Melatonin prevents experimental preterm labor and increases offspring survival

Abstract: Preterm delivery is the leading cause of neonatal mortality and contributes to delayed physical and cognitive development in children. At present, there is no efficient therapy to prevent preterm labor. A large body of evidence suggests that intra-amniotic infections may be a significant and potentially preventable cause of preterm birth. This work assessed the effect of melatonin in a murine model of inflammation-associated preterm delivery which mimics central features of preterm infection in humans. For this purpose, preterm labor was induced in BALB/c mice by intraperitoneal injections of bacterial lipopolysaccharide (LPS) at 10.00 hr (10 μg LPS) and 13.00 hr (20 μg LPS) on day 15 of pregnancy. On day 14 of pregnancy, a pellet of melatonin (25 mg) had been subcutaneously implanted into a group of animals. In the absence of melatonin, a 100% incidence of preterm birth was observed in LPS-treated animals, and the fetuses showed widespread damage. By comparison, treatment with melatonin prevented preterm birth in 50% of the cases, and all pups from melatonin-treated females were born alive and their body weight did not differ from control animals. Melatonin significantly prevented the LPS-induced rises in uterine prostaglandin (PG) E_2, $PGF_{2\alpha}$, and cyclooxygenase-2 protein levels. In addition, melatonin prevented the LPS-induced increase in uterine nitric oxide (NO) production, inducible NO synthase protein, and tumor necrosis factor-alpha (TNFα) levels. Collectively, our results suggest that melatonin could be a new therapeutic tool to prevent preterm labor and to increase offspring survival.

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Introduction

Preterm birth (i.e., birth before 37 wk gestation) is a serious health issue. In 2005, 13 million preterm babies were born worldwide; more than 1 million infants die every year because they are born preterm, and those who survive have an increased risk of morbidities, such as cerebral palsy, blindness, and hearing loss [1]. Despite the advances made in obstetrics and neonatology, the rate of premature delivery has not decreased over the past 20–30 yr. At present, there is no efficient therapy to prevent preterm labor and to increase offspring survival. Although the precise pathways that govern preterm parturition remain to be elucidated, epidemiological and experimental data indicate that maternal infection and inflammation are key factors in preterm birth. In fact, it has been suggested that at least 40% of all premature deliveries occur to mothers with intrauterine infection [2].

Bacterial endotoxins stimulate cytokine and chemokine responses by leukocytes and other cells in uterine, placental, and fetal tissues, leading to the release of prostaglandins (PGs), nitric oxide (NO), and matrix metalloproteinases [2]. It has been demonstrated that PGs, NO, and TNFα, are responsible for preterm labor in several rodent models [3–5], as well as in humans [6].

Melatonin, the main secretory product of the pineal gland, is an evolutionary conserved indolamine synthesized from tryptophan [7]. A number of anti-inflammatory and antioxidant effects have been reported when melatonin is administer exogenously in vivo or when added to cultured cells (for review [8, 9]. It is well known that melatonin acts on the immune system by regulating cytokine production of immunocompetent cells [10]. Experimental and clinical data show that melatonin reduces adhesion molecule and pro-inflammatory cytokine levels and modifies serum inflammatory parameters. As a consequence, melatonin improves the clinical course of illnesses which have an inflammatory etiology (for review [11]). Moreover, experimental evidence supports the actions of it and its metabolites as a direct and indirect antioxidant [9, 12], scavenging free radicals [12], stimulating antioxidant enzymes [13], and enhancing the activities of other antioxidants [13]. In addition, melatonin decreases nitric oxide synthase (NOS) activity [14] and inhibits PG biosynthesis in several tissues [15, 16].

Data from several sources provide evidence that melatonin is an important player in the physiology of reproduction [17]. The expression and function of melatonin receptors in the nonpregnant and pregnant human myometrium [18], and in rat uterus [19] have been

demonstrated. In addition, maternal plasma melatonin levels are elevated during late pregnancy and could contribute to gestation maintenance, by stimulating progesterone production [20, 21], and inhibiting PG synthesis [15, 16] and uterine contractility [22]. Over the last years, several studies have shown that melatonin has protective effects against LPS-induced damage in pregnancy. In that context, Chen et al. [23] reported that melatonin protects mice from LPS-induced fetal death and intrauterine growth restriction and alleviates LPS-induced cellular stress in the placenta [24], while Xu et al.[25] have demonstrated that maternally administered melatonin differentially regulates LPS-induced pro-inflammatory and anti-inflammatory cytokine levels in maternal serum, amniotic fluid, fetal liver, and fetal brain. Recently, we developed a murine model of preterm labor, induced by injections of LPS, and we showed that preterm delivery can be prevented by decreasing PG or NO production [26]. With this background, the aim of this work was to study the effect of melatonin on preterm labor and offspring survival in a murine model of inflammation-associated preterm delivery induced by LPS.

