therapy; it significantly reduced several parameters of sepsis, for example levels of lipid peroxidation products (Figure 6) and death in the treated neonates compared with controls. The mortality of newborns with sepsis is high, usually between 30% and 50% (Perez & Weiman 1997). In the study of Gitto et al (2001), three of 10 septic children who were not treated with melatonin died within 72 h after diagnosis of sepsis and, more importantly, none of the 10 septic newborns treated with melatonin died. In the study, a total of 20 mg of melatonin was used orally in two doses of 10 mg each, with a 1-h interval, within the first 12 h after diagnosis. This was the first study where melatonin was given to human newborns. The comparison of serum parameters

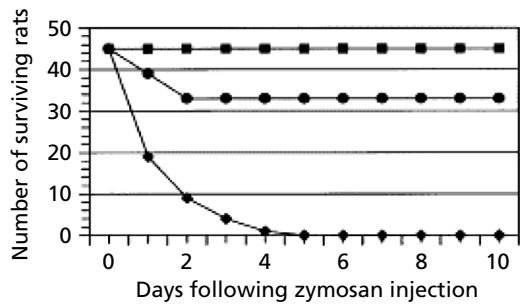

Figure 5 Effect of melatonin on sepsis in rats given a 500 mg kg^{-1} intraperitoneal injection of zymosan. Within 5 days, 45 of 45 rats given zymosan (plus diluent) (◆) died. Conversely, when zymosan-treated rats were also given daily injections of melatonin (0.8 mg kg^{-1} subcutaneously) (●), 33 of 45 rats (73%) survived for at least 10 days. No control rats (■) died during the experimental period (data from Reynolds et al 2003).

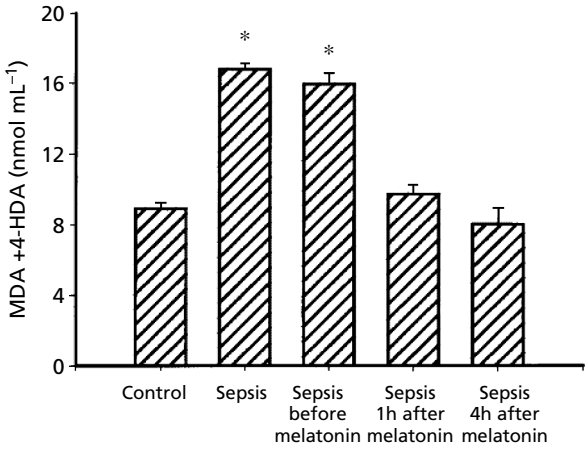

Figure 6 Mean ± s.d. serum levels of lipid peroxidation products (MDA, malondialdehyde; 4-HDA, 4 hydroxyalkanals) in septic human newborn without melatonin treatment, and 1 and 4 h after being given 20 mg melatonin orally. Ten septic newborns were given conventional therapy and 10 were given conventional therapy plus melatonin. *$P <$ 0.05 versus other groups (data from Gitto et al 2001).

between melatonin-treated and untreated septic newborns indeed confirmed the antioxidant (reduction in serum LPO) and the anti-inflammatory (reduction in C reactive protein) effects of melatonin. The white blood cell count and the absolute neutrophil count also significantly decreased in the melatonin-treated infants, but they remained elevated in the untreated newborns. Subsequently, melatonin was also used to treat newborn humans suffering from hypoxia or respiratory distress. In these studies as well, melatonin improved the clinical outcome (Fulia et al 2001; Gitto et al 2001). Finally, melatonin modified serum inflammatory and oxidative stress parameters and improved the clinical course of surgically treated neonates (Gitto et al 2004a, b).

A variety of studies, including those in children and adult humans (Molina-Carballo et al 1997; Seabra et al 2000; Jan et al 2000), have shown that melatonin has low toxicity. Studies conducted under the guidelines of US National Toxicity Program found little evidence of toxicity in rats treated throughout pregnancy with massive doses (10–200 mg kg^{-1} daily) of melatonin (Jahnke et al 1999). In addition to maternal health, this group examined prenatal survival, fetal bodyweight, and incidence of fetal malformations. None of these indices indicated that melatonin had any significant toxicity.

Conclusions and clinical implications

Sepsis is a major cause of mortality in critically ill patients and develops as a result of the host response to infection. Many mechanisms are involved in the pathophysiology of septic shock, including the release of cytokines and the activation of neutrophils, monocytes and microvascular endothelial cells. The decline in organ function is triggered by a reduction in intramitochondrial activity and oxidative phosphorylation, leading to reduced cellular metabolism. In sepsis, there is an association between NO overproduction, excessive free radical generation, antioxidant depletion, energy failure and mitochondrial inhibition.

Preserved organ function remains a fundamental principle in the care of septic patients. Although progress has been made in the treatment of sepsis, the continued high mortality rate in severe sepsis and septic shock is a sobering reflection of the insufficiency of current therapeutic approaches.

There is a significant mortality reduction when septic newborns are treated with melatonin. Besides counteracting mitochondrial oxidative stress, melatonin also suppresses iNOS/mtNOS expression and, thus, the use of melatonin alone or in combination with other antioxidants may improve the clinical outcome of septic patients as already shown by Gitto et al (2001).

The findings summarized here have important therapeutic implications for the potential use of melatonin. There are numerous published examples in which favourable and statistically significant positive effects have been observed with the use of melatonin in sepsis. These studies herald the promising therapeutic application of melatonin in the treatment of sepsis and septic shock in humans and we hope that this report will serve to initiate future clinical trials. The use of melatonin may assist considerably in reducing the mortality of this lethal condition.

