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Melatonin Protects Against Oxidative Organ Injury in a Rat Model of Sepsis

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Abstract

Purpose. Based on the potent antioxidant effects of melatonin, we investigated the putative protective role of melatonin against sepsis-induced oxidative organ damage in rats.

Methods. Sepsis was induced by cecal ligation and puncture (CLP) in Wistar albino rats. Animals subjected to CLP and sham-operated control rats were given saline or melatonin 10 mg/kg intraperitoneally 30 min before and 6 h after the operation. The rats were killed 16 h after the operation and the biochemical changes were investigated in the liver, kidney, heart, lung, diaphragm, and brain tissues by examining malondialdehyde (MDA) and glutathione (GSH) levels, and myeloperoxidase (MPO) activity. We also examined the tissues microscopically.

Results. Sepsis resulted in a significant decrease in GSH levels and a significant increase in MDA levels and MPO activity ($P < 0.05$–$P < 0.001$) showing oxidative damage, which was confirmed by histological examination. Melatonin clearly reversed these oxidant responses and the microscopic damage, demonstrating its protective effects against sepsis-induced oxidative organ injury.

Conclusion. The increase in MDA levels and MPO activity and the concomitant decrease in GSH levels demonstrate the role of oxidative mechanisms in sepsis-induced tissue damage. Melatonin, by its free radical scavenging and antioxidant properties, ameliorated oxidative organ injury. Thus, supplementing antiseptic shock treatment with melatonin may be beneficial in the clinical setting.

Key words Sepsis · Melatonin · Lipid peroxidation · Glutathione · Myeloperoxidase activity

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Introduction

Sepsis is a generalized inflammatory response, involving organ systems remote from the locus of the initial infectious insult.[1] Recent studies have shown that sepsis is associated with the enhanced generation of reactive oxygen metabolites (ROMs), leading to multiple organ dysfunction. The activation of macrophages and cytokines by endotoxin and the subsequent formation of reactive oxygen and nitrogen species are of central pathogenic importance in various inflammatory diseases, including sepsis. However, it is still unknown if different tissues behave in the same way during these pathological changes.[2]

The release of endotoxin (lipopolysaccharide; LPS) from bacteria is generally thought to be the initial event in the development of sepsis. Lipopolysaccharide activates inflammatory cells of the myeloid lineage, which subsequently amplify the inflammatory response by releasing various cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β). This systemic inflammatory cascade results in polymorphonuclear leukocyte (PMN) sequestration in various systemic organs, such as the heart and lungs. Subsequent PMN extravasation can lead to vascular dysfunction as well as parenchymal cell dysfunction.[1]

It was recently suggested that antioxidants might counteract the toxicity of oxygen radicals and ROMs,[3] and that free radical ablation for the treatment of sepsis could be useful in the clinical setting of sepsis-induced multiple organ failure.[4] Melatonin, a secretory product of the pineal gland and a potent free radical scavenger and antioxidant,[5] is thought to play a protective role in the initial and advanced stages of various diseases, with a pathogenesis involving damage by ROMs. Both in vitro and in vivo experiments have shown that melatonin protects tissues against oxidative damage in several ways. First, it scavenges hydrochlorous acid at a rate sufficient to protect catalase against inactivation by this

molecule;[6] second, it inhibits nitric oxide synthase and reduces tissue damage by peroxynitrite anion or its metabolites;[7] third, it directly scavenges peroxynitrite and detoxifies highly toxic hydroxyl radicals and peroxyl radicals in vitro;[8,9] and fourth, it stimulates the activity or genetic expression of several important endogenous antioxidant enzymes, including glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and glutatione reductase (GSH-Rd).[5] The remarkable protective effects of melatonin against oxidative stress are aided by its ability to cross all biological membranes. Thus, when melatonin reaches its highest concentration in the nucleus of the cell, it protects the DNA in the nucleus from free radical damage induced by ionizing radiation.[10] It may also influence hemotopoiesis, either by stimulating hemopoietic cytokines, including opioids, or by directly affecting specific progenitor cells, such as pre-B cells, monocytes, and natural killer cells. It was also proposed that melatonin may be used to stimulate the immune response, and strengthen immune reactivity, as a prophylactic procedure. The purpose of this study was to investigate the role of oxidative stress in sepsis-induced remote organ injury, and the putative protective effect of melatonin on the inflammatory responses of different organs to the initial infectious insult.

Materials and Methods

All experimental protocols were approved by the Marmara University School of Medicine Animal Care and Use Committee.

Animals and Protocol for the Induction of Sepsis

Wistar albino rats of both sexes, weighing 200–250 g, were fasted for 12 h but allowed free access to water before the experiments. The animals were kept in individual wire-bottomed cages, in a room at a constant temperature (22° ± 2°C) with 12-h light and dark cycles, and fed standard rat chow. The rats were divided into the following four groups of eight rats (four males and four females) each: a sham-operated control (C) group, a melatonin-treated sham-operated (Mel) group, a cecal ligation and perforation (CLP) group, and a melatonin-treated CLP (Mel + CLP) group.

