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A phase II, single-center, double-blind, randomized placebo-controlled trial to explore the efficacy and safety of intravenous melatonin in surgical patients with severe sepsis admitted to the intensive care unit

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Abstract

To determine whether IV melatonin therapy improves redox status and inflammatory responses in surgical patients with severe sepsis, a unicenter, phase II double-blind, randomized, placebo-controlled trial was carried out. The study included patients with severe sepsis marked by infectious systemic inflammatory response syndrome (SIRS), associated with organ dysfunction, hypoperfusion or hypotension requiring surgical intervention. IV melatonin at a daily dose of 60 mg, which was dissolved in 500 ml of 5% dextrose serum, was continuously administered to the patients for over 30 min starting on the day of the diagnoses during a 5-day period. A total of 14 patients received a placebo treatment and 15 melatonin doses. Redox status decreased in melatonin-treated patients during the 5 days of treatment as compared to the placebo-treated patients. Procalcitonin performed better in the melatonin group, whose neutrophil to lymphocyte ratio was also significantly reduced, resulting in an improved evolution of the disease. Moreover, hospital stays decreased by 19.60% from 26.64 days for the placebo group to 21.42 days for the melatonin group. The placebo group recorded five mortalities, as

compared to three for the melatonin group. IV melatonin administration improved the course of the disease in surgical patients with severe sepsis, with no side effects. Additional studies with higher doses of melatonin and a long duration of therapy need to be carried out to assess its clinical use.

KEYWORDS
hospital stay, inflammation, melatonin, mortality, oxidative stress, sepsis

1 | INTRODUCTION

Sepsis has been defined as an excessive inflammatory response of the host to infection[1] that triggers multiorgan dysfunction syndrome (MODS),[2,3] the main cause of death of postsurgical patients admitted to intensive care units (ICUs).[4] From 2016 and following The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3), sepsis has been defined as a "life-threatening organ dysfunction caused by a dysregulated host response to infection," which constitutes a threat to the patient's survival.[4]

During the first phase of sepsis, a systemic inflammatory response occurs, characterized by leukocyte and macrophage activation, as well as subsequent cytokine release, leading to the production of reactive oxygen and nitrogen species (ROS/RNS) responsible for endothelial injury and oxidative damage to cells and organs.[5] As specific treatments do not exist at present to control sepsis-inducing cytokine storms, oxidative stress and multiorgan failure, the mortality rate continues to be very high. The World Health Organization (WHO) has requested all countries to diagnose, prevent, and treat sepsis, and emphasized the urgent need to search for new therapies against sepsis.

Melatonin (N-acetyl-5-methoxytryptamine) is produced by the pineal gland that regulates circadian rhythms including those relating to the sleep/wake cycle.[6] However, melatonin is also produced in most body organs and tissues; with so-called extrapineal melatonin being accumulated in cells at concentrations one to two orders of magnitude more than pineal melatonin. Extrapineal melatonin, which does not exit the cell to the blood and does not follow a circadian pattern, is produced to protect the cell and mitochondria against oxidative and inflammatory injuries.[7] Given its high accumulation levels, extrapineal melatonin: (i) is an extremely potent radical scavenger against most ROS/RNS (8); (ii) acts as an excellent antioxidant which modulates the expression and activity of most endogenous antioxidant enzymes,[7,8] whose oxidative power, paradoxically, under oxidative stress conditions, may be reduced by melatonin diminishing the oxidative strength due to scavenging free radicals,[9] and (iii) maintains mitochondrial homeostasis and increases ATP production.[10] Melatonin is also a potent anti-inflammatory which reduces overactive innate immune system activity, decreases cytokine storms and modulates immune cells including T-cells; these properties are of relevance to conditions such as sepsis, in which the response of the host against inflammation can be improved.[11,12] Melatonin levels are impaired in critical septic patients,[13] while its administration reduces oxidative stress and improves outcomes in neonate surgical patients.[14] The therapeutic value of melatonin has been tested in several diseases using different doses and routes of administration, with no significant side effects.[15]

No well-controlled clinical trials have been carried out on melatonin, some of which have been performed with newborns and neonates using oral melatonin, with promising results.[16–18] Given the failure of intestinal absorption processes in sepsis,[19] we considered it worthwhile to carry out a clinical trial with a new formulation of injectable melatonin for intravenous administration to septic patients to analyze clinical outcomes, as well as the markers of redox status and immune responses. We hypothesize that melatonin should improve outcomes in septic patients due to its antioxidant and anti-inflammatory properties.

