Original Research

Antioxidant potential of different melatonin-loaded nanomedicines in an experimental model of sepsis

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Abstract

Oxidative stress has been shown to play a major role in the complex pathophysiological processes leading to organ failure during sepsis. The aim of the present research was to evaluate the effect of different melatonin nanoparticle (NP) carriers in an experimental animal model of sepsis. Poly-D,L-lactide-co-glycolide (PLGA [NP-A]) and polyethylene glycol-co-(poly-D,L-lactide-co-glycolide) (PLGA-PEG [NP-B]) were used to obtain melatonin-loaded nanocarriers (10 mg/kg). Oxidative stress was measured in tissue homogenates by measuring heme oxygenase-1 (HO-1) expression, total thiol groups and lipid hydroperoxides (LOOH). *In vitro* NPs showed a long lag time followed by a controlled release of melatonin. All the different melatonin formulations restored total thiol group levels to those of controls in all the examined organs, with no significant changes among them. Both melatonin NP formulations significantly decreased LOOH levels when compared with sepsis vehicle animals. The stealth formulation NP-B was able to produce a more significant reduction in LOOH levels in the heart, lung and liver when compared with NP-A. No significant changes were observed between the two NP formulations in the kidney. Interestingly, HO-1 expression was differently affected following treatment with various melatonin formulations. The NP-B formulation was more effective in inducing HO-1 protein compared with free melatonin and NP-A, with the exception of the kidney. Taken together, our results show that melatonin possesses a significant antioxidant activity during sepsis and that it is possible to improve this ability by delivering the compound with specific drug delivery systems.

Keywords: heme oxygenase, melatonin, sepsis, oxidative stress

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Introduction

Peritonitis is a severe infection that frequently leads to multiple organ dysfunction/failure, septic shock and mortality. The cascade of events from infection to septic shock and organ dysfunction is poorly understood. Prolonged activation of the immune system results in the excessive generation of proinflammatory cytokines and reactive oxygen species (ROS), leading to progressive damage of host tissue.[1,2] ROS include oxidants such as peroxynitrite, hydroxyl radicals and superoxide; these substances are toxic and may induce lipid peroxidation, as well as degradation of protein, sugar and DNA.[3] In this context, several lines of evidence suggest that antioxidants may play a major role in counteracting oxidative stress, leading to an improved mortality rate in different animal models of sepsis.[4] Among various antioxidants, melatonin has been shown to possess pleiotropic effects under various experimental conditions.[4,5]

The powerful antioxidant capacity of melatonin is usually attributed to its potential to eliminate free radicals by the donation of electrons.[4,6] In fact, melatonin may neutralize hydroxyl radicals by forming 3-hydroxymelatonin, which is excreted in the urine.[7,8] Furthermore, melatonin has been demonstrated to interact with toxic reactants like peroxyl radicals,[9] singlet oxygen species[10] and hydrogen peroxide.[11] In addition, metabolites of melatonin, including the major hepatic metabolite 6-hydroxymelatonin, as well as *N*-acetyl-*N*-formyl-5-methoxykynuramine and *N*-acetyl-5-methoxykynuramine, have been shown to detoxify radicals themselves.[12,13] In addition to these direct interactions with ROS, melatonin may induce upregulation of the activity of antioxidants and antioxidant enzymes, such as superoxide dismutase, glutathione, glutathione peroxidase, glutathione reductase and heme oxygenase-1 (HO-1).[14–16] HO isoforms catalyze the conversion of heme to carbon monoxide and bilirubin with a concurrent release of iron, which can drive the synthesis of ferritin for iron sequestration.[17]

A substantial body of evidence demonstrates that both bilirubin and carbon monoxide effectively play a pivotal role in sepsis.[18–21] To date, two HO isoforms have been shown to be catalytically active in heme degradation, and each is encoded by a different gene.[22] HO-1, the inducible isoform, is found ubiquitously in all organs and is rapidly and transiently expressed after various stimuli.[22] It is now widely accepted that induction of HO-1 expression represents an adaptive response that critically increases cell resistance to oxidative injury, and this pathway plays a critical role in acute injuries. In particular, previous reports have demonstrated that HO-1 plasma concentrations are significantly increased among the critically ill and are associated with the degree of organ dysfunction.[23] However, the clear molecular significance of HO-1 in sepsis remains to be elucidated.

Melatonin formulations are commercially available for oral administration, but alternative controlled and sustained release systems have been proposed, even though they are a long way away from clinical use. These include oral,[24] transdermal and transepidermal[25] and intranasal[26] delivery systems. Treatment of sepsis requires a systemic therapeutic approach; however, so far no injectable formulation has been studied for the controlled delivery of melatonin. In this context, colloidal drug carriers, such as polymeric nanoparticles (NPs), liposomes, micelles, etc can improve many pharmacological properties of conventional 'free' drugs.