Sepsis was induced by the CLP technique described previously.[11] Under brief ether anesthesia, a midline laparotomy was made using minimal dissection and the cecum was ligated just below the ileocaecal valve with 3-0 silk, so that intestinal continuity was maintained. The antimesenteric surface of the cecum was perforated with an 18-gauge needle at two locations 1 cm apart and the cecum was gently compressed until fecal matter was extruded. The bowel was then returned to the abdomen and the incision was closed. At the end of the operation, all rats were resuscitated with saline, 3 ml/100 g body weight, given subcutaneously. Postoperatively, the rats were deprived of food, but had free access to water for the next 16 h until they were killed. The sham-operated groups were given a laparotomy and the cecum was manipulated, but not ligated or perforated.

Melatonin Treatment

Melatonin (Sigma, St. Louis, MO, USA) was dissolved in absolute ethanol and diluted further with saline, until the final concentration of ethanol was 1%. Either melatonin 10 mg/kg or the vehicle of 1% alcohol in saline, 1 ml/kg was given intraperitoneally (i.p.) 30 min before and 6 h after the operation. The rats were decapitated 16 h after the CLP procedure, and trunk blood was collected for the determination of hepatic function, as aspartate transaminase (AST) and alanine transaminase (ALT), and renal function, as creatinine and blood urea nitrogen (BUN) levels. Liver, kidney, heart, lung, diaphragm, and brain tissue samples were immediately taken and stored at −70°C. The levels of malondialdehyde (MDA), an end product of lipid peroxidation, and glutathione (GSH), a key antioxidant, were measured later. Tissue-associated myeloperoxidase (MPO) activity, as indirect evidence of neutrophil infiltration, was measured in all tissue samples.

Hepatic and Renal Function Tests

Blood urea nitrogen and creatinine concentrations were studied to assess renal function.[12] Serum AST and ALT levels were studied to assess liver function, using AST and ALT (Roche Diagnostic, Mannheim, Germany) commercial kits from Roche Diagnostic (Roche-Hitachi Modular Autoanalyzer).

Malondialdehyde and Glutathione Assays

Tissue samples were homogenized with 150 mM ice-cold KC1 for the determination of MDA and glutathione levels. The MDA levels were assayed for products of lipid peroxidation[13] and results are expressed as μmol MDA/g tissue. Glutathione was determined by the spectrophotometric method, based on the use of Ellman's reagent,[14] and results are expressed as μmol GSH/g tissue.

Myeloperoxidase Activity

Myeloperoxidase activity in tissues was measured by a procedure similar to that described by Hillegass et al.[15]

Samples of liver, lung, or intestinal tissue were homogenized in 50 mM potassium phosphate buffer (PB), pH 6.0, and centrifuged at 41 400 × *g* for 10 min. The pellets were then suspended in 50 mM PB containing 0.5% hexadecyltrimethylammonium bromide. After three freeze-and-thaw cycles, with sonication between cycles, the samples were centrifuged at 41 400 × *g* for 10 min. Aliquots (0.3 ml) were added to 2.3 ml of reaction mixture containing 50 mM PB, *o*-dianisidine, and 20 mM H_2O_2 solution. One unit of enzyme activity was defined as the amount of MPO present that caused a change in absorbance, measured at 460 nm for 3 min. Myeloperoxidase activity was expressed as U/g tissue.

Histological Preparation and Analysis

Samples of lung, liver, kidney, diaphragm, heart, and brain tissue were fixed in 10% formaldehyde and processed routinely for embedding in paraffin. Paraffin sections were stained with hematoxylin–eosin and examined under a light microscope. Microscopic scoring was done by experienced histologists, unaware of which treatment the animal was subjected to. The histological score of the organ was calculated as the sum of the scores (0 to 3) given for each criterion, using the semiquantitative scale outlined in Table 1.[16,17] The maximum score calculated was 9 for the lung, liver, and kidney, and 6 for the diaphragm, heart, and brain.

Statistical Analysis

Statistical analysis was done using a GraphPad Prism 3.0 (GraphPad Software, San Diego, CA, USA). All data are expressed as mean ± SEM. Groups of data were compared with an analysis of variance followed by Tukey's multiple comparison tests. Values of $P < 0.05$ were considered significant.

Results

Malondialdehyde Levels

The MDA levels in the liver, kidney, heart, lung, diaphragm, and brain were significantly higher in the CLP group than in the control group ($P < 0.05$–$P < 0.001$). Treatment with melatonin significantly reversed the elevations in MDA levels in all the tissues ($P < 0.05$–$P < 0.001$; Fig. 1).

Glutathione Levels

Sepsis caused a significant decrease ($P < 0.05$–$P < 0.001$) in GSH levels in all tissues, compared with the control group. Melatonin treatment significantly ($P < 0.001$) reversed the GSH level back to the control value in the live, kidney, diaphragm, and brain, but the decreased GSH levels in the cardiac and pulmonary tissues were not altered by melatonin (Fig. 2).