2 | MATERIALS AND METHODS

2.1 | Trial design and patients

This is a phase IV clinical trial of the product to be administered, melatonin, which is already in the market, but with a design based on based on a prospective, randomized, double-blind, parallel, and controlled phase II trial, to examine the effect of intravenous (IV) melatonin on outcomes in severe sepsis patients. The trial was carried out in a single hospital (Virgen de las Nieves University Hospital) between January 2013 and April 2016.

Eligible participants, who were recruited from the hospital, met the following inclusion criteria: patients with severe sepsis based on the American College of Chest Physicians/Society of Critical Care Medicine diagnostic criteria,[20] in other words with infectious SIRS associated with organ dysfunction, hypoperfusion or hypotension due to a disease requiring surgical intervention by the General Surgery and Digestive Department of the Virgen de las Nieves University Hospital. As the study began in 2013, it was not possible to use the current Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) released in 2016.[4] The patients enrolled in the trial were diagnosed with sepsis requiring surgical intervention when admitted to the hospital. Exclusion criteria included being under 18 years of age, pregnancy, medical or surgical terminal illness (incurable advanced neoplasia, chronic diseases such as liver cirrhosis or chronic renal failure), as the infectious process in these patients could increase the risk of mortality per se, impaired mental abilities, psychiatric illness under treatment, melatonin hypersensitivity, patients who took melatonin 2 days before surgery, or refusal to take part in the trial. Table 1 shows the main characteristics of the groups of patients participating in the trial. Neither homogenous group showed any significant differences in terms of number, sex, age, or diagnosis. Table 2 contains the CONSORTIUM reporting criteria checklist of the trial.

A written consent form was signed by the patients or their relatives. The clinical trial, which was approved by the Ethical Committee of Clinical Research of the Virgen de las Nieves University Hospital, was entered in the Spanish Clinical Trials Register on 24/9/2013, and all trial procedures were in accordance with the 1975 Helsinki Declaration. The clinical trial involved orphan drugs authorized by the Spanish Agency of Medicines and Health Products (AEMPS; EudraCT number 2008-006782-83). The clinical trial was conducted in accordance the regulations contained in Royal Decree 223/2004. Patient medical information is protected by Spanish data protection legislation (15/1999 and 41/2002), which regulates patient autonomy, rights and obligations regarding information and clinical documentation. The patients participating in the trial were insured by HDI-Seguros (Madrid, Spain; policy number: 130/001/008419).

TABLE 1 Demographic and disease characteristiccs

	Melatonin treated group (MTG)		Placebo treated group (PTG)	
n	15		14	
Age	65.5 (43–88) years		71.6 (51/87) years	
Sex	Male 10/female 5		Male 8/female 6	
Number of patients				
Diagnoses	Community secondary peritonitis	6	Community secondary peritonitis	7
	Cholecistitis/ cholangitis	5	Cholecistitis/ cholangitis	1
	Dehiscence/ anastomosis	2	Dehiscence/ anastomosis	3
	Intestinal ischemia	1	Secondary hospital peritonitis	2
	Isquiorrectal abscess	1	Fournier gangrene	1

2.2 | Randomization and blinding

Randomization was carried out by assigning a consecutive ascending number to each patient according to a list issued by the Epidemiology and Biostatistics Unit of the Virgen de las Nieves University Hospital. None of the personnel involved in the trial knew which patients belonged to either the melatonin-treated group (MTG) or the placebo-treated group (PTG). Once the trial was over, the research group received a sealed envelope with the list of patients and their assigned melatonin and placebo groups. As this was the first clinical trial in which IV melatonin administration was used in septic patients, the AEMPS only gave us permission to use a small number of patents. A total of 29 patients were randomized in two groups: (a) the MTG, a group of 15 patients to whom melatonin was administered, and (b) the PTG, a group of 14 patients who were administered a placebo. Information regarding any adverse effects caused by the administration of IV melatonin or the placebo, whether reported voluntarily by a patient or discovered by a researcher, was collected through a general questionnaire and entered in the data collection register. No side effects of melatonin were detected in any patient, suggesting the safety of melatonin therapy.