In particular, in the present work, we tested the properties of NPs obtained from two different polymers: poly(D,L-lactide-co-glycolide), PLGA, and diblock poly[(D,L-lactide-co-glycolide)-co-PEG], PLGA-PEG. PLGA polymers are among the most used biomaterials in the field of drug nanocarriers because of their biodegradability and biocompatibility; they are approved by the Food and Drug Administration (FDA) for human studies and allow a controlled release of the loaded drugs to be obtained. However, after intravenous injection, conventional nanocarriers are rapidly cleaned from the systemic circulation by the mononuclear phagocyte system, mainly by the macrophages present in the liver and spleen. For this reason, in the last few decades, there has been an increasing interest in developing long-circulating (Stealth®) NPs as drug carriers. One of the principal methods to obtain such formulations consists in modifying the surface with a hydrophilic and non-ionic polymer, such as poly(ethyleneglycol) (PEG) or poloxamer derivatives. For instance, PLGA-PEG polymers are able to avoid macrophagic uptake, thus allowing the NPs to circulate for longer times.[27]

The aim of this work was to determine the effect of different melatonin NP carriers in an experimental animal model of sepsis and to elucidate how the HO system is involved in such an effect.

Materials and methods

Materials

The copolymers Resomer® RG 502 H (polylactide-co-glycolide 50:50, free carboxyl endgroup) (PLGA) and RESOMER® RGP d 50105 (poly[(D,L-lactide-co-glycolide)-co-PEG] diblock; D,L-lactide:glycolide 48:52 to 52:48 mole % and PEG portion 8–12%) (PLGA-PEG) were purchased from Boeringher Ingelheim KG (Ingelheim am Rhein, Germany). Melatonin and polysorbate (Tween®) 80 were obtained from Sigma-Aldrich Chimica srl (Milan, Italy). All the other materials were of analytical reagent grades. PLGA- and PLGA-PEG-unloaded NPs were prepared as previously described (respectively blank A and blank B).[28] The solvent displacement method was used with Tween® 80 (0.5% w/V) as surfactant. Drug-loaded NPs (NP-A and NP-B) were obtained by adding melatonin (1% w/w drug/polymer) to the aqueous phase. The purification process (three times) consisted of ultracentrifugation ($15{,}000 \times g$) for one hour at 5°C (Beckman [Fullerton, CA, USA] model J2–21 centrifuge equipped with a Beckman JA-20.01 fixed-angle rotor) for PLGA NP (blank A and NP-A). The obtained supernatants were collected and the pellets were resuspended in 50 mL water. PLGA-PEG NP (blank B and NP-B) were centrifuged at $4000 \times g$ for four hours at room temperature with bimolecular separation disposables (Microsep 30k Omega; Life Sciences, Pall Italia, Milan, Italy). NP mean size was determined by photon correlation spectroscopy (Zetamaster, Malvern Instruments Ltd, Malvern, UK). Size measurements were carried out at a fixed scattering angle of 90°. To obtain the mean diameter and polydispersity index of colloidal suspensions, a third-order cumulant fitting correlation function was performed by the analyzer. Each suspension was suitably diluted with filtered water (10 mL) to avoid multiscattering phenomena and placed in a quartz cuvette. The size analysis of each sample consisted of 30 measurements, and the result was expressed as mean size $\pm$ SD. The zeta potential distribution was measured with the Zetamaster particle electrophoresis analyzer set up equipped with a 5 mW HeNe laser (633 nm). Measurements were performed on the same samples prepared for size analysis (100 μL) diluted with 10 mL of filtered water. Zeta limits ranged from -120 to $+120$ mV. For measurement of osmolarity and pH, the NP pellet was resuspended in bidistilled water. Osmotic values were calculated with a digital osmometer (Osmomat 030; Gonotec, Berlin, Germany); pH was determined at 25°C with a glass pH meter (Basic 20; Crison, Milan, Italy). NPs (50 mg) were dissolved in 5 mL of acetonitrile and the drug amount was determined by spectrophotometric analysis (UV-VIS 1601 spectrophotometer; Shimadzu Italia, Milan, Italy) at melatonin λ_{max} (278 nm). The calibration curve for the quantitative evaluation of the drug was linear in the following ranges: (1) 38.15–2.29 μg/mL of melatonin (r^2, 0.9995) for analyses in acetonitrile; and (2) 65.47–3.95 μg/mL of melatonin (r^2, 0.9820) in phosphate-buffered saline (PBS). The entrapment of melatonin in NP-A and NP-B was expressed as encapsulation efficiency and drug content. The encapsulation efficiency was determined as the mass ratio of entrapped melatonin in the NP to the theoretical amount of drug used in their preparation ([recovered melatonin/theoretical melatonin] $\times$ 100). Drug content was expressed as (recovered melatonin/mg of NP) $\times$ 100. For the *in vitro* release studies, the NP pellet was resuspended in 1.5 mL of PBS, pH 7.4 and placed inside a dialysis bag (Spectra/Por® membrane tubing, 11.5 mm diameter; Spectrum Europe BV, Breda, Netherlands). The membrane