Myeloperoxidase Activity

Myeloperoxidase activity is an indicator of tissue neutrophil infiltration.[18] The MPO activity was significantly higher ($P < 0.001$) in all the tissues of the CLP group (except the cardiac tissue) than in the control group. On the other hand, the neutrophil infiltration induced by sepsis was decreased by melatonin treatment ($P < 0.01$–$P < 0.001$; Fig. 3).

Plasma ALT, AST, BUN, and Creatinine Levels

As shown in Table 2, plasma ALT, AST, BUN, and creatinine levels were significantly ($P < 0.05$–$P < 0.001$) higher in the CLP group than in the control group. Giving melatonin to the CLP group significantly ($P <$

Table 1. Criteria for the microscopic scoring of tissue damage

Tissue	Appearance
Lung	Vascular congestion and interstitial edema
	Alveolar structural disturbance
	Inflammatory cell infiltration
Liver	Vacuolization of hepatocytes and pyknotic hepatocyte nuclei
	Enlargement of sinusoids
	Kupffer cell infiltration
Kidney	Degeneration of Bowman space and glomeruli
	Degeneration of proximal and distal tubules
	Vascular congestion and interstitial edema
Diaphragm and heart	Inflammatory cell infiltration
	Degeneration of muscle fibers
Brain	Degeneration of neurons
	Vascular edema and hemorrhage

Scores for each criterion are given as 0, none; 1, mild; 2, moderate; 3, severe. At least five microscopic areas were examined to score each specimen

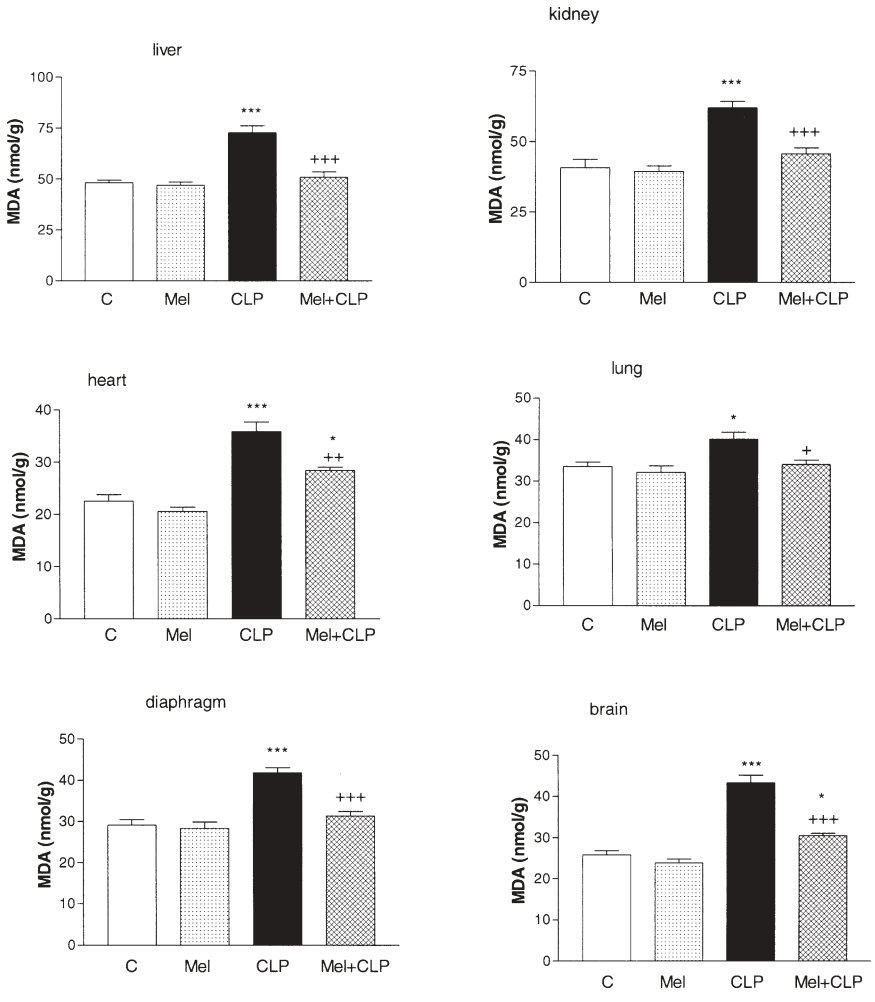

Fig. 1. Liver, kidney, heart, lung, diaphragm, and brain malondialdehyde (*MDA*) levels in the melatonin- and vehicle-treated groups ($n = 8$ in each group). *C*, control; *Mel*, melatonin; *CLP*, cecal ligation and perforation. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the control group; $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$, melatonin-treated CLP group compared with the respective vehicle-treated group

0.05–$P < 0.001$) abolished the elevations in these values.