Materials and methods

Animals

Female BALB/c mice from our own colony were housed in a standard animal room with food and water ad libitum, in controlled conditions of humidity, temperature (21 ± 2°C), and luminosity (200 lux), under a 12-hr light/dark lighting schedule (lights on at 7:00 hr). Virgin female mice were mated with fertile males of the same strain. Timing of pregnancy was determined by visual inspection of the vaginal plug, which was defined as day 0 of pregnancy. Under our animal facility conditions, normal term labor occurs on day 19 of gestation.

Animals were sacrificed in a carbon dioxide chamber, and all efforts were made to minimize animal suffering. The experimental procedures reported here were approved by the Animal Care Committee of the Center for Pharmacological and Botanical Studies, National Research Council, and by the Institutional Committee for the Care and Use of Laboratory Animals (CICUAL) from the School of Medicine, University of Buenos Aires, and were carried out in accordance.

Treatments

Four groups of mice were included in this study, as depicted in Fig. 1. Mice received two doses of vehicle or LPS on day 15 of pregnancy, the first one (0.26 mg/kg, 10 μg in 0.1 mL of sterile saline solution) at 10.00 hr and the second at 13.00 hr (0.52 mg/kg, 20 μg in 0.1 mL vehicle). For the intraperitoneal (i.p.) administration of vehicle or LPS, animals were manually restrained and no anesthesia was used. One day earlier (day 14 of pregnancy), animals were anesthetized with ketamine hydrochloride (110 mg/kg) and xylazine hydrochloride (10 mg/kg), and subcutaneously implanted with a single pellet of melatonin (25 mg with 3% w/v vegetable oil) compressed in a metallic punch of 4 mm diameter and 3 mm length, or were sham-operated without pellet implantation. Sham-operated animals were used as control, because in preliminary studies, we found that in comparison with intact animals, the sham procedure did not affect the pregnancy length or the pup number.

Animals were closely observed for any signs of morbidity (piloerection, decreased movement, and diarrhea), vaginal bleeding, and preterm delivery. The beginning of preterm delivery was defined by the delivery of the first pup. The body weight of pups was recorded at day 1, 8, 15, and 22. Another group of mice was euthanized in a carbon dioxide chamber on day 15 of pregnancy, at 2 and
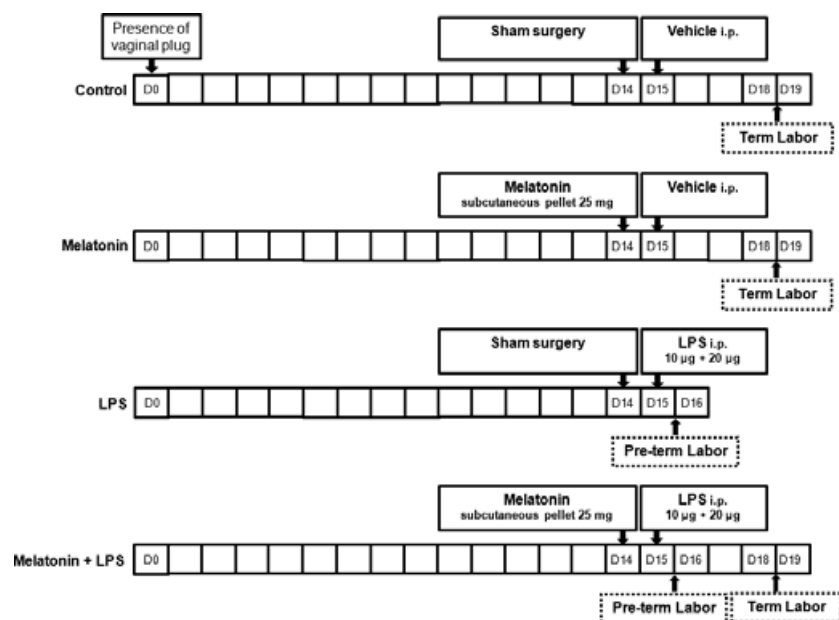

Fig. 1. Experimental groups. On day 14 of pregnancy, BALB/c females were subcutaneously implanted with a single pellet of melatonin or were sham-operated without pellet implanting at 16.00 hr. On day 15 of pregnancy, pregnant mice were i.p. injected with two doses of LPS or vehicle. Control animals or those treated with melatonin in the presence or absence of LPS delivered during the night of day 18 or the early morning of day 19.