2.3 | Trial procedure

We developed a new melatonin formulation for IV administration in collaboration with the Virgen de las Nieves University Hospital Pharmacy Service and the University of Granada Institute of Biotechnology (Spain). The formulation was patented worldwide (PCT/ES2015/070236) and authorized by AEMPS for use in this clinical trial. The formulation was pharmaceutically presented in 10 ml ampoules of the patented solution containing

TABLE 2 CONSORTIUM reporting criteria checklist

Item	Description	Reported on page number
Title	A phase II, single-canter, double-blind, randomized placebo-controlled trial to explore the efficacy and safety of intravenous melatonin in surgical patients with severe sepsis admitted to the intensive care unit	Page 1
Authors	Alfonso Mansilla-Roselló, MD, PhD, Jorge Hernández-Magdalena, Mireia Domínguez-Bastante, MD, Carmen Olmedo-Martín, Ana Comino-Pardo, MD, Germaine Escames, MD, PhD, Darío Acuña-Castroviejo, MD, PhD	Page 1
Methods		
Participants	A total of 29 patients were randomized in two groups: (a) Melatonin-treated group (MTG), a group of 15 patients that received melatonin; (b) Placebo-treated group (PTG), a group of 14 patients that received placebo	Page 7
Interventions	The MTG group of 15 patients was perfused with an IV melatonin dose of 60 mg/24 h from the time of the sepsis diagnosis following surgery and for 5 subsequent days (days T0 to T5). The patients also received appropriate treatment for their pathology and surgery. The 14 PTG patients received the placebo treatment (by means of an excipient similar in volume to that used for the other group) at the same rate as the melatonin group (days T0 to T5), together with appropriate treatment for their pathology and surgery.	Pages 7–8
Objective	We hypothesize that melatonin should improve outcomes in septic patients due to its antioxidant and anti-inflammatory properties.	Page 4
Outcome	The primary outcome was any incidence of organ dysfunction which was determined every 24 h using the Sequential Order Failure Assessment (SOFA) scale throughout the trial. Secondary outcomes included all causes of mortality and hospital stays such as clinical features, oxidative stress status and inflammatory responses such as biochemical markers of the disease. Patients were monitored until hospital discharge or death for a maximum period of 40 days following surgery. The MTG group recorded three deaths which occurred at day 11, 18, and 27 following surgery, while the PTG group recorded 5 deaths which occurred at Day 6, 9, 10, 16, and 38 after surgery. The trial ended 40 days after surgery.	Page 8
Randomization	Randomization was carried out by assigning a consecutive ascending number to each patient according to a list issued by the Epidemiology and Biostatistics Unit of the Virgen de las Nieves University Hospital.	Page 6
Binding (masking)	None of the personnel involved in the trial knew which patients belonged to either the melatonin-treated group (MTG) or the placebo-treated group (PTG).	Page 6
Results		
Numbers randomized	A total of 29 patients were randomized in two groups: (a) the MTG, a group of 15 patients to whom melatonin was administered, and (b) the PTG, a group of 14 patients who were administered a placebo.	Page 7
Recruitment	Finalized	Page 9
Numbers analyzed	The results cover all the patients in the MTG (15 patients) and PCG (14 patients) groups.	Page 9
Outcome	The results cover all the patients in the MTG (15 patients) and PCG (14 patients) groups.	Page 9
Harms	No side effects of melatonin were detected in any patient, suggesting the safety of melatonin therapy.	Page 7
Conclusions	In summary, the antioxidant and anti-inflammatory properties of melatonin improved the redox/inflammatory responses in septic patients, which were associated with a reduction in the mortality and the length of hospitalization.	Page 14

TABLE 2 (Continued)

Item	Description	Reported on page number
	Treatment with melatonin was not associated with any adverse reaction. Nevertheless, we consider that 5 days of treatment, the number of patients, and the dose used, 60 mg/day, are too low to observe more significant effects of melatonin. This is because the human equivalent dose calculated from preclinical studies is 5 mg/kg bw/day, and the half-life of melatonin in blood is too short, 30–40 min. However, given that this was the first intravenous melatonin trial in patients, the Spanish Agency of Medicines and Health Products only gave us permission for a low dose, that is, 60 mg/day and only for 5 days, to check for toxicity and adverse effects. Consequently, intravenous melatonin should be further investigated in a longer treatment duration, a larger sample size, and higher dose, because it represents a possible option for the treatment of sepsis as suggested by this phase II study. No side effects of melatonin were detected in any patient, suggesting the safety of melatonin therapy.	
Trial registration	EUDRACT number 2008-006782-83	Page 6
Funding	The trial was funded by the Ministry of Health and Social Politic of the Spanish Government, through the call for the Promotion of Independent Clinical Research, ref. EC 10-181, and for the ISCIII through the grants PI08-1664 and RD12/0043/0005	Page 14

6 mg/ml melatonin. A continuous IV perfusion of 60 mg, dissolved in 500 ml 5% dextrose serum, was administered to the patients.