bags were poured in a known volume in 8.5 mL of the same buffer solution PBS. The samples were magnetically stirred at 100 rpm in a water bath maintained at $37 \pm 0.5^{\circ}C$. The release of encapsulated melatonin from NPs was monitored for 15 days. At predefined time intervals, 1.5 mL aliquots were withdrawn and replaced with an equal volume of fresh buffer, prewarmed at 37°C.

Animals and experimental sepsis model

All procedures were carried out in accordance with the Italian Guidelines for the Care and Use of Laboratory Animals. The rats were housed in a temperature-controlled room maintained in 12 h light/dark cycles. The standard laboratory food and water were freely available *ad libitum* except for an overnight fasting before inducing experimental sepsis in rats as previously described.[29] Anesthesia was obtained with Zoletil (combination of zolazepam, with the dissociative anesthetic tiletamine; VIRBAC srl, Milan, Italy). The adequacy of the anesthetic was ascertained by the lack of a dodge reaction to hind paw pinching and by pupil diameter. Additional anesthesia (5% of the initial dose) was administered when necessary. A small incision was made in the abdomen of anesthetized rats for intraperitoneal instillation of fecal suspension at a dosage of 1 mL/kg body weight of animals. The fecal suspension was prepared by dissolving fresh feces (1:1 w/v in normal saline) obtained surgically from the caecum of non-fasted healthy rats, and was used within two hours. Control animals received pre-autoclaved (135°C for one hour) fecal suspension. The wound was closed and the animals returned to their home cages. The surgery was performed aseptically. No fluid therapy was administered. This procedure results in a fecal peritonitis septicemia resembling the model of cecal ligation and puncture. Wistar male rats were divided into five groups of five animals each ($n = 5$). Group I was treated with preautoclaved fecal suspension (non-septic animals); group II and III received fresh fecal suspension and were treated concomitantly with vehicle and free melatonin (10 mg/kg), respectively; and groups IV and V received fresh fecal suspension and were treated concomitantly with an amount of NP-A and NP-B suspension, respectively, containing an equivalent concentration of melatonin. All formulations were delivered intraperitoneally.

Following 24 h from surgical procedure and pharmacological treatment, animals were anesthetized and perfused with cold saline. Heart, lung, liver and kidney were rapidly removed and processed for biochemical analysis.

Total thiol group determination

Tissue levels of total thiol groups (RSH) were measured in 200 μL of tissue homogenate using a spectrophotometric assay based on the reaction of thiol groups with 2,2-dithio-bis-nitrobenzoic acid (DTNB) at $\lambda = 412$ nm ($\varepsilon_M = 13{,}600\ mol/L^{-1}\ cm^{-1}$, where ε_M is a wavelength-dependent molar absorbtivity coefficient). The limit of detection for this assay is approximately 15 nmol/L. The intra-assay coefficient of variation is 4% whereas the inter-assay coefficient of variation is 5.6%.

Determination of lipid hydroperoxide levels

Lipid hydroperoxide (LOOH) levels were evaluated following the oxidation of Fe^{2+} to Fe^{3+} in the presence of xylenol orange at $\lambda = 560$ nm.[30] The assay mixture contained, in a total volume of 1 mL/100 μL of tissue homogenate, 100 μmol/L xylenol orange, 250 μmol/L ammonium ferrous sulfate, 90% methanol, 4 mmol/L butylated hydroxytoluene and 25 mmol/L H_2SO_4. After a 30-min incubation at room temperature, the absorbance was measured using a U2000 Hitachi spectrophotometer (Hitachi, Tokyo, Japan). Calibration was obtained using hydrogen peroxide (0.2–20 μmol/L). The limit of detection for this assay is approximately 0.25 nmol/L.