5 hr postinjection of vehicle or LPS, and the uterus and fetuses were immediately removed, cleaned of fat, and frozen until used.

NOS activity assessment

NOS activity was quantified in uterine strips by the modified method of Bredt and Snyder [27], which measures the conversion of $[^{14}C]$-L-arginine to $[^{14}C]$-L-citrulline. Briefly, samples were weighed and homogenized in a buffer containing 20 mM HEPES, 4.5 μM $CaCl_2$, 100 mM DTT, and 25 mM valine. After homogenization, 10 μM $[^{14}C]$-arginine (0.3 μCi, purity > 98%) and 0.12 mM NADPH were added. Samples were incubated for 15 min in a 5% CO_2 atmosphere at 37°C and immediately centrifuged at 7800 × g for 10 min (4°C). Then, the supernatants were applied to 1 mL DOWEX AG50W-X8 columns (Na$^+$ form), equilibrated with HEPES medium and citrulline. Finally, $[^{14}C]$-citrulline was eluted in 3 mL of water. The radioactivity was measured by liquid scintillation counting. Enzyme activity is reported in fmol of $[^{14}C]$-citrulline/mg of tissue in 15 min. Nonenzymatic conversion of $[^{14}C]$-L-arginine to $[^{14}C]$-citrulline was tested by adding buffer instead of the enzyme source.

Prostaglandin radioimmunoassay

PGE_2 and $PGF_{2\alpha}$ were measured in uterine samples as described by Ribeiro et al. [28]. Uterine strips were incubated for 1 hr in Krebs-Ringer bicarbonate solution in a 5% CO_2 atmosphere at 37°C. After incubation, the medium was acidified, and PGs were extracted twice with ethyl acetate. PG concentrations were determined by radioimmunoassay. Sensitivity was 5–10 pg per tube, and values were expressed as pg PGs/mg of tissue (wet weight).

Assay for TNFα level assessment

Each uterine fragment (100 mg) was homogenized in 120 μL of PBS buffer containing 10% fetal bovine serum and supplemented with 20 μL of a cocktail of protease inhibitors. Samples were cleared by centrifugation for 20 min at 15,000 g. TNFα levels were estimated with enzyme-linked immunosorbent assay (ELISA) kit (TNF [Mono/Mono] Set of BD Biosciences Pharmingen (San Diego, CA, USA), according to the manufacturer's instructions. The reaction was stopped and absorption was measured in an ELISA reader at 450 nm. All measurements were performed in duplicate.

Western blot

Uteri were homogenized in an Ultra-Turrax homogenizer in 20 mM Tris-buffer (pH 7.4), containing 1 mM EDTA, 2 μg/mL aprotinin, 10 μg/mL leupeptin, 10 μg/mL DTT, 100 μg/mL streptomycin, 1 mg/mL caproic acid, and 1 mg/mL benzamidine. The homogenates were sonicated and centrifuged at 3000 × g for 10 min to remove cellular debris. The supernatants were collected and kept at −70°C until the Western blot was performed. The protein concentration was measured by the Bradford method [29], and 100 μg of protein was loaded into each lane. The samples were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a nitrocellulose membrane (Sigma Chemical Co, St. Louis, MI, USA). The membrane was reacted with a rabbit antiserum against iNOS, COX-2 (Cayman, Ann Arbor, MI, USA), or β-actin (Sigma Chemical Co, St. Louis, MI, USA), followed by a horse radish peroxidase-conjugated anti-rabbit IgG as the secondary antibody, and developed by chemiluminescence (ECL Western Blotting Analysis System; GE Healthcare, Amersham, UK). The protein bands were identified by molecular weight markers (BIO-RAD, Hercules, CA, USA). Each blot was repeated three times with different samples. Blots were scanned using a scanning densitometer, and the intensity of bands determined using the Image J (NIH) program.