References

Acuña-Castroviejo, D., Coto-Montes, A., Monti, G., Ortiz, G. G., Reiter R. J. (1997) Melatonin is protective against MPTP-induced striatal and hippocampal lesions. *Life. Sci*. **60**: PL23–PL29

Acuña-Castroviejo, D., Martín, M., Macías, M., Escames, G., León, J., Khaldy, H., Reiter, R. J. (2001) Melatonin, mitochondria, and cellular bioenergetics. *J. Pineal Res*. **30**: 65–74

Acuña-Castroviejo, D., Escames, G., Carazo, A., León, J., Khaldy, H., Reiter, R. J. (2002) Melatonin, mitochondrial homeostasis and mitochondrial-related diseases. *Curr. Top. Med. Chem*. **2**: 133–151

Acuña-Castroviejo, D., Escames, G., León, J., Carazo A., Khaldy, H. (2003) Mitochondrial regulation by melatonin and its metabolites. *Adv. Exp. Med. Biol*. **527**: 549–557

Acuña-Castroviejo, D., Escames, G., López, L. C., Hitos, A. B., León, J. (2005) Melatonin and nitric oxide. *Endocrine* **27**: 159–168

Aldridge, A. J. (2002) Role of the neutrophil in septic shock and the adult respiratory distress syndrome. *Eur. J. Surg*. **168**: 204–214

Allegra, M., Reiter, R. J., Tan, D. X., Gentile, C., Tesoriere, L., Livrea, M. A. (2003) The chemistry of melatonin's interaction with reactive species. *J. Pineal Res*. **34**: 1–10

Andrabi, S. A., Sayeed, I., Siemen, D., Wolf, G., Horn, T. F. (2004) Direct inhibition of the mitochondrial permeability transition pore: a possible mechanism responsible for anti-apoptotic effects of melatonin. *FASEB J*. **18**: 869–871

Angus, D. C., Wax, R. S. (2001) Epidemiology of sepsis: an update. *Crit. Care Med*. **29**: S109–S116

Angus, D. C., Linde-Zwirble, W. T., Lidicker, J., Clemont, G., Garcillo, J., Pinsky, M. R. (2001) Epidemiology of severe sepsis in the United States. *Crit. Care Med*. **29**: 1303–1310

Annane, D., Bellisant, E., Bollaert, P., Briegel, J., Keh, D., Kupfer, Y. (2004) Corticosteroids for severe sepsis and septic shock: a systematic review and meta-analysis. *Br. Med. J*. **329**: 480

Annane, D., Bellissant, E., Cavaillon, J. M. (2005) Septic shock. *Lancet* **365**: 63–78

Baggionili, M. (1995) Activation and recruitment of neutrophil leukocytes. *Clin. Exp. Immunol*. **171**: 472–475

Baggiolini, M., Dewald, B., Moser, B. (1994) Interleukin-8 and related chemotactic cytokines. *Adv. Immunol*. **55**: 97–179

Baker, C. C., Huynh, T. (1995) Sepsis in the critically ill patient. *Curr. Probl. Surg*. **32**: 1013–1092

Baykal, A., Iskit, A. B., Hamaloglu, E., Guc, M. O., Hascelik, G., Sayek, I. (2000a) Melatonin modulates mesenteric blood flow and TNFalpha concentrations after lipopolysaccharide challenge. *Eur. J. Surg*. **166**: 722–727

Baykal, A., Kaynaroglu, V., Iskit, A. B., Guc, M. O., Hascelik, G., Sayek, I., Sanac, Y. (2000b) Adrenaline tolerance does not prevent bacterial translocation in a murine burn model. *Int. Surg*. **85**: 18–22

Beckman, J. S., Beckman, T. W., Chen, J., Marshall, P. A., Freeman, B. A. (1990) Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc. Natl Acad. Sci.USA* **87**: 1620–1624

Bernard, G. R., Wheeler, A. P., Russel, J. A., Schein, R., Summer, W. R., Steinberg, K. P., Fulkerson, W. J., Wright, P. E., Christman, B. W., Dupont, W. D., Higgins, S. B., Swindell, B. B. (1997) The ibuprofen in sepsis study group. The effects of ibuprofen on the physiology and survival of patients with sepsis. *N. Engl. J. Med*. **336**: 912–918

Bernard, G. R., Vincent, J. L., Laterre, P. F., La Rosa, S. P., Dhainaut, J. F., Lopez-Rodriguez, A., Steingrub, J. S., Garber, G. E., Helterbrand, J. D., Ely, E. W., Fisher, C. J. Jr (2001) Recombinant human Protein C Worldwide Evaluation in Severe Sepsis (PROWESS) study group. Efficacy and safety of recombinant human activated protein C for severe sepsis. *N. Engl. J. Med*. **344**: 699–709

Bhatia, M., Moochhala, S. (2004) Role of inflammatory mediators in the pathophysiology of acute respiratory distress syndrome. *J. Pathol*. **202**: 145–156

Blanchard, B., Pompon, D., Ducroq, C. (2000) Nitrosation of melatonin by nitric oxide and peroxinitrite. *J. Pineal Res*. **29**: 184–192

Boveris, A., Costa, L. E., Poderoso, J. J., Carreras, M. C., Cadenas, E. (2001) Regulation of mitochondrial respiration by oxygen and nitric oxide. *Ann. NY Acad. Sci*. **899**: 121–135

Boveris, A., Alvarez, S., Navarro, A. (2002) The role of mitochondrial nitric oxide synthase in inflammation and septic shock. *Free Radic. Biol. Med*. **33**: 1186–1193

Brackett, D. J., Hamburger, S. A., Lerner, M. R., Jones, S. B., Schaefer, C. F., Henry D. P., Wilson, M. F. (1990) An assessment of plasma histamine concentrations during documented endotoxic shock. *Agents Actions* **31**: 263–274

Brown, G. C. (1995) Reversible binding and inhibition of catalase by nitric oxide. *Eur. J. Biochem*. **232**: 188–191

Brown, G. C. (1999) Nitric oxide and mitochondrial respiration. *Biochem. Biophys. Acta* **1411**: 351–369

Brown, G. C. (2001) Regulation of mitochondrial respiration by nitric oxide inhibition of cytochrome *c* oxidase. *Biochim. Biophys. Acta* **1504**: 46–57

Cadenas, E., Poderoso, J. J., Antunes, F., Boveris, A. (2000) Analysis of the pathways of nitric oxide utilization in mitochondria. *Free Radic. Res*. **33**: 747–756

Calandra, T., Cohen, J. (2005) The international sepsis forum consensus conference on definitions of infection in the intensive care unit. *Crit. Care Med*. **33**: 1538–1548