HO-1 measurement

A commercially available enzyme-linked immunosorbent assay (ELISA) kit (Stressgen, Victoria, Canada) was used to measure HO-1 protein concentration. The assay was performed in accordance with the protocol provided by the manufacturer and as previously described.[31] Briefly, each milk sample was incubated with anti-HO-1, anti-rabbit immunoglobulin G and horseradish peroxidase conjugates, in successive order. Absorbance at 450 nmol/L was measured, and HO-1 concentration was calculated from a standard curve generated with purified HO-1. The limits of detection provided by the manufacturer were 0.78–25 ng/mL. Each measurement was performed in triplicate, and averages were reported. The intra-assay coefficient of variation and the inter-assay coefficient of variation of the HO-1 ELISA kit have been determined to be <10%.

Statistical analysis

One-way analysis of variance followed by Bonferroni's *t*-test was performed to estimate significant differences among groups, after the Levene's test evaluation. Data are presented as means $\pm$ SD, and differences between groups were considered to be significant at $P < 0.05$.

Results

Effect of polymer on the physicochemical characteristics of NPs

Polymeric NPs were obtained by two biodegradable copolymers (PLGA and PLGA-PEG), using a solvent displacement method. The obtained NPs were spherical and homogeneous with smooth surfaces when assessed by scanning electron microscopy (data not shown). PLGA-PEG NPs were smaller than PLGA NPs, and showed a greater size homogeneity (polydispersity index, PDI) (Table 1). Melatonin-loaded nanosuspensions were isotonic and isohydric with biological fluids, and they presented a unimodal mean size distribution with low PDI values (<0.300). The PLGA-PEG system (NP-B) showed higher encapsulation efficiency for the drug.

In Figure 1, we report the zeta potential values. No significant variations between unloaded and melatonin-loaded NPs were registered for both polymers. In particular, NP-A showed a value of about −30 mV, more negative than the corresponding PEGylated NP-B system.

Table 1 Physicochemical characterization of polymeric NPs unloaded and loaded with melatonin (at 1%, w/w initial drug/polymer ratio)

Sample	Mean size (nm)	PDI	pH	Osmolarity (mOsm/kg)	EE (%)	DC (%)
Blank A	120.90 ± 0.6	0.147 ± 0.010	6.37 ± 0.03	–	–	
NP-A	122.50 ± 14.3	0.168 ± 0.090	6.24 ± 0.02	0.344 ± 0.004	44.15 ± 4.7	0.44 ± 0.9
Blank B	67.50 ± 2.80	0.253 ± 0.020	7.48 ± 0.05	–	–	
NP-B	83.80 ± 1.10	0.260 ± 0.016	7.42 ± 0.02	0.330 ± 0.008	78.20 ± 3.4	0.78 ± 0.4

Values are the mean of three measurements (± SD)
PDI, polydispersity index; EE, encapsulation efficiency; DC, drug content; NPs, nanoparticles

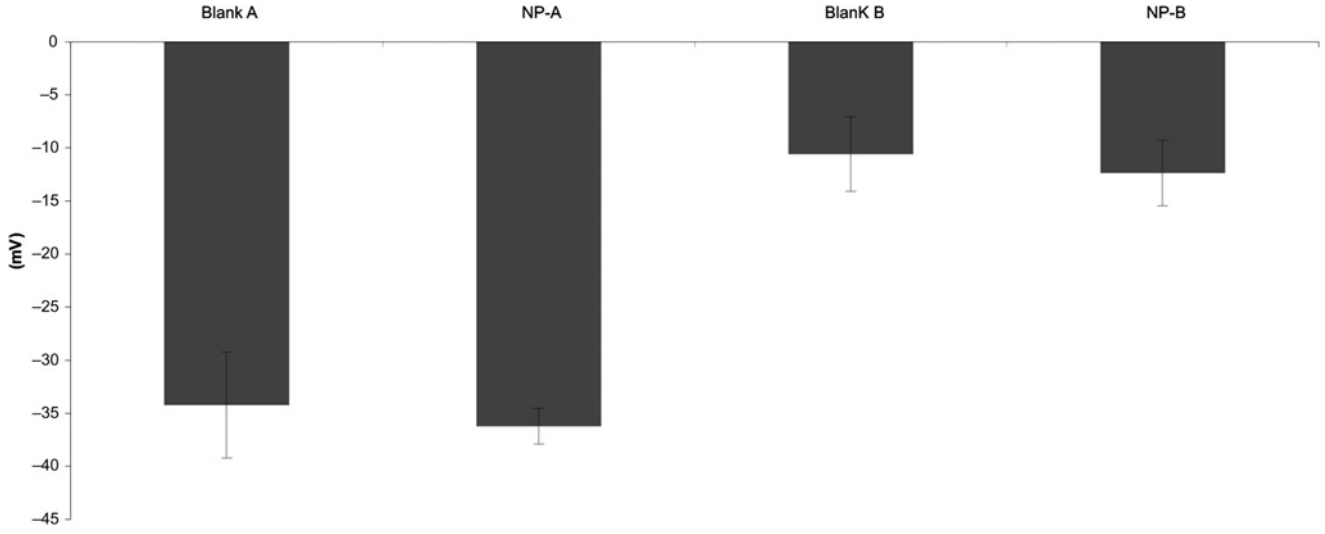

Figure 1 Zeta potential value (mV) of nanoparticles (NPs) unloaded and loaded with melatonin (1% w/w initial drug/polymer amount)