Data analysis and statistical procedures

The results (except newborn mice weight) were analyzed by two-way ANOVA in a completely randomized design (LPS and melatonin). Comparisons were made with the Tukey's test. Results were considered significant at $P < 0.05$. The assumptions of normality and homoscedasticity were studied analytically by Shapiro–Wilks test and the Levene test, respectively. The newborn mice weight was analyzed by three-way ANOVA in a completely randomized design with repeated measures (treatment, litter, and time). Comparisons were made with the Tukey's test. Results were considered significant at $P < 0.05$. The assumptions of normality, homogeneity of variances, and sphericity were studied analytically by Shapiro–Wilks test, Box test, and Mauchly test, respectively. All statistical analyses were performed using the statistical program InfoStat (FCA, University of Cordoba, Cordoba, Argentina).

Results

Table 1 shows the effect of a subcutaneous pellet of melatonin on LPS-induced preterm delivery and on viable pup number. Preterm delivery was induced in all animals (100%) treated with LPS, whereas melatonin, which did not alter pregnancy length per se, prevented LPS-induced

Table 1. Litter sizes and percentage of preterm and term labor

	% Preterm labor	% Term labor	Viable pup number/mother
Control	0	100	10 ± 1
Melatonin	0	100	9 ± 2
LPS	100	0	0
Melatonin + LPS	50	50	8 ± 2

The treatment with LPS induced preterm labor in 100% of cases, whereas melatonin prevented LPS-induced preterm delivery in 50% of cases. Control animals or animals treated with melatonin delivered at term in 100% of cases. The number of viable pups did not differ among vehicle-injected animals untreated or treated with melatonin, and LPS-injected animals in the presence of melatonin. Data are mean ± S.E.M. (n = 10, $P > 0.05$).

preterm delivery in 50% of cases. The number of viable pups/mother did not differ between control animals and those treated with LPS in the presence of melatonin. Macroscopic examination of the antepartum cervix in LPS-treated mice showed increased opening and bleeding when compared with the closed cervix observed in gestational age-matched animals injected with vehicle or in animals treated with LPS in the presence of melatonin (Fig. 2A). Uteri from animals sacrificed 5 hr after the last injection of LPS appeared less irrigated and the intersites less defined (Fig. 2B).

In the absence of melatonin, the fetuses from LPS-treated animals showed widespread damage, the most relevant being the lack of cerebral irrigation (Fig. 3A), and number of stillborn pups, whereas in the presence of melatonin, fetuses did not differ from those of control animals in 100% of cases, even in those from mothers treated with melatonin + LPS in which the birth occurred prematurely (Fig. 3A). In the cases where melatonin prevented LPS-induced preterm delivery (50%), the pups were born alive and their body weight did not differ from control animals at 1, 8, 15, and 22 days, as shown in Fig. 3B. The offspring from females treated with melatonin + LPS did not differ at the time of eye-opening, hair growth, and nail and tooth eruption from those of control animals. Macroscopic observation of these pups on day 8 did not show any sign of distress (Fig. 3C).

To get insight into the mechanism involved in the protective effect of melatonin on LPS-induced preterm birth, uterine PG levels and COX-2 expression were assessed at 5 hr after the last administration of LPS. As shown in Fig. 4, melatonin significantly prevented the increase in uterine PGE_2 (Fig. 4A) and $PGF_{2\alpha}$ (Fig. 4B) production induced by LPS. Concurrently, melatonin significantly reduces the effect of LPS on uterine COX-2 levels (Fig. 4C). Fig. 4C shows representative Western blots of COX-2 from control and LPS-treated animals in the absence or presence of melatonin.

Uterine NOS activity and iNOS levels were assessed in control and LPS-injected mice untreated or treated with melatonin. Melatonin, which was ineffective per se, significantly prevented the increase in NOS activity and iNOS protein levels induced by LPS (Fig. 5A and 5B, respectively). To evaluate the effect of melatonin on pro-inflammatory cytokine production, pregnant animals were sacrificed 2 hr after the last injection of vehicle or LPS, and uterine levels of TNFα were assessed. TNFα levels significantly increased in LPS-treated animals, whereas melatonin significantly reduced the effect of LPS on this parameter (Fig. 6).

Discussion

The present results indicate that melatonin prevented preterm labor and increased offspring survival in an LPS-induced experimental model which mimics the central features of infection-induced preterm delivery in humans [30, 31]. The administration of LPS to pregnant BALB/c mice induced premature parturition, cervix bleeding and opening, damage to fetuses, and offspring mortality, whereas melatonin significantly prevented preterm labor induced by LPS. Moreover, in this experimental model, all pups from mice treated with melatonin were born alive and their body weights did not differ from control animals.