Histological Scores

Sepsis-induced cellular damage was seen in all the tissues (Table 3), demonstrating structural degeneration, vasocongestion, and edema, accompanied by inflammatory cell infiltration. Melatonin treatment reduced the scores significantly ($P < 0.001$) in all tissues examined.

Discussion

Our results clearly showed that sepsis causes oxidative damage in the liver, kidney, heart, lungs, diaphragm, and brain tissues, as demonstrated by the increased lipid peroxidation and decreased GSH levels. Melatonin treatment depressed lipid peroxidation in all of the tissues, verifying the protective effect of melatonin against oxidative injury. Melatonin replenished the GSH content in all except the cardiac and pulmonary tissues. Moreover, oxidative injury of the tissues studied, apart from cardiac tissue, was accompanied by neutrophil infiltration, but melatonin treatment suppressed the sepsis-induced elevation of MPO activities in these organs. It also ameliorated the hepatic and renal dysfunction according to the biochemical parameters. These data, together with the histological findings, suggest that sepsis-induced oxidative injuries in most organs are neutrophil-dependent and can be attenuated by melatonin.

Septic shock, a severe form of sepsis associated with the development of progressive damage to multiple organs, is an important cause of mortality in intensive care units.[19,20] Many reports suggest that ROMs play an important role in the pathogenesis of sepsis and its complications. Experimental and clinical studies have shown that any harmful tissue event, such as infection, trauma, or anoxia, is perceived by macrophages and monocytes, which in turn secrete cytokines, such as IL-1 and TNF-α. These cytokines activate inflammatory cells, such as

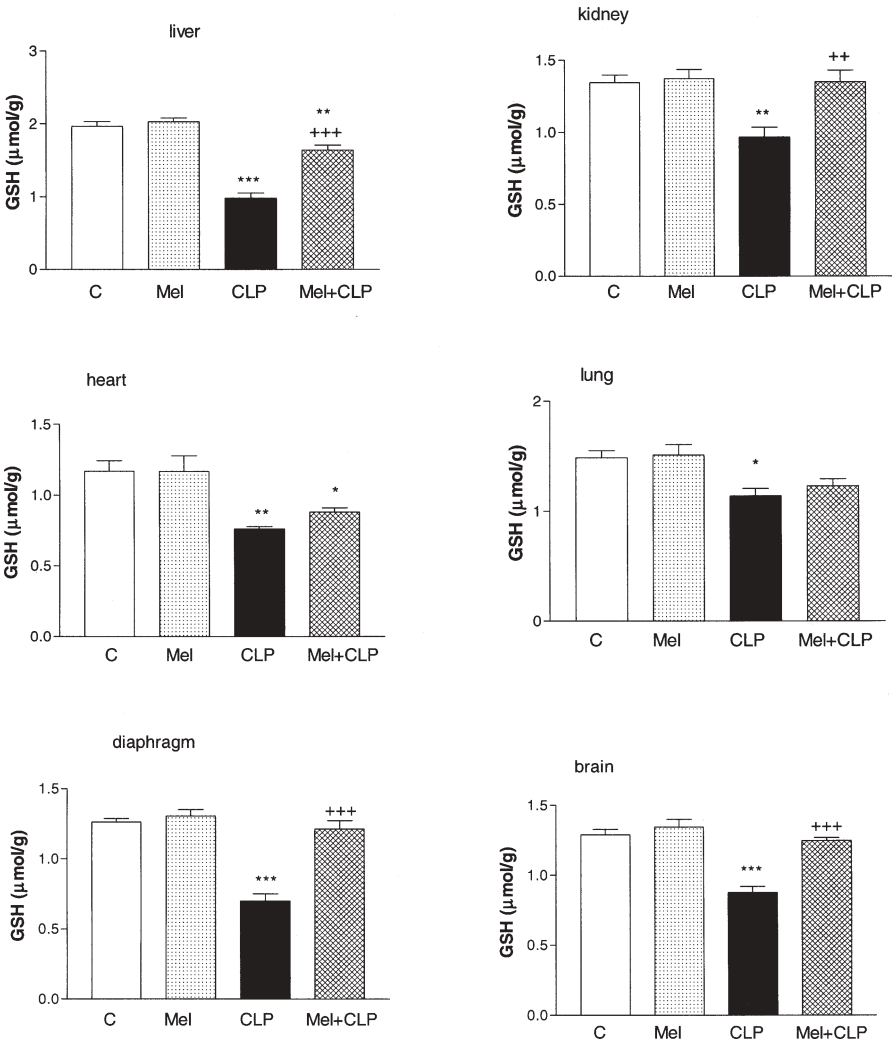

Fig. 2. Liver, kidney, heart, lung, diaphragm, and brain glutathione (*GSH*) levels in melatonin- and vehicle-treated groups ($n = 8$ in each group). *C*, control; *Mel*, melatonin; *CLP*, cecal ligation and perforation. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the control group; $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$, melatonin-treated CLP group compared with the respective vehicle-treated group

neutrophils, macrophages or monocytes, platelets, and mastocytes, releasing large amounts of the toxic oxidizing ROMs, which cause cellular injury via several mechanisms including the peroxidation of membrane lipids and the oxidative damage of proteins and DNA.[21]