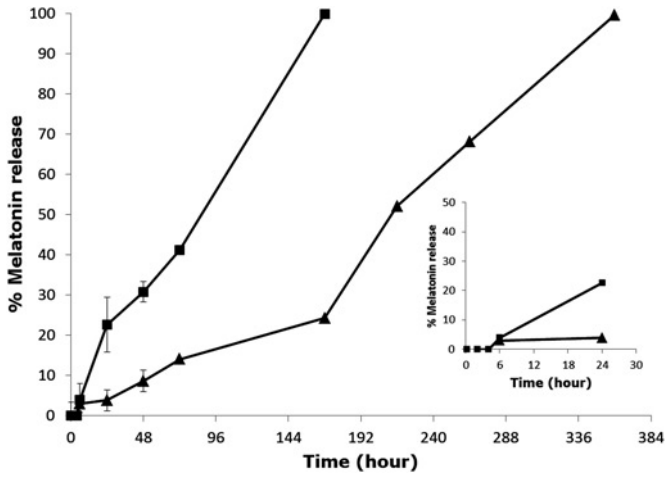

Figure 2 *In vitro* release profiles of melatonin from poly-D,L-lactide-co-glycolide (PLGA) and PLGA-PEG nanoparticles (NPs) until 24 h (inset) and 360 h. The inset profile further evidences the lag time during the first 24 h from the beginning of the experiment. Release experiments were carried out at 37 ± 0.5°C in isotonic phosphate buffer solution (phosphate-buffered saline, pH 7.4), under constant stirring. Each point represents the mean value of five different experiments ± SD

With respect to the *in vitro* melatonin release, both formulations showed a lag time of about 24 h (Figure 2, insert), followed by a controlled release profile. In this case, we also demonstrated that the drug is completely incorporated into polymeric matrix. Besides, NP-A gave a zero order release profile with a complete release of the encapsulated drug in six days, while NP-B showed a more complex triphasic profile, with a slower melatonin release. Drug release remained slow up to seven days, followed by a linear release that approached a zero-order kinetic until the end of the experiment (Figure 2).

Effects of different melatonin formulations on plasma and tissue RSH levels

Vehicle treatment resulted in a significant decrease of plasma RSH levels when compared with non-septic animals (Figure 3). In particular, RSH depletion was more evident in the liver compared with other organs. In all examined organs, all melatonin formulations restored RSH levels to those of the control group. To this regard, no significant changes were observed among different formulations.

Effects of different melatonin formulations on plasma and tissue LOOH levels

Our results showed a notable and statistically significant increase in plasma LOOH levels in the sepsis vehicle-treated group when compared with non-septic animals (Figure 4). LOOH levels were differently affected following different melatonin formulations. In particular, melatonin treatment resulted in a slight decrease of LOOH levels when compared with sepsis vehicle-treated animals. Interestingly, both melatonin NP formulations significantly decreased LOOH levels