The ideal tocolytic agent should be myometrium specific, easy to administer, inexpensive, effective in preventing preterm birth, and improving neonatal outcomes, with few maternal, fetal, and neonatal side effects, and without long-term adverse effects [32]. At present, nonsteroid
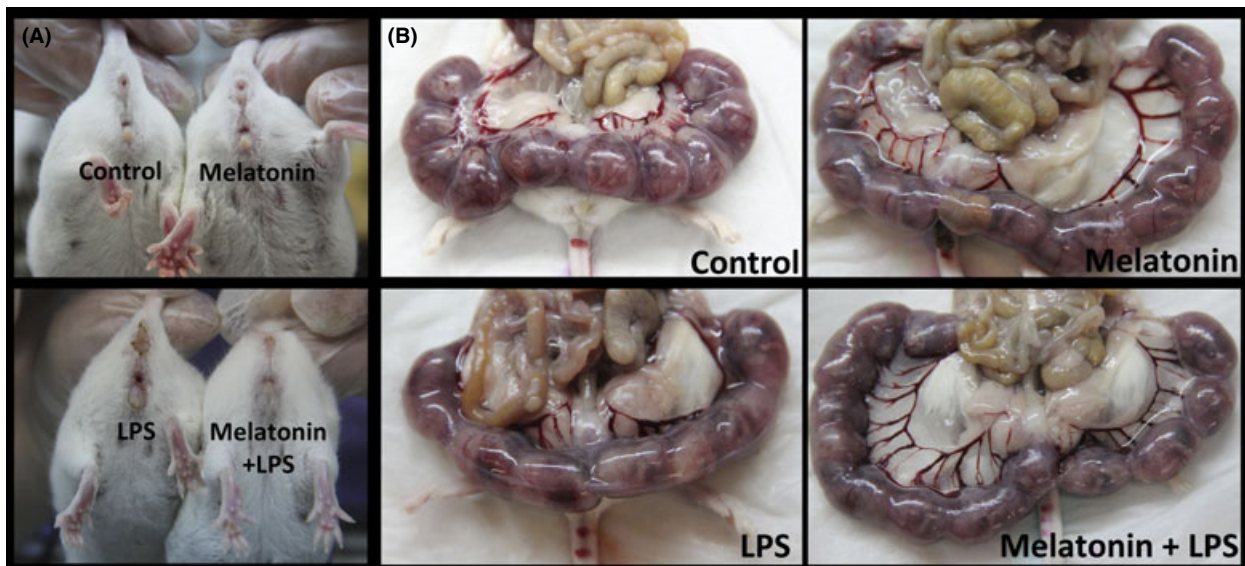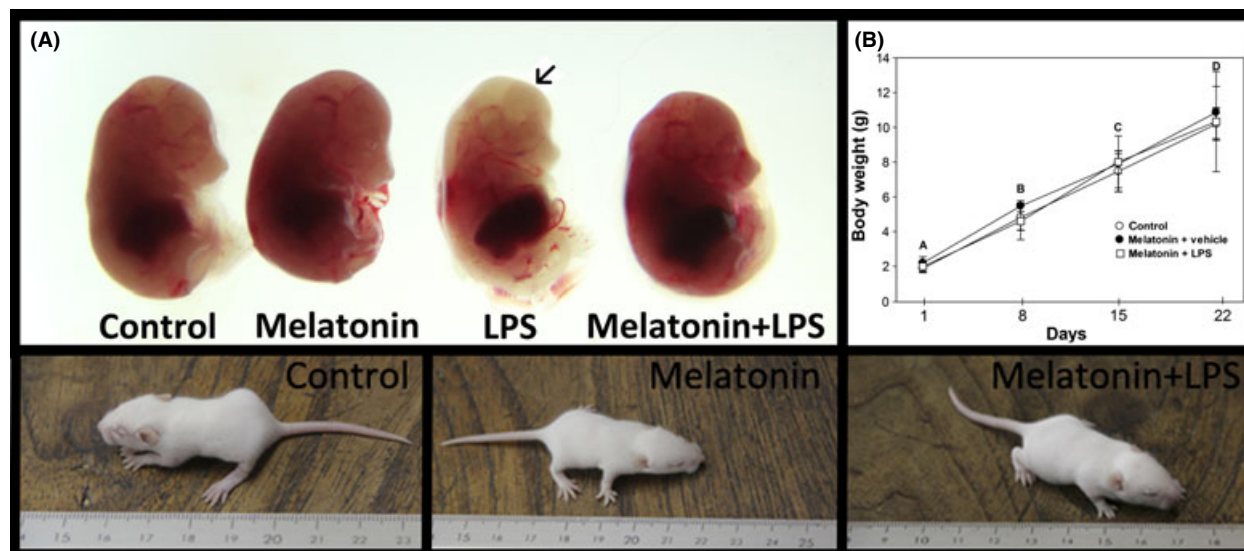