Melatonin, the chief secretory product of the pineal gland, was recently found to be a potent free radical scavenger and antioxidant,[22–25] and is thought to play a protective role in the initial and advanced stages of diseases with a pathogenesis involving damage by reactive oxygen metabolites. Both in vitro and in vivo experiments have shown that melatonin protects tissues against oxidative damage induced by many free radical generating agents and processes, including the chemical carcinogen safrol, LPS, kainic acid, Fenton reagents, potassium cyanide, L-cysteine, excessive exercise, ischemia-reperfusion, and ionizing radiation.[26,27] Melatonin reduces oxidative stress by scavenging hydrochlorous acid at a rate sufficient to protect catalase against inactivation by this molecule.[28] It also detoxifies highly toxic hydroxyl radical and peroxyl radical in vitro,[23,24] and directly scavenges and stimulates the activity of the endogenous antioxidant enzyme, glutathione peroxidase, possibly caused by its ability to remove hydrogen peroxide.[29] A previous study found that melatonin reduces circulatory failure in animals with endotoxic shock,[22] which was attributed to its anti-inflammatory effect on cytokines, such as TNF-α, its suppression on nitric oxide synthase expression, and its antioxidant properties.

Lipid peroxidation can cause changes in membrane fluidity and permeability and increase the rate of protein degradation, which will eventually lead to cell lysis.[8] In the present study the levels of MDA, an end product of lipid peroxidation, were significantly increased in all of the tissues studied. This observation is in agreement with previous studies, which showed increased levels of lipid products as a result of oxidative stress.[30–32] Furthermore, we found that the antioxidant melatonin abolished MDA elevations, which suggests that by

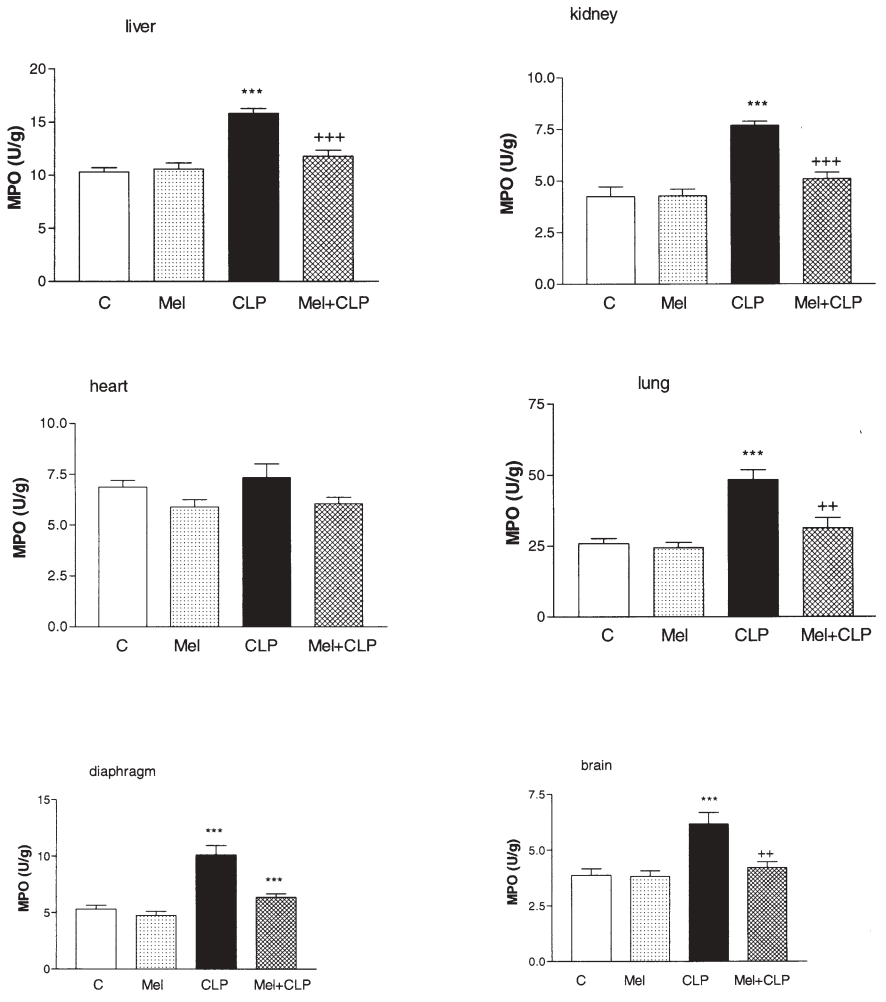

Fig. 3. Liver, kidney, heart, lung, diaphragm, and brain myeloperoxidase (*MPO*) activity in the melatonin- and vehicle-treated groups ($n = 8$ in each groups). *C*, control; *Mel*, melatonin; *CLP*, cecal ligation and perforation. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the control group; $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$, melatonin-treated CLP group compared with the respective vehicle-treated group