The MTG group of 15 patients was perfused with an IV melatonin dose of 60 mg/24 h from the time of the sepsis diagnosis following surgery and for 5 subsequent days (days T0 to T5). The patients also received appropriate treatment for their pathology and surgery. The 14 PTG patients received the placebo treatment (by means of an excipient similar in volume to that used for the other group) at the same rate as the melatonin group (days T0 to T5), together with appropriate treatment for their pathology and surgery.

2.4 | Clinical outcomes

The primary outcome was any incidence of organ dysfunction which was determined every 24 h using the Sequential Order Failure Assessment (SOFA) scale throughout the trial. Secondary outcomes included all causes of mortality and hospital stays such as clinical features, oxidative stress status and inflammatory responses such as biochemical markers of the disease. Patients were monitored until hospital discharge or death for a maximum period of 40 days following surgery. The MTG group recorded three deaths which occurred at day 11, 18, and 27 following surgery, while the PTG group recorded 5 deaths which occurred at Day 6, 9, 10, 16, and 38 after surgery. The trial ended 40 days after surgery.

2.5 | Biochemical and hematological outcomes

Blood samples were taken from each subject at Day 0 (just before surgery and melatonin therapy), and at Day 1, 2, 3, 4, and 5 after surgery. Other EDTA-containing blood samples were centrifugated to separate plasma and aliquots were frozen −20°C for later biochemical analysis. Both lactate and pH were measured using arterial blood gases. Procalcitonin, neutrophils, and lymphocytes were also determined. Oxidative stress status was measured in plasma samples. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reductase (GRd) activities, and malondialdehyde (MDA) levels as the lipid peroxidation index, were spectrophotometrically measured using a Beckman DU-650 spectrophotometer.

2.6 | Statistical analysis

Data are expressed as means ± SEM Parametric one-way ANOVA analysis of variance and the non-parametric Friedmann test were used for data trend analysis. To compare the groups over time (0 to 5 days), one-way ANOVA analysis and the Kruskal–Wallace test were also used. Mortality was analyzed with the aid of the Fisher exact test. SPSS software was used for statistical analysis, with $p < .05$ being considered statistically significant.

3 | RESULTS

The results cover all the patients in the MTG (15 patients) and PCG (14 patients) groups.

Figure 1A shows that hospital stays decreased by 19.60%, from 26.64 days for the PTG patients to 21.42 days for the MTG participants. Of the 15 MTG patients, 12 survived and 3 died, while, of the 14 PTG patients, 9 survived and 5 died (Figure 1B).

The SOFA score progressively decreased in both groups of patients and was significantly lower at the end of the trial (Figure 2, $p < .001$), with the reduction being significantly higher among the MTG patients than that for the PTG patients.

Lactate and pH values were recorded for the subjects involved in the clinical trial. The values for pH increased significantly in MTG patients (Figure 3A, $p < .05$), while PTG patients showed an increasing trend up to the end of the trial, when they began to decrease. A significant difference between MTG and PTG pH values was also observed at T4 and T5. Lactate levels were higher in MTG subjects than in PTG subjects at T0, with the former showing a decrease after 1 day of melatonin therapy to the levels observed for the PTG patients (Figure 3B).

Superoxide dismutase (SOD), which transforms superoxide anions into hydrogen peroxide, behaved in a similar fashion in both groups. Lower SOD activity was observed in melatonin group at day T0 and T1 as compared to the placebo group (Figure 4A, $p < .05$). Like SOD, CAT activity as higher in PTG patients as compared to MTG patients at day T0, T1, and T3 (Figure 4B, $p < .05$). Both CAT and SOD activity increased at day T2. Like CAT, GPx converts peroxides into water; GPx was higher in PTG patients as compared to MTG patients at T1 and T4 (Figure 4C, $p < .05$). In both groups, GPx