Carbonell, L. F., Nadal, J. A., Llanos, M. C., Hernandez, I., Nava, E., Diaz, J. (2000) Depletion of liver glutathione potentiates the oxidative stress and decreases nitric oxide synthesis in a rat endotoxin shock model. *Crit. Care Med*. **28**: 2002–2006

Cardinali, D. P., Brusco, L. I., Liberczuk, C., Furio, A. M. (2002) The use of melatonin in Alzheimer's disease. *Neuroendocrinol. Lett*. **1**: 20–23

Carreras, M. C., Franco, M. C., Peralta, J. G., Poderoso, J. J. (2004) Nitric oxide, complex I, and the modulation of mitochondrial reactive species in biology and disease. *Mol. Asp. Med*. **25**: 125–139

Chen, L. J., Gao, Y. Q., Li, X. J., Shen, D. H., Sun, F. Y. (2005) Melatonin protects against MPTP/MPP+-induced mitochondrial DNA oxidative damage in vivo and in vitro. *J. Pineal Res*. **39**: 34–42

Cohen, J. (2002) The immunopathogenesis of sepsis. *Nature* **420**: 885–891

Cowley, H. C., Bacon, P. J., Goode, H. F., Webster, N. R., Jones, J. G., Menon, D. K. (1996) Plasma antioxidant potential in severe sepsis: a comparison of survivors and nonsurvivors. *Crit. Care Med*. **24**: 1179–1183

Crespo, E., Macías, M., Pozo, D., Escames, G., Martin, M., Vives, F., Guerrero, J. M., Acuña-Castroviejo, D. (1999) Melatonin inhibits expression of the inducible NO synthase II in liver and lung and prevents endotoxemia in lipopolysaccharide-induced multiple organ dysfunction in rats. *FASEB J*. **13**: 1537–1546

Cui, X., Parent, C., Macarthur, H., Ochs, S. D., Gerstenberg, E., Solomon, S., Fitz, Y., Danner, R. L., Banks, S. M., Natanson, C., Salvemini, D., Eichacker, P. Q. (2004) Severity of sepsis alters the effects of superoxide anion inhibition in a rat sepsis model. *J. Appl. Physiol*. **97**: 1349–1357

Cuzzocrea, S., Caputi, A. (1999) Protective effects of melatonin on zymosan-induced cellular damage. *Biol. Signals Recept*. **8**: 136–142

Cuzzocrea, S., Costantino, G., Caputi, A. (1998) Protective effect of melatonin on cellular energy depletion mediated by peroxynitrite and poly (ADP-ribose) synthetase activation in a non-septic shock model induced by zymosan in the rat. *J. Pineal Res*. **25**: 78–85

Dabbeni-Sala, F., Floreani, M., Franceshini, D., Skaper, S. D., Giusti, P. (2001) Kainic acid induces selective mitochondrial oxidative phosphorylation enzyme dysfunction in cerebellar granule neurons: protective effects of melatonin and GSH ethyl ester. *FASEB J*. **15**: 1786–1788

d'Emmanuele di Villa Bianca, R., Marzocco, S., Di Paola, R., Autore, G., Pinto, A., Cuzzocrea, S., Sorrentino, R. (2004) Melatonin prevents lipopolysaccharide-induced hyporeactivity in rat. *J. Pineal Res*. **36**: 146–154

Eichacker, P. Q., Parent, C., Kalil, A., Esposito, C., Cui, X., Banks, S. M., Gerstenberger, E. P., Fitz, Y., Danner, R. L., Natanson, C. (2002) Risk and the efficacy of antiinflammatory agents: retrospective and confirmatory studies of sepsis. *Am. J. Respir. Crit. Care Med*. **166**: 1197–1205

El-Sokkary, G. H., Reiter, R. J., Cuzzocrea, S., Caputi, A. P., Hassanein, A.-F. M. M., Tan, D. X. (1999) Role of melatonin in reduction of lipid peroxidation and peroxynitrite formation in non-septic shock induced by zymosan. *Shock* **12**: 402–408

Elfering, S. L., Sarkela, T. M., Giulivi, C. (2002) Biochemistry of mitochondrial nitric-oxide synthase. *J. Biol. Chem*. **277**: 38 079–38 086

Escames, G., Guerrero, J. M., Reiter, R. J., Garcia, J. J., Muñoz, A., Ortiz, G. G., Oh, C. S. (1997) Melatonin and vitamin E prevent nitric oxide-induced lipid peroxidation in rat brain homogenates. *Neurosci. Lett*. **230**: 147–150

Escames, G., León, J., Macías, M., Khaldy, H., Acuña-Castroviejo, D. (2003) Melatonin counteracts lipopolysaccharide-induced expression and activity of mitochondrial nitric oxide synthase in rats. *FASEB J*. **17**: 932–934

Escames, G., León, J., López, L. C., Acuña-Castroviejo, D. (2004) Mechanism of the NMDA receptor inhibition by melatonin in the rat brain striatum. *J. Neuroendocrinol*. **16**: 929–935

Escames, G., López, L. C., Tapias, V., Utrilla, P., Reiter, R. J., Hitos, A. B., León, J., Rodríguez, M. I., Acuña-Castroviejo, D. (2006) Melatonin counteracts inducible mitochondrial nitric oxide

synthase dependent mitochondrial dysfunction in skeletal muscle of septic mice. *J. Pineal Res*. **40**: 71–78

Esparza, J. L., Gomez, M., Rosa Nogues, M., Paternain, J. L., Mallol, J., Domingo, J. L. (2005) Melatonin reduces oxidative stress and increases gene expression in the cerebral cortex and cerebellum of aluminum-exposed rats. *J. Pineal Res*. **39**: 129–136

Feng, Z., Chang, Y., Cheng, Y., Zhang, B. L., Qu, Z. W., Qin, C., Zhang, J. T. (2004) Melatonin alleviates behavioral deficits associated with apoptosis and cholinergic system dysfunction in the APP 695 transgenic mouse model of Alzheimer's disease. *J. Pineal Res*. **37**: 129–136

Fink, M. P. (2002) Reactive oxygen species as mediators of organ dysfunction caused by sepsis, acute respiratory distress syndrome, or hemorrhagic shock: potential benefits of resuscitation with Ronger's ethyl pyruvate solution. *Curr. Opin. Clin. Nutr. Metab. Care* **5**: 167–174