Table 2. Plasma aspartate transaminase, alanine transaminase, blood urea nitrogen, and creatinine levels in the control (C), melatonin (Mel), cecal ligation and puncture (CLP), and Mel + CLP groups ($n = 8$ per group)

	C	Mel	CLP	Mel + CLP
AST (U/l)	134.6 ± 15.3	132.5 ± 11.4	574.2 ± 44.6***	361.5 ± 5.7***,++
ALT (U/l)	69.7 ± 8.5	57.8 ± 3.5	153.7 ± 34.2*	64.2 ± 10.7+
BUN (mg/dl)	20.2 ± 1.5	19.0 ± 1.3	56.8 ± 5.4***	30.0 ± 4.17+++
Creatinine (mg/dl)	0.29 ± 0.2	0.28 ± 0.1	0.9 ± 0.2***	0.36 ± 0.1++

AST, aspartate transaminase; ALT, alanine transaminase; BUN, blood urea nitrogen
$^{*}P < 0.05$, $^{***}P < 0.001$ vs control; $^{++}P < 0.01$, $^{+++}P < 0.001$ CLP vs Mel + CLP

preserving cellular integrity, melatonin can protect against sepsis-induced organ damage. Previous studies also showed that melatonin prevented oxidative damage in rats treated with lipopolysaccharide[33] or carrageenan.[34] Similarly, melatonin improved the clinical outcome of infants with sepsis, while elevated serum MDA levels in septic newborns were reduced significantly by melatonin treatment.[21]

In many diseases and acute inflammatory disorders, important components of the pathological process are linked to the ability of neutrophils to release a complex assortment of agents that can destroy normal cells and dissolve connective tissue.[35,36] Observations suggest that ROMs play a role in the recruitment of neutrophils into injured tissues, but activated neutrophils are also a potential source of ROMs.[37] Reactive oxygen metabolites

Table 3. Total histological scores of the liver, kidney, heart, lung, diaphragm, and brain tissues of the control (C), melatonin (Mel), cecal ligation and puncture (CLP), and Mel + CLP groups ($n = 8$ per group)

	C	Mel	CLP	Mel + CLP
Liver	0.13 ± 0.1	0.13 ± 0.1	9.00 ± 0.0***	3.75 ± 0.1***,+++
Kidney	0.38 ± 0.3	0.25 ± 0.2	9.00 ± 0.0***	5.87 ± 0.6***,+++
Lung	0.13 ± 0.1	0.00 ± 0.0	9.00 ± 0.0***	4.87 ± 0.9***,+++
Diaphragm	0.00 ± 0.0	0.13 ± 0.1	6.00 ± 0.0***	3.50 ± 0.4***,+++
Heart	0.25 ± 0.2	0.25 ± 0.3	5.75 ± 0.2***	3.87 ± 0.4***,+++
Brain	0.13 ± 0.1	0.13 ± 0.1	5.60 ± 0.1***	2.80 ± 0.5***,+++

The maximum score given was 9 for the lung, liver, and kidney, and 6 for the diaphragm, heart, and brain
*** $P < 0.001$ vs control; +++ $P < 0.001$ CLP vs Mel + CLP

can generate hypochlorous acid in the presence of neutrophil-derived MPO, and initiate the deactivation of antiproteases and activation of latent proteases, which cause tissue damage.[29] Evidence suggests that neutrophils release chemotactic substances, such as IL-8, which promote neutrophil migration to the tissue, activate neutrophils, and increase the damage.[38,39] Our results suggest that sepsis-induced tissue damage is neutrophil-dependent, except in the heart. Moreover, the anti-inflammatory effect of melatonin on all these tissues involves the inhibition of tissue neutrophil accumulation, which appears to limit the sepsis-induced oxidant injury. Our findings also provide evidence that cardiac oxidative injury does not involve the recruitment of neutrophils in rats, implicating another source for ROMs in the heart.

Glutathione is an important constituent of the intracellular protective mechanisms against various noxious stimuli including oxidative stress. However, reduced GSH, as the main component of the endogenous nonprotein sulfhydryl pool, is known to be a major low-molecular-weight scavenger of free radicals in the cytoplasm.[40,41] Because of their exposed sulfhydryl groups, nonprotein sulfhydryls bind to a variety of electrophilic radicals and metabolites that may be damaging to cells.[42] It has been proposed that antioxidants, which maintain the concentration of reduced GSH, may restore the cellular defense mechanisms and block lipid peroxidation, thereby protecting against oxidative tissue damage. Glutathione depletion, which is often associated with sepsis, was suggested to be detrimental, impairing the host response to infection.[43] In accordance with these findings, our results also verify that oxidative organ injury in animals with sepsis is accompanied by GSH depletion. Our findings also indicate that melatonin protects against sepsis-induced organ failure through a GSH-repleting effect. Conversely, the antioxidant melatonin had no impact on sepsis-induced GSH depletion in cardiac and pulmonary tissue, suggesting that the GSH-repleting effect of melatonin is not anonymous to all injured tissues.