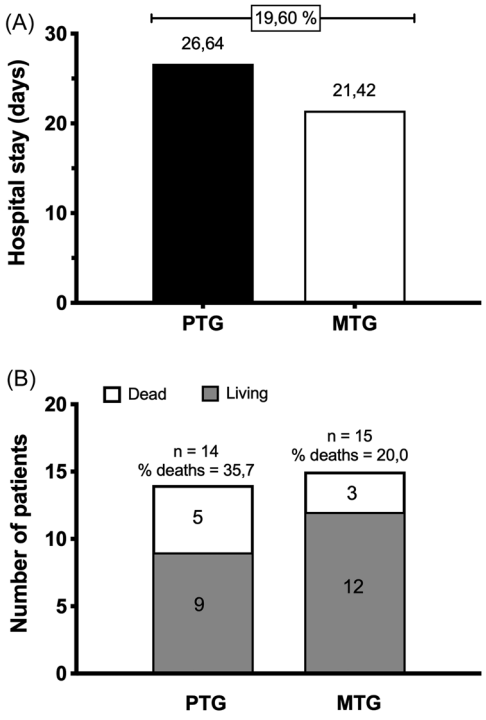

FIGURE 1 Hospital stays (A) and mortality (B) in the placebo-treated group and melatonin-treated group of ICU septic patients

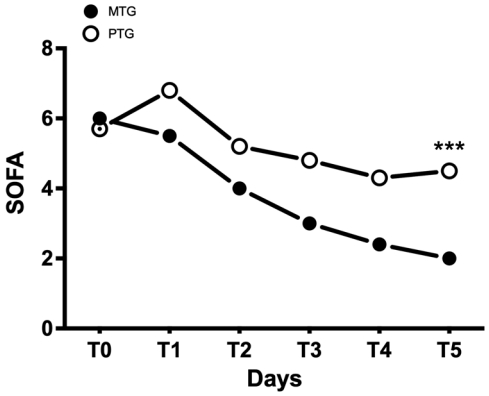

FIGURE 2 Evolution of the Sequential Order Failure Assessment score in the placebo-treated group and melatonin-treated group groups of intensive care unit septic patients. ***$p < .001$ versus T0.

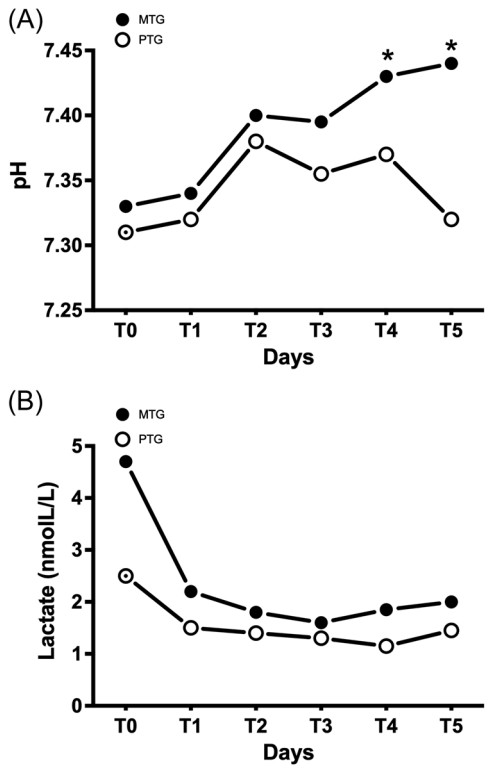

FIGURE 3 Changes in pH (A) and lactate (B) levels in placebo-treated group and melatonin-treated group patients during the trial