Fulia, F., Gitto, E., Cuzzocrea, S., Reiter, R. J., Dugo, L., Gitto, P., Barberi, S., Cordaro, S., Barberi, I. (2001) Increased levels of malondialdehyde and nitrite/nitrate in the blood of asphyxiated newborns: reduction by melatonin. *J. Pineal Res*. **31**: 343–349

Garcia, J. J., Reiter, R. J., Guerrero, J. M., Escames, G., Yu, B. P., Oh, C. S., Muñoz-Hoyos, A. (1997) Melatonin prevents changes in microsomal membrane fluidity during induced lipid peroxidation. *FEBS Lett*. **408**: 297–300

Ghafourifar, P., Richter, C. (1997) Nitric oxide synthase activity in mitochondria. *FEBS Lett*. **418**: 291–296

Gitto, E., Karbownik, M., Reiter, R. J., Tan, D. X., Cuzzocrea, S., Chiurazzi, P., Cordaro, S., Corona, G., Trimarchi, G., Barberi, I. (2001) Effects of melatonin treatment in septic newborns. *Pediatr. Res*. **50**: 756–760

Gitto, E., Reiter, J. R., Cordaro, S. P., La Rosa, M., Chiurazzi, P, Trimarchi, G, Gitto, P, Calabro, M. P., Barberi, I. (2004a) Oxidative and inflammatory parameters in respiratory distress syndrome of preterm newborns: beneficial effects of melatonin. *Am. J. Perinatol*. **21**: 209–216

Gitto, E., Romeo, C., Reiter, R. J., Impellizzeri, P., Pesce, S., Basile, M., Antonuccio, P., Trimarchi, G., Gentile, C., Barberi, I., Zuccarello, B. (2004b) Melatonin reduces oxidative stress in surgical neonates. *J. Pediatr. Surg*. **39**: 184–189

Giulivi, C., Poderoso, J. J., Boveris, A. (1998) Production of nitric oxide by mitochondria. *J. Biol. Chem*. **273**: 11 038–11 043

Gluck, T., Opal, S. M. (2004) Advances in sepsis therapy. *Drugs* **64**: 837–859

Greenberg, R. N., Wilson, K. M., Kunz, A. Y., Wedel, N. I., Gorelick, K. J. (1991) Randomized, double-blind phase II study of anti-endotoxin antibody (E5) as adjuvant therapy in humans with serious gram-negative infections. *Prog. Clin. Biol. Res*. **367**: 179–186

Guha, M., Mackman, N. (2001) LPS induction of gene expression in human monocytes. *Cell Signal*. **13**: 85–94

Gutteridge, J. M., Mitchell, J. (1999) Redox imbalance in the critically ill. *Br. Med. Bull*. **55**: 49–75

Ha, H. C., Snider, S. H. (1999) Poly (ADP-ribose) polymerase-1 is a mediator of necrotic cell death by ATP depletion. *Proc. Natl Acad. Sci. USA* **96**: 13 978–13 982

Hack, C. E., Zeerleder, S. (2001) The endothelium in sepsis: source of and a target for inflammation. *Crit. Care Med*. **29** (Suppl.): S21–S27

Hack, C. E., Hart, M., Van Schijndel, R. J., Eerenberg, A. J., Nuijens, J. H., Thijs, L. G., Aarden, L. A. (1992) Interleukin-8 in sepsis: relation to shock and inflammatory mediators. *Infect. Immun*. **60**: 2835–2842

Hardeland, R., Fuhrberg, B. (1996) Ubiquitous melatonin presence and effects in unicells, plants and animals. *Trends Comp. Biochem. Physiol.* **2**: 25–44

Hardeland, R., Poeggeler, B. (2003) Non-vertebrate melatonin. *J. Pineal Res*. **34**: 233–241

Heikkila, R. E. (1985) In: Greenwald, R. A. (ed.) *Handbook of methods for oxygen radicals research*. CRC, Boca Raton, FL

Henneke, P., Golenbock, D. T. (2002) Innate immune recognition of lipopolysaccharide by endothelial cells. *Crit. Care Med*. **30**: S207–S213

Hobbs, A. J., Higgs, A., Moncada, S. (1999) Inhibition of nitric oxide synthase as a potential therapeutic target. *Annu. Rev. Pharmacol. Toxicol*. **39**: 191–220

Horton, J. W. (2003) Free radicals and lipid peroxidation mediated injury in burn trauma: the role of antioxidant therapy. *Toxicology* **189**: 75–88

Iskit, A. B., Guc, O. (2003) Effects of endothelin and nitric oxide on organ injury, mesenteric ischemia, and survival in experimental models of septic shock. *Acta Pharmacol. Sin*. **24**: 953–957

Iskit, A. B., Guc, O. (2004) A new therapeutic approach for the treatment of sepsis. *Med. Hypotheses* **62**: 342–345

Ivanova, T. N., Iuvone, P. M. (2002) Melatonin synthesis in retina: circadian regulation of arylalkylamine *N*-acetyltransferase activity in cultured photoreceptor cells of embryonic chick retina. *Brain Res*. **973**: 56–63

Jack, R. S., Fan, X., Bernheiden, M., Rune, G., Ehlers, M., Weber, A., Kirsch, G., Mentel, R., Furll, B., Freudenberg, M., Schmitz, G., Stelter, F., Schutt, C. (1997) Lipopolysaccharide binding protein is required to combat a murine gram-negative bacterial infection. *Nature* **389**: 742–745

Jahnke, G., Marr, M., Myers, C., Wilson, R., Travlos, G., Price, C. (1999) Maternal and developmental toxicity evaluation of melatonin administered orally to pregnant Sprague-Dawley rats. *Toxicol. Res*. **50**: 271–274

Jan, J. E., Hamilton, D., Seward, N., Fast, D. K., Freeman, R. D., Laudon, M. (2000) Clinical trials of controlled-release melatonin in children with sleep-wake cycle disorders. *J. Pineal Res*. **29**: 34–39

Jiang-Shieh, Y. F., Wu, C. H., Chien, H. F., Wei, I. H., Chang, M. L., Shieh, J. Y., Wen, C. Y. (2005) Reactive changes of interstitial glia and pinealocytes in the rat pineal gland challenged with cell wall components from gram-positive and -negative bacteria. *J. Pineal Res*. **38**: 17–26