We used the most clinically relevant sepsis model to monitor sepsis-induced multiple organ failure[44] and found that melatonin prevented sepsis-induced multiple organ failure by its antioxidative properties, which involve the inhibition of neutrophil migration to the affected tissues. Thus, considering its low toxicity,[45] our results support the clinical use of melatonin to treat conditions in which oxidative organ failure may be present.

References

1. Neviere RR, Ceepinskas G, Madorin WS, Hoque N, Karmazyn M, Sibbald WJ, et al. LPS pretreatment ameliorates peritonitis-induced myocardial inflammation and dysfunction: role of myocytes. Am J Physiol (Heart Circ Physiol 46) 1999;277:H885–92.
2. Bian K, Murad F. Diversity of endotoxin-induced nitrotyrosine formation in macrophage-endothelium-rich organs. Free Radic Biol Med 2001;31:421–9.
3. Bohles H. Antioxidative vitamins in prematurely and maturely born infants. Int J Vit Nutr Res 1997;67:321–8.
4. Heller AR, Groth G, Heller SC, Breitkreutz R, Nebe T, Quintel M, et al. N-Acetylcysteine reduces respiratory burst but augments neutrophil phagocytosis in intensive care unit patients. Crit Care Med 2001;29:272–6.
5. Tan DX, Chen LD, Poeggeler B, Manchester LC, Reiter RJ. Melatonin: a potent endogenous hydroxyl radical scavenger. Endocr J 1993;1:57–60.
6. Pierrefiche G, Topall G, Courboin G, Henriet I, Laborit H. Anti-oxidant activity of melatonin in mice. Res Commun Chem Pathol Pharmacol 1993;80:211–23.
7. Poeggeler B, Saarela S, Reiter RJ, Tan DX, Chen LD, Manchester LC, et al. Melatonin — a highly potent endogenous radical scavenger and electron donor: new aspects of the oxidation chemistry of this indole assessed in vitro. Ann NY Acad Sci 1994; 738:419–20.
8. Carcia JJ, Reiter RJ, Guerrero JM, Escames G, Yu BP, Oh CS, et al. Melatonin prevents changes in microsomal membrane fluidity during induced lipid peroxidation. FEBS Lett 1997;408: 297–300.
9. Reiter RJ, Tan DX, Manchester LC, Qi W. Biochemical reactivity of melatonin with reactive oxygen and nitrogen species. A review of the evidence. Cell Biochem Biophys 2001;34:237–56.
10. Vijayalaxmi, Reiter RJ, Herman TS, Meltz ML. Melatonin reduces gamma radiation-induced primary DNA damage in human blood lymphocytes. Mutat Res 1998;397:203–8.