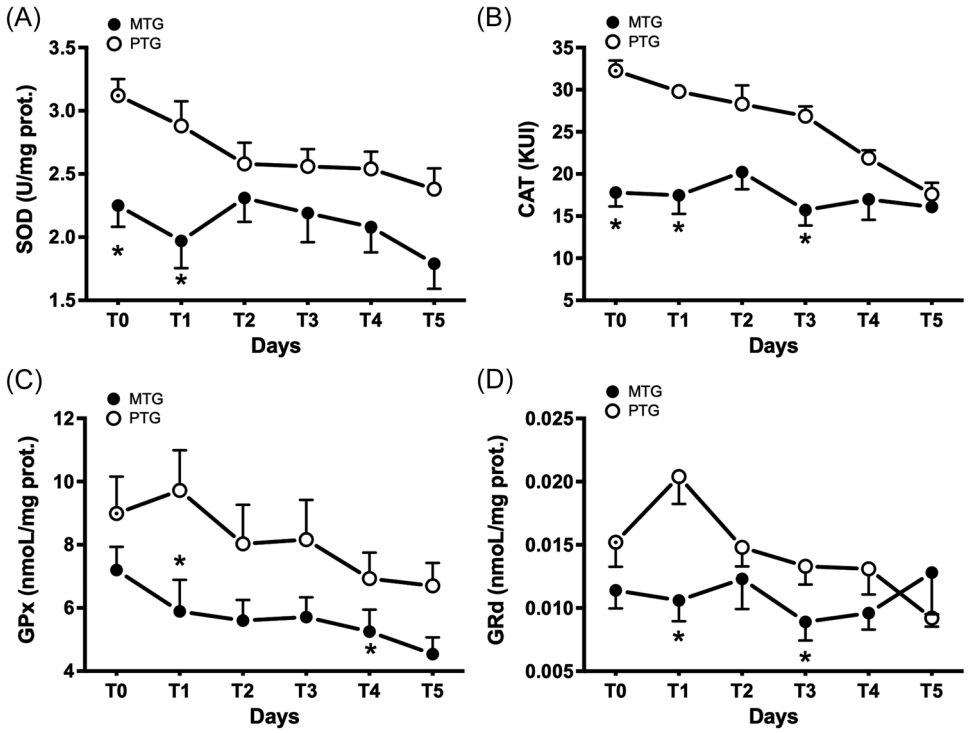

FIGURE 4 Changes in endogenous antioxidant defenses during the trial in the placebo-treated group and melatonin-treated group groups of septic patients. (A) superoxide dismutase; (B) catalase; (C) glutathione peroxidase; (D) glutathione reductase. *$p < .05$ between placebo-treated group and melatonin-treated group values.

activity decreased throughout the trial. GRd, which regenerates GSH from its oxidized form, GSSG, produced during the GPx enzymatic activity. GRd activity was higher among PTG patients at T1 and T3 (Figure 4D, $p < .05$). Like SOD and CAT, a transient increase in GRd activity was observed at T2.

MDA, which is a reliable index of lipid peroxidation, showed a pattern of changes throughout the trial similar to that observed in the antioxidant enzymes. MDA levels were higher in MTG patients at T0 and then decreased up to T5, with a transient increase observed at T4. MDA was significantly higher in MTG patients as compared to PTG patients at T0, T2, and T4 (Figure 5, $p < .05$).

Neutrophils, lymphocytes, and procalcitonin were measured as markers of inflammatory responses. A more pronounced reduction in neutrophils was observed in MTG patients. The lymphocyte count was higher in the melatonin group than in the placebo group. A significant reduction in the neutrophil to lymphocyte ratio (NLR) was found in MTG patients, mainly at T1, T3, and T5 (Figure 6C, $p < .05$). The NLR decreased from 10.83 at T0 to 9.50 at T5 in the placebo group, while the melatonin group showed a reduction from 10.02 at T0 to 5.15 at T5.

Procalcitonin was higher at T0 in MTG patients than in PTG patients. Interestingly, the reduction in procalcitonin levels was much more pronounced in the former

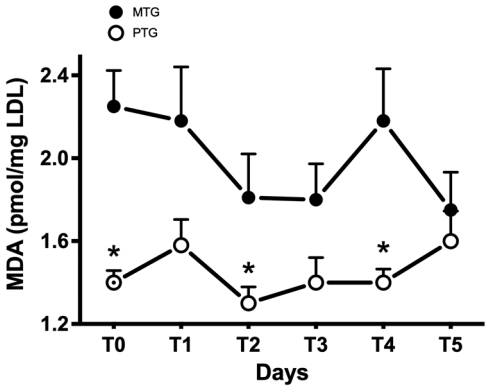

FIGURE 5 Evolution of malondialdehyde levels throughout the trial in the placebo-treated group and melatonin-treated group groups of patients. *$p < .05$ between the values for both groups.

group, reaching significantly lower levels at T4 and T5 than at T0 (Figure 6D, $p < .05$).