Jou, M. J., Peng, T. I., Reiter, R. J., Jou, S. B., Wu, H. Y., Wen, S. T. (2004) Visualization of the antioxidative effects of melatonin at the mitochondrial level during oxidative stress-induced apoptosis of rat brain astrocytes. *J. Pineal Res*. **37**: 55–70

Kanai, A. J., Pearce, L. L., Clemens, P. R., Birder, L. A., VanBibber, M. M., Choi, S. Y., de Groat, W., Peterson, J. (2001) Identification of a neuronal nitric oxide synthase in isolated cardiac mitochondria using electrochemical detection. *Proc. Natl Acad. Sci. USA* **98**: 14 126–14 131

Karbownik, M., Reiter, R. J., Garcia, J. J., Tan, D. X., Manchester, L. C. (2000) Melatonin reduces rat hepatic macromolecular damage due to oxidative stress caused by delta-aminolevulinic acid. *Biochem. Biophys. Acta* **1523**: 140–146

Khaldy, H., Escames, G., León, J., Vives, F., Luna, J. D., Acuña-Castroviejo, D. (2000) Comparative effects of melatonin, L-deprenyl, Trolox and ascorbate in the suppression of hydroxyl radical formation during dopamine autoxidation in vitro. *J. Pineal Res*. **29**: 100–107

Khaldy, H., Escames, G., León, J., Bikjdaouene, L., Acuña-Castroviejo, D. (2003) Synergistic effects of melatonin and deprenyl against MPTP-induced mitochondrial damage and DA depletion. *Neurobiol. Aging* **24**: 491–500

Kiefer, W., Dannhardt, G. (2002) COX-2 inhibition and the control of pain. *Curr. Opin. Investig. Drugs* **3**: 1348–1358

Kilic, E., Kilic, U., Reiter, R. J., Basseti, C. L., Hermann, D. M. (2004) Prophylactic use of melatonin protects against focal cerebral ischemia in mice: role of endothelin converting enzyme-1. *J. Pineal Res*. **37**: 247–251

Kolar, J., Machackova, I. (2005) Melatonin in higher plants: occurrence and possible functions. *J. Pineal Res*. **39**: 333–341

Kumar, A., Thota, V., Dee, L., Olson, J., Uretz, E., Parrillo, J. E. (1996) Tumor necrosis factor α and interleukin 1β are responsible

for in vitro myocardial cell depression induced by human septic shock serum. *J. Exp. Med*. **183**: 949–958

Laflamme, N., Rivest, S. (2001) Toll-like receptor 4: the missing link of the cerebral innate immune response triggered by circulating gram-negative bacterial cell wall components. *FASEB J*. **15**: 155–163

Lahiri, D. K., Chen, D., Ge, Y. W., Bondy, S. C., Sharman, E. H. (2004) Dietary supplementation with melatonin reduces levels of amyloid beta-peptides in the murine cerebral cortex. *J. Pineal Res*. **36**: 224–231

Lanone, S., Mebazaa, A., Heymes, C., Henin, D., Poderoso, J. J., Panis, Y., Zedda, C., Billiar, T., Payen, D., Aubier, M., Boczkowski, J. (2000) Muscular contractile failure in septic patients: role of the inducible nitric oxide synthase pathway. *Am. J. Respir. Crit. Care Med*. **162**: 2308–2315

León, J., Acuña-Castroviejo, D., Sainz, R. M., Mayo, J. C., Tan, D. X., Reiter, R. J. (2004) Melatonin and mitochondrial function. *Life Sci*. **75**: 765–790

León, J., Acuña-Castroviejo, D., Escames, G., Tan, D. X., Reiter, R. J. (2005) Melatonin mitigates mitochondrial malfunction. *J. Pineal Res*. **38**: 1–9

Levi, M. (2001) Pathogenesis and treatment of disseminated intravascular coagulation in the sepsis patient. *J. Crit. Care* **16**: 166–177

Liaudet, L., Pacher, P., Mabley, J. G., Virag, L., Soriano, F. G., Hasko, G., Szabo, C. (2002) Activation of poly(ADP-ribose) polymerase-1 is a central mechanism of lipopolysaccharide-induced acute lung inflammation. *Am. J. Respir. Crit. Care Med*. **165**: 373–377

Lipton, S. A., Choi, Y. B., Pan, Z. H., Lei, S. Z., Chen, H. S., Sucher, N. J., Loscalzo, J., Singel, D. J., Stamler, J. S. (1993) A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. *Nature* **364**: 626–632

Lizasoain, L., Moro, M. A., Knowles, R. G., Darley-Usmar, V., Moncada, S. (1996) Nitric oxide and peroxynitrite exert distinct effects on mitochondrial respiration which are differentially blocked by glutathione or glucose. *Biochem. J*. **314**: 877–880

López, L. C., Escames, G., Utrilla, M. P., Hitos, A. B., Rodríguez, M. I., Rivas, I., Acuña-Castroviejo, D. (2005) Heart mitochondrial failure in CLP-induced sepsis: the role of inducible mtNOS and prevention by melatonin. *XXXIII Cong. Spanish Soc. Physiol. Sci*. **61**: 294

Macarthur, H., Westfall, T. C., Riley, D. P., Misko, P. T., Salvemini, D. (2000) Inactivation of catecholamines by superoxide gives new insights on the pathogenesis of septic shock. *Proc. Natl Acad. Sci. USA* **97**: 9753–9758

Maestroni, G. J. M. (1996) Melatonin as a therapeutic agent in experimental endotoxic shock. *J. Pineal Res*. **20**: 284–289

Martin, G. S., Mannino, D. M., Eaton, S., Moss, M. (2003) The epidemiology of sepsis in United States from 1979 through 2000. *N. Engl J. Med*. **348**: 1546–1554

Martín, M., Macías, M., Escames, G., León, J., Acuña-Castroviejo, D. (2000a) Melatonin but not vitamins C and E maintains glutathione homeostasis in t-butyl hydroperoxide-induced mitochondrial oxidative stress. *FASEB J*. **14**: 1677–1679