11. Fujimura N, Sumita S, Narimatsu E, Nakayama Y, Shitinohe Y. Effects of isoproterenol on diapragmatic contractility in septic peritonitis. Am J Respir Crit Care Med 2000;161:440–6.
12. Bauer JD, Ackermann PG, Toro G. Bray's clinical methods. St. Louis: Mosby; 1968. p. 308–15.
13. Beuge JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 1978;53:302.
14. Beutler E. Glutathione in red blood cell metabolism. A manual of biochemical methods. New York: Grune and Stratton; 1975. p. 112.
15. Hillegass LM, Griswold DE, Brickson B, Albrightson-Winslow C. Assessment of myeloperoxidase activity in whole rat kidney. J Pharmacol Methods 1990;24:285–95.
16. Ozveri ES, Bozkurt A, Haklar G, Cetinel S, Arbak S, Yegen C, et al. Estrogens ameliorate remote organ inflammation induced by burn injury in rats. Inflamm Res 2001;50:585–91.
17. Sener G, Satıroğlu H, Kabasakal L, Arbak S, Oner S, Ercan F, et al. The protective effect of melatonin on cisplatin nephrotoxicity. Fundam Clin Pharmacol 2000;14;553–60.
18. Kettle AJ, Winterbourn CC. Myeloperoxidase: a key regulator of neutrophil oxidant production. Redox Rep 1997;3:3–15.
19. Feihl F, Waeber B, Liaudet L. Is nitric oxide overproduction the target of choice for the management of septic shock? Pharmacol Ther 2001;91:179–213.
20. Wickel DJ, Cheadle WG, Mercer-Jones MA, Garrison RN. Poor outcome from peritonitis is caused by disease acuity and organ failure, not recurrent peritoneal infection. Ann Surg 1997;225: 744–53.
21. Gitto E, Karbownik M, Reiter RJ, Tan DX, Cuzzocrea S, Chiurazzi P, et al. Effects of melatonin treatment in septic newborns. Pediatr Res 2001;50:756–60.
22. Wu CC, Chiao CW, Hsiao G, Chen A, Yen MH. Melatonin prevents endotoxin-induced circulatory failure in rats. J Pineal Res 2001;30:147–56.
23. Reiter RJ, Melchiorri D, Sewerynek E, Poeggeler B, Barlow-Walden L, Chuang J, et al. A review of the evidence supporting melatonin's role as an antioxidant. J Pineal Res 1995;18:1–11.
24. Reiter RJ, Acuna-Castroviejo D, Tan DX, Burkhart S. Free radical-mediated molecular damage. Ann N Y Acad Sci 2001; 939:200–15.
25. Reiter RJ, Maestroni GJM. Melatonin in relation to the antioxidative defense and immune systems: possible implications for cell and organ transplantation. J Mol Med 1999;77:36–9.
26. Lin AT, Chen MT, Yang CH, Chang LS. Oxygen free radical-induced lipid peroxidation in overdistention of the rabbit urinary bladders. Neurourol Urodyn 1995;14:553–4.
27. Saito M, Wada K, Kamisaki Y, Miyagawa I. Effect of ischemia-reperfusion on contractile function of rat urinary bladder: possible role of nitric oxide. Life Sci 1998;62:149–56.
28. Lefer AM. Pharmacology of the endothelium in ischemia-reperfusion and circulatory shock. Annu Rev Pharmacol Toxicol 1993;33:71–90.
29. Reiter RJ, Tan DX, Osuna C, Gitto E. Actions of melatonin in the reduction of oxidative stress. J Biomed Sci 2000;7:444–58.
30. Supinski G, Stofan D, Callahan LA, Nethery D, Nosek TM, DiMarco A. Peroxynitrite induces contractile dysfunction and lipid peroxidation in the diaphragm. J Appl Physiol 1999;87:783–91.
31. Sener G, Sehırlı AO, Keyer-Uysal M, Arbak S, Ersoy Y, Yegen BC. The protective effect of melatonin on renal ischemia-reperfusion in rat. J Pineal Res 2002;32:120–6.
32. Şener G, Şehirli AO, Şatıroğlu H, Keyer-Uysal M, Yegen BC. Melatonin prevents oxidative kidney damage in a rat model of thermal injury. Life Sci 2002;70:2977–85.
33. Sewerynek E, Abe M, Reiter RJ, Barlow-Walden LR, Chen L, McCabe TJ, et al. Melatonin administration prevents lipopolysaccharide-induced oxidative damage in phenobarbital-treated rats. J Cell Biochem 1995;58:436–44.
34. Cuzzocrea S, Tan DX, Costantino G, Mazzon E, Caputi AP, Reiter RJ. The protective role of endogenous melatonin in carrageenan-induced pleurisy in the rat. FASEB J 1999;13:1930–8.
35. Sullivan GW, Sarembock IJ, Linden J. The role of inflammation in vascular diseases. J Leukocyte Biol 2000;67:591–602.
36. Cuzzocrea S, Reiter RJ. Pharmacological action of melatonin in shock, inflammation and ischemia/reperfusion injury. Eur J Pharmacol 2001;426:1–10.
37. Zimmerman BJ, Grisham MB, Granger DN. Role of oxidants in ischemia-reperfusion induced granulocyte infiltration. Am J Physiol 1990;258:G185–90.
38. Donnahoo KK, Meng X, Ayala A, Cain MP, Harken AH, Meldrum DR. Early kidney TNF-α expression mediates neutrophil infiltration and injury after renal ischemia-reperfusion. Am J Physiol 1999;277:R922–9.
39. Cuzzocrea S, Costantino G, Mazzon E, Micali A, De Sarro A, Caputi AP. Beneficial effects of melatonin in a rat model of splanchnic artery occlusion and reperfusion. J Pineal Res 2000;28:52–63.
40. Shaw S, Herbert V, Colman N, Jayatılleke E. Effect of ethanol-generated free radicals on gastric intrinsic factor and glutathione. Alcohol 1990;7:153–7.
41. Ross D. Glutathione, free radicals and chemotherapeutic agents. Pharmacol Ther 1988;37:231–49.
42. Szabo S, Nagy L, Plebani M. Glutathione, protein sulfhydryls and cysteine proteases in gastric mucosal injury and protection. Clin Chim Acta 1992;206:95–105.
43. Villa P, Saccani A, Sica A. Glutathione protects mice from lethal sepsis by limiting inflammation and potentiating host defense. J Infect Dis 2002;185:1115–20.
44. Tanjoh K, Shima A, Aida M, Tomita R, Kurosu Y. Nitric oxide and active oxygen species in severe sepsis and surgically stressed patients. Surg Today 1995;25:774–7.
45. Jan JE, Hamilton D, Seward N, Fast DK, Freman RD, Laudon M. Clinical trials of controlled-release melatonin in children with sleep-wake disorders. J Pineal Res 2000;29:34–9.