4 | DISCUSSION

For the first time, this clinical trial showed the beneficial effects of intravenous melatonin administration in ICU surgical septic patients. The rationale for using

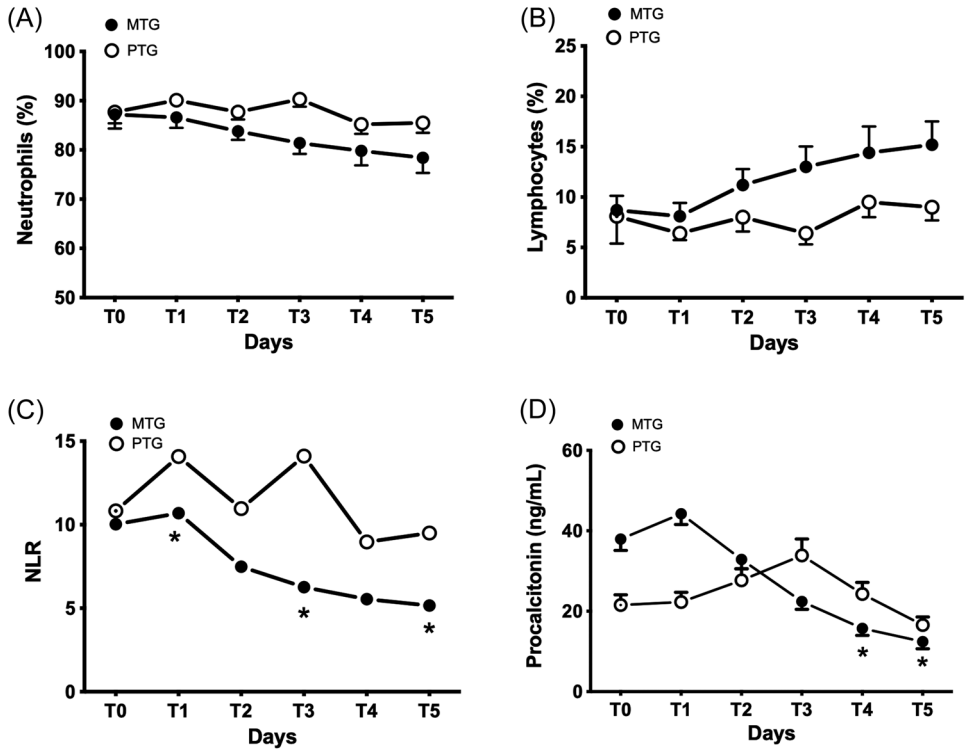

FIGURE 6 Values for neutrophils (A) lymphocytes (B) the neutrophils/lymphocytes ratio (NLR) (C) and procalcitonin levels (D) in the placebo-treated group and melatonin-treated group groups. *$p < .05$ between both groups.

melatonin is a response to the search for innovative therapies for sepsis, a disease with no effective treatment.[21] In this paper, we show that melatonin reduced hospital stays for MTG patients by 19.60% with respect to PTG patients, while the mortality rates for MTG and PTG patients were 20% and 35.7%, respectively.

The excessive inflammatory response during sepsis is mediated by the activation of innate immunity through the sequential triggering of the NF-κB and NLRP3 inflammasome pathways.[22,23] NF-κB upregulates the expression of pro-inflammatory molecules including pro-cytokines and the inducible enzymes, COX-2 and iNOS,[24] which, in turn, produce ROS and RNS that are capable of damaging cells and mitochondria.[25–27] Damaged mitochondria release ROS and mtDNA into the cytosol,[28,29] which activate the NLRP3 inflammasome,[30] inducing caspase 1 which processes pro-IL-1β into IL-1β.[28,31] IL-1β enhances NF-κB translocation to the nucleus in a positive feedback loop that boosts the inflammatory response responsible for the high mortality and morbidity of sepsis.[32,33]

Melatonin, an indirect antioxidant that induces the expression and activity of antioxidant enzymes such as SOD, CAT, GPx, and GRd, is also a direct scavenger of most free radicals.[34,35] At high concentrations, such as those obtained by IV administration, melatonin removes these radicals and reduces their oxidative capacity whereby endogenous antioxidant system activation is no longer required,[9] as we reported here.