Martín, M., Macías, M., Escames, G., Reiter, R. J., Agapito, M. T., Ortiz. G. G., Acuña-Castroviejo, D. (2000b) Melatonin-induced increased activity of the respiratory chain complexes I and IV can prevent mitochondrial damage induced by ruthenium red in vivo. *J. Pineal Res*. **28**: 242–248

Martín, M., Macías, M., León, J., Escames, G., Khaldy, H., Acuña-Castroviejo, D. (2002) Melatonin increases the activity of the oxidative phosphorylation enzymes and the production of ATP in rat brain and liver mitochondria. *Int. J. Biochem. Cell. Biol*. **34**: 348–357

Matsubara, E., Bryant-Thomas, T., Pacheco Quinto, J., Henry, T. L., Poeggeler, B., Herbert, D., Cruz-Sanchez, F., Chyan, Y. J., Smith, M. A., Perry, G., Shoji, M., Abe, K., Leone, A., Grundke-Ikbal, I., Wilson, G. L., Ghiso, J., Williams, C., Refolo, L. M., Pappolla, M. A., Chain D. G., Neria, E. (2003) Melatonin increases survival and inhibits oxidative and amyloid pathology in a transgenic model of Alzheimer's disease. *J. Neurochem*. **85**: 1101–1108

Mayo, J. C., Sainz, R. M., Tan, D. X., Hardeland, R., León, J., Rodríguez, C., Reiter, R. J. (2005) Anti-inflammatory actions of melatonin and its metabolites, N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) and N1-acetyl-5-methoxykynuramine (AMK), in macrophages. *J. Neuroimmunol*. **165**: 139–149

McCloskey, R. V., Straube, R. C., Sanders, C., Smith, S. M., Smith, C. R. (1994) Treatment of septic shock with human monoclonal antibody HA-1A: a randomized, double-blind, placebo-controlled trial. CHESS Trial Study Group. *Ann. Intern. Med*. **121**: 1–5

Mela, L., Bacalzo, L. V., White, R. R., Miller, L. D. (1970) Shock induced alterations of mitochondrial energy-linked functions. *Surg. Forum* **21**: 6–8

Menendez-Pelaez, A., Reiter, R. J. (1993) Distribution of melatonin in mammalian tissues: the relative importance of nuclear versus cytosolic localization. *J. Pineal Res*. **15**: 59–69

Mirochnitchenko, O., Prokopenko, O., Painitkar, U., Kisler, I., Powell, W. S., Inouye, M. (2000) Endotoxemia in transgenic mice overexpressing human glutathione peroxidases. *Circ. Res*. **87**: 289–295

Molina-Carballo, A., Muñoz-Hoyos, A., Reiter, R. J., Sanchez-Forte, M., Moreno-Madrid, F., Rufo-Campos, M., Molina-Font, J. A., Acuña-Castroviejo, D. (1997) Utility of high doses of melatonin as adjunctive anticonvulsant therapy in a child with severe myoclonic epilepsy: two years' experience. *J. Pineal Res*. **23**: 97–105

Morrison, D. C., Ulevitch, R. J. (1978) The effects of bacterial endotoxins on host mediation systems. A review. *Am. J. Pathol*. **93**: 526–617

Motilva, V., Cabeza, J., Alarcon de la Lastra, C. (2001) New issues about melatonin and its effects on the digestive system. *Curr. Pharm. Des*. **7**: 909–931

Muller, A. M., Cronen, C., Muller, K. M., Kirkpatrick, C. J. (2002) Heterogeneous expression of cell adhesion molecules by endothelial cells in ARDS. *J. Pathol*. **198**: 270–275

Mundigler, G., Delle-Karth, G., Koreny, M., Zehetgruber, M., Steindl-Munda, P, Marktl, W., Ferti, L., Siostrzonek, P. (2002) Impaired circadian rhythm of melatonin secretion in sedated critically ill patients with severe sepsis. *Crit. Care Med*. **30**: 536–540

Opal, S., Laterre, P. F., Abraham, E., Francois, B., Wittebole, X., Lowry, S., Dhainaut, J. F., Warren, B., Dugernier, T., Lopez, A., Sanchez, M., Demeyer, I., Jauregui, L., Lorente, J. A., McGee, W., Reinhart, K., Kljucar, S., Souza, S., Pribble, J. (2004) Recombinant human platelet-activating factor acetylhydrolase for treatment of severe sepsis: results of a phase III, multicenter, randomized, double-blind, placebo-controlled, clinical trial. *Crit. Care Med*. **32**: 332–341

Ozdulger, A., Cinel, I., Koksel, O., Cinel L., Avlan, D., Unlu, A., Okcu, H., Dikmengil, M., Oral, U. (2003) The protective effect of N-acetylcysteine on apoptotic lung injury in cecal ligation and puncture-induced sepsis model. *Shock* **19**: 366–372

Parrillo, J. E. (1993) Pathogenetic mechanisms of septic shock. *N. Engl J. Med*. **328**: 1471–1477

Paskaloğlu, K., Sener, G., Kapucu, C., Ayanoğlu-Dülger, G. (2004) Melatonin treatment protects against sepsis-induced functional and biochemical changes in rat ileum and urinary bladder. *Life Sci*. **74**: 1093–1204

Perez, E. M., Weisman, L. E. (1997) Novel approaches to the prevention and therapy of neonatal bacterial sepsis. *Clin. Perinatol*. **24**: 213–225

Peters, K., Unger, R. E., Brunner, J., Kirkpatrick, J. (2003) Molecular basis of endothelial dysfunction in sepsis. *Cardiovasc. Res*. **60**: 49–57

Petrucci, N., Lacovelli, W. (2004) Ventilation with lower tidal volumes versus traditional tidal volumes in adults for acute lung injury and acute respiratory distress syndrome. *Cochrane Database Syst. Rev*. **2**: CD003844

Pinsky, M. R. (2001) Sepsis: a pro- and anti-inflammatory disequilibrium syndrome. *Contrib. Nephrol*. **132**: 354–366

Polderman, K. H., Girbes, A. R. J. (2004) Drug intervention trials in sepsis: divergent results. *Lancet* **363**: 1721–1723

Putensen, C., Wrigge, H. (2000) Ventilator-associated systemic inflammation in acute lung injury. *Intensive Care Med*. **26**: 1411–1413