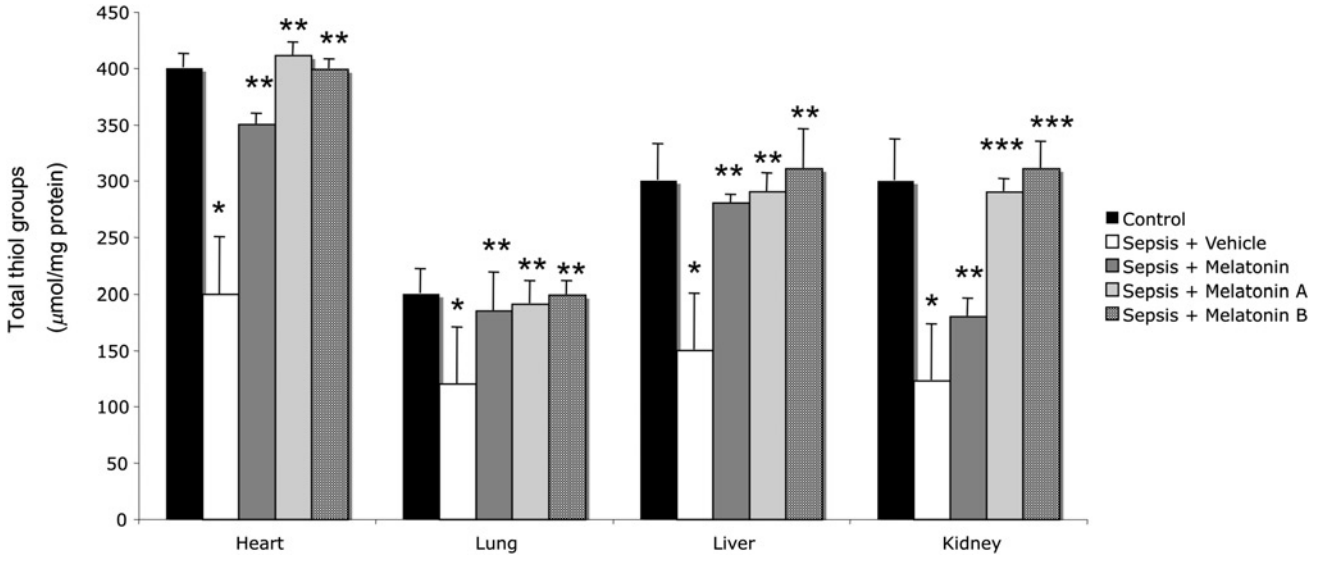

Figure 3 Levels of non-proteic thiol groups were measured in 200 μL of tissue homogenate. This spectrophotometric assay is based on the reaction of thiol groups with 2,2-dithio-bis-nitrobenzoic acid (DTNB) in absolute ethanol to give a colored compound absorbing at $\lambda = 412$ nm. Each value represents the mean $\pm$ SD of three experimental determinations for each sample. Results were expressed as micromoles per mg of protein. ($^{*}P < 0.01$ when compared with control; $^{**}P < 0.05$ when compared with sepsis+vehicle; $^{***}P < 0.01$ when compared with sepsis+melatonin or vehicle)

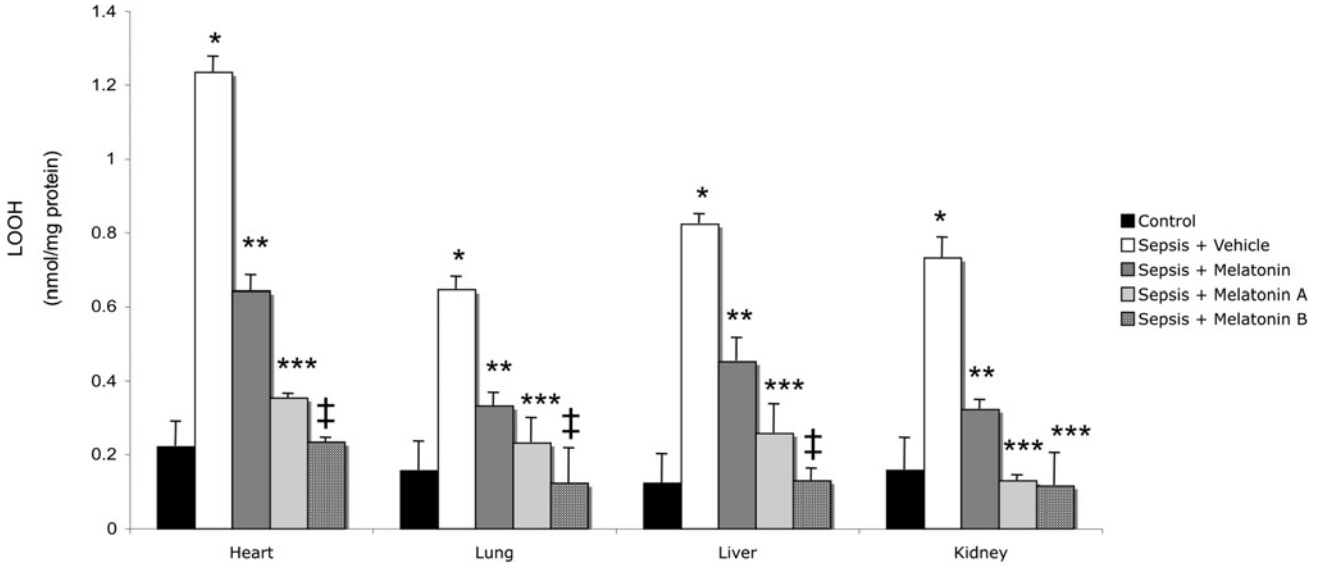

Figure 4 Tissue levels of lipid hydroperoxides (LOOH) were evaluated by oxidation of Fe^{+2} to Fe^{+3} in the presence of xylenol orange at $\lambda = 560$ nm. Calibration was obtained using hydrogen peroxide (0.2 to 20 μmol/L). Each value represents the mean $\pm$ SD of three experimental determinations for each sample. Results were expressed as nanomoles per mg of protein ($^{*}P < 0.01$ when compared with control; $^{**}P < 0.01$ when compared with sepsis+vehicle; $^{***}P < 0.05$ when compared with sepsis+melatonin or vehicle; $^{\ddagger}P < 0.05$ when compared with sepsis+melatonin A, melatonin or vehicle)

when compared with sepsis vehicle animals. The stealth formulation NP-B was able to produce a more significant LOOH level reduction in the heart, lung and liver when compared with NP-A. No significant changes were observed between the two NP formulations in the kidney.