Our results highlight significant changes in the oxidative status of septic patients. SOD showed a similar pattern in both the placebo and melatonin groups. Lower initial SOD activity in the MTG patients reflects its lower effectiveness in removing superoxides. It is particularly worth noting that there is clearly a transitory peak in SOD levels 48 h after the commencement of melatonin therapy in MTG patients, suggesting that melatonin induces SOD activity. However, as melatonin also scavenges superoxides, elevated SOD activity is no longer needed, which decreases to below PTG levels to reach a minimal level at the end of the trial in MTG patients. Like SOD, both CAT and GPx enzymes metabolize peroxide to water. Likewise, melatonin scavenges peroxides, rendering it unnecessary to maintain CAT and GPx activities at elevated levels, both of which remain lower than those in the placebo group. GRd levels in the melatonin group also remained below those in the placebo group, which points to low GSH consumption during the antioxidant response due to a reduction in oxidative status caused by high melatonin levels. The increase in ROS caused lipid peroxidation, leading to an overproduction of MDA which was higher in the melatonin group than in the placebo group; however, MDA began to decrease rapidly 24 h after melatonin

therapy, which maintains optimal membrane fluidity and confirms its scavenger status discussed above.[36] Together, these data indicate that the placebo group has greater redox potential than the melatonin group.

The inflammatory response during sepsis involves changes in the NLR. While neutrophils are responsible for the acute deleterious inflammatory response, lymphocytes are associated with adaptive immunity. It has been reported that higher NLRs are directly related to unfavorable prognoses and mortality rates for different diseases including sepsis.[37–39] Our results show that NLRs were significantly lower in the melatonin group than in the placebo group, which may be related to the reduction in mortality in the former and confirms the beneficial effect of melatonin on the immune system.[40] Procalcitonin levels decreased in MTG patients as compared to PTG patients; despite its tendency to increase in the latter group, melatonin treatment reduced procalcitonin levels significantly at the end of the trial. The SOFA index for sepsis showed a sharper reduction in the melatonin group than in the placebo group, with minimal levels being observed at the end of the trial. The difference in SOFA scores at T5 between PTG and MTG suggest that the condition of patients in the latter group had improved. An improvement in the physiological condition of patients was also reflected in changes in pH, which increased in the melatonin group as compared to placebo group, mainly at the end of the clinical trial. No differences were observed, however, in lactate levels.

Two clinical trials were carried out to evaluate the efficacy of antioxidant vitamin C treatment in septic patients. In one trial, vitamin C was orally administered at a dose of 450 mg/day for 6 days,[41] and, in the other, a 1000 mg bolus intravenous injection of vitamin C was administered for 30 min, followed by a continuous infusion of 250 mg/h/96 h.[42] The results suggest that the routine clinical use of vitamin C does not benefit septic patients and that molecules containing only antioxidative activity is not sufficient to protect against sepsis.

Interestingly, a retrospective cohort study reported the effect of oral melatonin in septic patients. The study was conducted with data from the US Department of Veterans Affairs, including patients from the years 2000/2001 who were exposed to melatonin from the first day of hospitalization. The results show that melatonin reduced hospital stays and mortality rates, except for ICU patients, who showed no significant changes in these items.[43] Our clinical trial, which was carried out on in septic ICU patients, suggests that IV melatonin administration may be better than the oral method.

5 | CONCLUSIONS

In summary, the antioxidant and anti-inflammatory properties of melatonin improved the redox/inflammatory responses in septic patients,[44,45] which were associated with a reduction in mortality and length of hospitalization. Treatment with melatonin was not associated with any adverse reactions. Nevertheless, in our view, 5 days of treatment, the number of patients, and the dose used, 60 mg/day, were too limited to evaluate any more significant effects of melatonin. This is because the human equivalent dose calculated from preclinical studies is 5 mg/kg bw/day,[46] and the half-life of melatonin in blood, at 30–40 min, is too short.[47] However, as this was the first intravenous melatonin trial carried out in patients, the Spanish Agency of Medicines and Health Products only gave us permission for a low dose (60 mg/day) and for 5 days to check for toxicity and adverse effects. Consequently, intravenous melatonin needs to be further investigated over a longer treatment period, for a larger sample size, and at a higher dose, as IV melatonin constitutes a possible option for the treatment of sepsis, as suggested by this phase II clinical trial.

AUTHOR CONTRIBUTIONS

Alfonso Mansilla-Roselló: Conceptualization; original draft; review and editing; and funding acquisition. **Darío Acuña-Castroviejo**: Conceptualization; original draft; review and editing; and funding acquisition. **Germaine Escames**: Conceptualization; original draft; review and editing; and funding acquisition. **Jorge Hernández-Magdalena**: Methodology and validation. **Mireia Domínguez-Bastante**: Supervision; formal analysis. **Carmen Olmedo-Martín**: Research; data curation. **Ana Comino-Pardo**: Research; data curation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data is available on request to the corresponding author. The data that support the findings of this study are available on request from the corresponding author.

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