Reiter, R. J. (1991) Pineal melatonin: cell biology of its synthesis and of its physiological interactions. *Endocr. Rev*. **12**: 151–180

Reiter, R. J. (1995) Oxidative processes and antioxidative defense mechanisms in the aging brain. *FASEB J*. **9**: 526–533

Reiter, R. J., Tan, D. X. (2002) Melatonin: an antioxidant in edible plants. *Ann. N. Y. Acad. Sci*. **957**: 341–344

Reiter, R. J., Tan, D. X. (2003) What constitutes a physiological concentration of melatonin? *J. Pineal Res*. **34**: 79–80

Reiter, R. J., Tan, D. X., Osuna, C., Gitto, E. (2000) Actions of melatonin in the reduction of oxidative stress: a review. *J. Biomed. Sci*. **7**: 444–458

Reiter, R. J., Tan, D. S., Manchester, L. C., Qi, W. (2001) Biochemical reactivity of melatonin with reactive oxygen and nitrogen species. *Cell. Biochem. Biophys*. **34**: 237–256

Reiter, R. J., Tan, D. X., Pappolla, M. A. (2004) Melatonin relieves the neural oxidative burden that contributes to dementia. *Ann. N. Y. Acad. Sci*. **1035**: 179–196

Ressmeyer, A. R., Mayo, J. C., Zelosko, V., Sainz, R. M., Tan, D. X., Poeggeler, B., Antolin, I., Zsizsik, B. K., Reiter, R. J., Hardeland, R. (2003) Antioxidant properties of the melatonin metabolite N1-acetyl-5-methoxykynuramine (AMK): scavenging of free radicals and prevention of protein destruction. *Redox Rep*. **8**: 205–213

Reyes Toso, C. F., Ricci, C. R., de Mignone, I. R., Reyes, P., Linares, L. M., Albornoz, L. E., Cardinali, D. P., Zaninovich, A. (2003) In vitro effect of melatonin on oxygen consumption in liver mitochondria of rats. *Neuroendocrinol. Lett*. **24**: 341–344

Reynolds, F. D., Dauchy, R., Blask, D., Dietz, P. A., Lynch, D., Zuckerman, R. (2003) The pineal gland hormone melatonin improves survival in a rat model of sepsis/shock induced by zymosan A. *Surgery* **134**: 474–479

Rivas, I., León, J., López, L. C., Ruiz, M., Carretero, M., Escames, G., Valera, M. D., Acuña-Castroviejo, D. (2005) Melatonin prevents rotenone-induced mitochondrial complex I inhibition, oxidative damage and cell death in PC12 cells. *J. Physiol. Biochem*. **61**: 141–141

Rodriguez, C., Mayo, J. C., Sainz, R. M., Antolin, I., Herrera, F., Martin, V., Reiter, R. J. (2004) Regulation of antioxidant enzymes: a significant role for melatonin. *J. Pineal Res*. **36**: 1–9

Ruetten, H., Thiemermann, C. (2000) Nitric oxide and septic shock. In: Ignarro, L. (ed.) *Nitric oxide: biology and pathobiology*. Academic Press, San Diego, pp 747–758

Salvemini, D., Cuzzocrea, S. (2003) Therapeutic potential of superoxide mimetics as therapeutic agents in critical care medicine. *Crit. Care Med*. **31**: S29–38

Sands, K. E., Bates, D. W., Lanken, P. N., Graman, P. S., Hibberd, P. L., Kahn, K. L., Parsonnet, J., Panzer, R., Orav, E. J., Snydman, D. R. (1997) Epidemiology of sepsis syndrome in 8 academic medical centers. *J. Am. Med. Assoc*. **278**: 234–240

Schrier, R. W., Wang, W. (2004) Acute renal failure and sepsis. *N. Engl. J. Med*. **351**: 159–169

Seabra, M. L., Bignotto, M., Pinto, L. R. Jr, Tufik, S. (2000) Randomized double-blind clinical trial, controlled with placebo, of the toxicology of chronic melatonin treatment. *J. Pineal Res*. **29**: 193–200

Sener, G., Toklu, H., Kapucu, C., Ercan, F., Erkanli, G., KaÇmaz, A., Tilki, M., Yeğen, B. Ç. (2005) Melatonin protects against organ injury in rat model of sepsis. *Surg. Today* **35**: 52–59

Sewerynek, E., Abe, M., Reiter, R. J., Barlow-Walden, L. R., Chen, L., McCabe, T. J., Roman, L. J., Diaz-Lopez, B. (1995a) Melatonin administration prevents lipopolysaccharide-induced oxidative damage in phenobarbital-treated animals. *J. Cell Biochem*. **58**: 436–444

Sewerynek, E., Melchiorri, D., Chen, L. D., Reiter, R. J. (1995b) Melatonin reduces both basal and bacterial lipopolysaccharide-induced lipid peroxidation in vitro. *Free Radic. Biol. Med*. **19**: 903–909

Sewerynek, E., Wiktorska, J., Lewinski, A. (1999) Effects of melatonin on the oxidative stress induced by thyrotoxicosis in rats. *Neuroendocrinol Lett*. **20**: 157–161

Skinner, D. C., Malpaux B. (1999) High melatonin concentrations in third ventricular cerebrospinal fluid are not due to Galen vein blood recirculating through the choroids plexus. *Endocrinology* **140**: 4399–4405

Slominski, A., Fischer, T., Zmijewski, M. A., Wortsman, J., Sergov, I., Zhytek, B., Slominski, R. M., Tobin, D. T. (2005) On the role of melatonin in skin physiology and pathology. *Endocrine* **27**: 137–148

Sriskandan, S., Cohen, J. (1995) The pathogenesis of septic shock. *J. Infect*. **30**: 201–206

Sullivan, D. J., Shelby, J., Shao, Y., Affleck, D. G., Hinson, D. M., Barton, R. G. (1996) Melatonin and a 21-aminonosteroid attenuate shock after hemorrhage but differentially affect serum cytokines. *J. Surg. Res*. **64**: 13–18

Tan, D. X., Chen, L. D., Poeggeler, B., Manchenster, L. C., Reiter, R. J. (1993) Melatonin: a potent endogenous hydroxyl radical scavenger. *Endocr. J*. **1**: 57–60