Effects of different melatonin formulations on HO-1 expression

HO-1 expression was significantly increased in the sepsis vehicle-treated group when compared with non-septic animals (Figure 5), especially in the liver. Interestingly, HO-1 expression was differently affected following treatment with various melatonin formulations. In particular, treatment with free melatonin decreased HO-1 levels when compared with sepsis vehicle-treated animals in the heart, liver and kidney. No significant changes were observed in the lung. NP-A formulation-treated animals displayed a similar HO-1 pattern when compared with free melatonin, although it significantly reduced HO-1 in the lung. NP-B formulation was more effective in inducing HO-1 protein compared with free melatonin and NP-A, with the exception of the kidney. This pattern could be ascribed to the presence of the hydrophilic PEG corona on

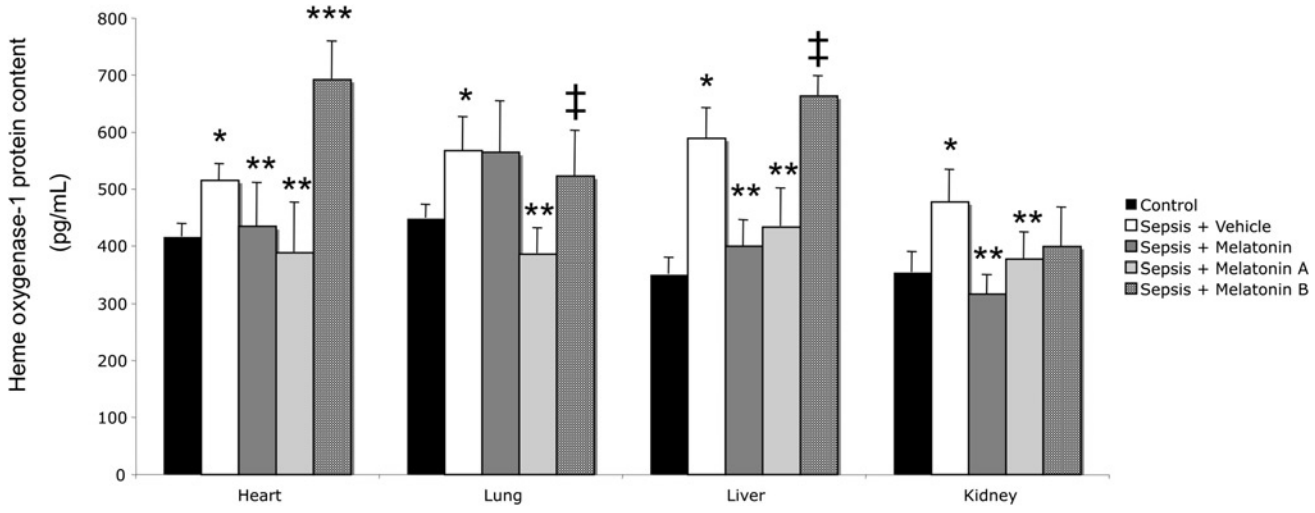

Figure 5 Tissue heme oxygenase-1 (HO-1) protein levels measured by a commercially available enzyme-linked immunosorbent assay kit. Each value represents the mean ± SD of three experimental determinations for each sample. Results were expressed as pg/mg of protein. ($^{*}P < 0.05$ when compared with control; $^{**}P < 0.05$ when compared with sepsis+vehicle; $^{***}P < 0.01$ when compared with sepsis+melatonin A, melatonin or vehicle; $^{‡}P < 0.05$ when compared with melatonin A)

the NP-B surface that hindered the accumulation of these NPs in various organs, especially in the lungs and liver, while prolonging their permanence in the bloodstream.

Discussion

The type of used polymer influenced the physicochemical properties of the NP produced, as well as their biological fate and efficacy. The lower mean size of the stealth nanosuspension (NP-B) was due to the presence of the hydrophilic PEG chains covalently linked to the more hydrophobic PLGA backbone. A microphase separation of the components should occur during NP production for the immiscibility of these two blocks. As a consequence, smaller PLGA-PEG NPs were formed, since the PEG crown did not allow the growth of particle size caused by the further deposition of other copolymers.[32] The zeta potential value, around −30 mV, indicates a good stability for the colloidal suspensions. Analogous surface charge values were measured for both loaded and unloaded NPs, indicating that melatonin was uniformly dispersed within the polymer matrix.

In vitro release studies confirmed this hypothesis: in fact, the first part of the release curves for both formulations showed a long lag time and the absence of a burst effect, which would have indicated the presence of drug adsorbed on the NP surface. In the presence of the PEG crown, the resulting external hydration layer hindered the penetration of water towards the NP surface and the counter-diffusion of melatonin from inside the particles, thus reducing the drug release time. Moreover, since PLGA copolymer can undergo a progressive degradation under the relatively long (24 h) conditions used for the test, this phenomenon could also be slowed down by the presence of PEG chains on the particle surface.

Several lines of evidence suggest that melatonin may act as a protective substance, for example, when administered in models of oxidative stress.[4] Furthermore, previous *in vitro* studies have shown that in addition to melatonin, other structurally related indoleamine compounds may exhibit beneficial effects.[33] In the present research, we aimed to test the efficacy of different melatonin nanocarriers on this antioxidant property. Our findings are consistent with previous results demonstrating the antioxidant properties of melatonin.[4] Furthermore, we showed that melatonin-loaded nanomedicines are particularly effective in counteracting oxidative injury in different organs.

Septic animals receiving vehicle are characterized by increased oxidative stress as measured by a significant reduction of RSH and an increase of LOOH formation in various organs. Consistent with this increased oxidative stress, we showed that HO-1 levels increased in all organs. Intraperitoneal injection of melatonin in septic animals restored the RSH levels to those of the control but not in the kidney, where RSH were increased compared with vehicle-treated septic animals but remained significantly lower compared with healthy animals. Furthermore, melatonin administration resulted in a significant decrease in LOOH content in the heart, lung and liver compared with vehicle-treated septic animals. Similarly, melatonin decreased LOOH formation in the kidney even though they remained at higher levels when compared with healthy animals.

Interestingly, melatonin treatment prevented HO-1 increase in the heart, liver and kidney but not in the lung. This result suggests that in the lung, melatonin significantly improves oxidative stress in septic animals independently of HO-1 levels. In this regard, it is conceivable that HO-1 exerts its effects in septic animals in an organ-specific manner. Furthermore, the antioxidant effects may be dependent on the scavenging property of melatonin *per se*. Finally this effect may be dependent on the pharmacokinetics of melatonin. To address this issue, we used two septic animal groups treated with different melatonin NP formulations.

This set of experiments showed that NP-A exerted the same effects of melatonin on RSH in the heart, lung and

liver, while it was more effective than the free drug in the kidney. NP-A also prevented HO-1 induction to the same extent as the free drug in all the examined organs. Treatment with NP-B showed the same pattern of RSH restoration of NP-A; however, it caused a significantly lower LOOH formation in the heart, lung and liver when compared with the vehicle, free melatonin or NP-A. In this regard, it is possible to explain these observations considering that NP-B formulation is a stealth carrier due to its hydrophilic PEG corona that avoids entrapment in the reticular endothelial system (i.e. liver and spleen).

NP-B induced HO-1 expression in the same organs where LOOH formation was significantly lowered. These data suggest that HO-1 may provide an additional protection against oxidative stress under our experimental conditions.

One of the possible limits of the present research, with particular respect to its possible translation into a clinical setting, is related to the safety of NP introduced in the human body or released into the environment. Many efforts are underway at world level to clarify these aspects. Only a relatively limited number of published papers report *in vitro* or *in vivo* data on the toxicological aspects of nanomaterials, compared with the huge amount of research in the field. However, consulting and expert groups are at work to delineate efficient rules. For instance, the US FDA, in 2006, created a Nanotechnology Task Force (http://www.fda.gov/ScienceResearch/SpecialTopics/Nanotechnology/NanotechnologyTaskForce/default.htm) that will drive a regulatory body for the development of safe and effective nanomedicines.

Taken all together, our results showed that melatonin possesses a significant antioxidant activity during sepsis and that it is possible to improve this ability by delivering the compound with specific drug delivery systems.

Author contributions: All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript. GLV conceived and designed the study, performed experiments, analyzed data and wrote the manuscript; TM performed experiments, analyzed data and critically reviewed the manuscript; RP designed the study, performed experiments, analyzed data and critically reviewed the manuscript; IB, PM and CC performed experiments and analyzed data; AG analyzed data and critically reviewed the manuscript; and GP designed the study, analyzed data and contributed to the writing of the manuscript.

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