Tan, D. X., Reiter, R. J., Chen, L. D., Poeggeler, B., Manchester, L. C., Barlow-Walden, L. R. (1994) Both physiological and pharmacological levels of melatonin reduce DNA adduct formation induced by the carcinogen safrole. *Carcinogenesis* **15**: 215–218

Tan, D. X., Manchester, L. C., Reiter, R. J., Qi, W., Zhang, W., Weintraub, S., Cabrera, J., Sainz, R. M., Mayo, J. C. (1999) Identification of highly elevated levels of melatonin in bone marrow: its origin and physiological significance. *Biochim. Biophys. Acta* **1472**: 206–214

Tan, D. X., Manchester, L. C., Sainz, R. M., Mayo, J. C., Alvarez, F. L., Reiter, R. J. (2003) Antioxidant strategies in protection against neurodegeneration disorders. *Expert Opin. Ther. Patents* **13**: 1513–1543

Tatoyan, A., Giulivi, C. (1998) Purification and characterization of a nitric oxide synthase from rat liver mitochondria. *J. Biol. Chem*. **273**: 11 044–11 048

Tay, S. Y., O'Brien, T. E., McNulty, J. A. (2001) Microglia play a role in mediating the effects of cytokines on the structure and function of the rat pineal gland. *Cell Tissue Res*. **303**: 423–431

Thiemermann, C. (2003) Membrane-permeable radical scavenger (tempol) for shock, ischemia-reperfusion injury, and inflammation. *Crit. Care Med*. **31**: S76–84

Thiemermann, C., Vane, J. R. (1990) Inhibition of nitric oxide synthesis reduces the hypotension induced by bacterial lipopolysaccharide in the rat in vivo. *Eur. J. Pharmacol*. **182**: 591–595

Titheradge, M. A. (1999) Nitric oxide in septic shock. *Biochim. Biophys. Acta*. **1411**: 437–455

Tsiotou, A. G., Sakorafas, G. H., Anagnostopoulos, G., Bramis, J. (2005) Septic shock; current pathogenetic concepts from a clinical perspective. *Med. Sci. Monit*. **11**: RA76–RA85

Urata, Y., Honma, S., Goto, S., Todoroki, S., Tida, T., Cho, S., Honma, K., Kondo, T. (1999) Melatonin induces gamma-glutamylcysteine synthetase mediated by activator protein-1 in human vascular endothelial cells. *Free Radic. Biol. Med*. **27**: 838–847

Vane, J. R., Bakhle, Y. S., Botting, R. M. (1998) Cyclooxygenases 1 and 2. *Annu. Rev. Pharmacol. Toxicol*. **38**: 97–120.

Victor, V. M., Rocha, M., De la Fuente, M. (2004) Immune cells: free radicals and antioxidants in sepsis. *Int. Immunopharmacol.* **4**: 327–347

Wakatsuki, A., Okatani, Y., Shinohara, K., Ikenoue, N., Kaneda, C., Fukaya, T. (2001) Melatonin protects fetal rat brain against oxidative mitochondrial damage. *J. Pineal Res.* **30**: 22–28

Wang, D. L., Ling, Z. Q., Cao, F. Y., Zhu, L. Q., Wang, J. Z. (2004) Melatonin attenuates isoproterenol-induced protein kinase A overactivation and tau hyperphosphorylation in rat brain. *J. Pineal Res.* **37**: 11–16

Warren, H. S., Amato, S. F., Fitting, C., Black, K. M., Loiselle, P. M., Pasternack, M. S., Cavaillon, J. M. (1993) Assessment of ability of murine and human anti-lipid A monoclonal antibodies to bind and neutralize lipopolysaccharide. *J. Exp. Med.* **177**: 89–97

Webster, N. R., Nunn, J. F. (1988) Molecular structure of free radicals and their importance in biological reactions. *Br. J. Anaesth.* **60**: 98–108

Weitzberg, E. (2005) L-arginine transport and sepsis. *Acta Anaesthesiol. Scand.* **49**: 434–436

Wichman, M. W., Haisken, J. M., Ayala, A., Chaudry, I. H. (1996) Melatonin administration following hemorrhagic shock decreases mortality from subsequent septic challenge. *J. Surg. Res.* **65**: 109–114

Wu, C. C., Chiao, C. W., Hsiao, G., Chen, A., Yen, M. H. (2001) Melatonin prevents endotoxin-induced circulatory failure in rats. *J. Pineal Res.* **30**: 147–156

Wu, Y. H., Swaab, D. F. (2005) The human pineal gland and melatonin in aging and Alzheimer's disease. *J. Pineal Res.* **38**: 145–152

Yerer, M. B., Aydogan, S., Yapislar, H., Yalcin, O., Kuru, O., Baskurt, O. K. (2003) Melatonin increases glutathione peroxidase activity and deformability of erythrocytes in septic rats. *J. Pineal Res.* **35**: 138–139

Yerer, M. B., Yapislar, H., Aydogan, S., Yalcin, O., Baskurt, O. (2004) Lipid peroxidation and deformability of red blood cells in experimental sepsis in rats: the protective effects of melatonin. *Clin. Hemorheol. Microcirc.* **30**: 77–82

Zavodnik, I. B., Lapshina, E. A., Zavodnik, L. B., Labieniec, M., Bryszewska, M., Reiter, R. J. (2004) Hypochlorous acid-induced oxidative stress in Chinese hamster B14 cells: viability, DNA and protein damage and the protective action of melatonin. *Mutat. Res.* **559**: 39–48

Ziegler, E. J., Fisher, C. J., Sprung, C. L., Straube, R. C., Sadoff, J. C., Foulke, G. E., Wortel, C. H., Fink, M. P., Dellinger, R. P., Ten, N. N. (1991) Treatment of gram-negative bacteremia and septic shock with HA-1A human monoclonal antibody against endotoxin: a randomized, double-blind, placebo-controlled trial. The HA-1A sepsis study group. *N. Engl J. Med.* **324**: 429–436

Zimmerman, J. J. (1995) Defining the role of oxyradicals in the pathogenesis of sepsis. *Crit. Care Med.* **23**: 646–651