Fig. 2. Effect of melatonin and LPS on cervix dilation and uterus morphology. Panel A: representative photographs showing the cervix dilation at 5 hr after the second injection of LPS in the absence or presence of melatonin. Note the vaginal bleeding induced by LPS which was not evident in animals treated with melatonin. Panel B: representative photographs of uterus morphology at 5 hr after the second injection of vehicle or LPS. Note the less defined intersites (arrow) in uteri from mice treated with LPS in the absence of melatonin.

Fig. 3. Effect of melatonin and LPS on fetuses and offspring body weight. Panel A: representative photographs of fetuses from day 15, at 5 hr after the second injection of vehicle or LPS. Note the decrease in cerebral irrigation (arrow) induced by LPS, which was not observed in fetuses from mice injected with LPS in the presence of melatonin. Panel B: time course of pup body weight from pregnant mice treated with vehicle, melatonin + vehicle, or melatonin + LPS. The growth rate of pups from mice treated with melatonin + LPS did not differ from that observed in pups from control females, or those treated with melatonin in the absence of LPS. Data are mean ± S.E.M. (n = 7). For all the treatments, the pup body weight significantly differed among weeks. $P < 0.05$, by Tukey's test, a ≠ b ≠ c ≠ d. Panel C: representative photographs of 8-day pups from mice injected with vehicle in the absence or presence of melatonin, or LPS in the presence of melatonin. The offspring from females treated with melatonin + LPS showed a similar appearance to that from control mice untreated or treated with melatonin.

anti-inflammatory drugs (NSAIDs) are considered the most effective tocolytics, but there are concerns regarding possible fetal and neonatal effects (for review [33]). In our experimental conditions, melatonin prevented preterm labor in 50% of LPS-treated mice. As it has been demonstrated that even at very high concentrations given during pregnancy, melatonin had no maternal or fetal toxicity [34], ongoing studies are in progress to improve the outcome of melatonin by increasing the dose.

There remain to be established the mechanism/s involved in the protection induced by melatonin. Many of melatonin's actions are mediated through interaction with type 1 and type 2 G-protein-coupled membrane melatonin receptors. The expression of these receptors in rodent uterus during estrous cycle and pregnancy [19, 35], human myometrium [36], has been reported. Therefore, it seems likely that the effects of melatonin on LPS-induced preterm delivery may be, at least in part, mediated by specific receptors in mouse uterus. In fact, melatonin prevented cervix opening and bleeding induced by LPS, supporting a local action of melatonin at uterus level. Moreover, uteri from LPS-treated mice had their intersites less defined, which can be considered an index of upcoming expulsion of fetuses, whereas melatonin prevented this effect of LPS. Besides receptor-mediated effects of melatonin, a number of non-receptor-mediated actions of melatonin have been reported, particularly its free radical scavenging action. Melatonin is a noteworthy free radical scavenger and a broad-spectrum antioxidant [9]. Free radical damage is commonplace during pregnancy and has negative effects on the mother, placenta, and fetus [17]. Thus, the antioxidant actions of melatonin could also account for the protective effect of melatonin on preterm delivery. The immediate motivation for addressing the regulation of parturition relates to the importance of the clinical consequences of being born too early. Although survival rates of premature babies have increased in the last decade, there are still high rates of morbidity, even under a tocolytic treatment. Exposure to prenatal inflammation is believed to be an important causal factor in adverse outcomes for children born preterm or at term. Notably, it was recently shown that intrauterine inflammation, which is insufficient to cause parturition, is sufficient to induce fetal injury in both the preterm and term period and that a maternal immune response does not appear necessary for fetal injury to occur [37]. These results could indicate that besides regulating maternal inflammation, additional protective mechanisms are needed to protect fetuses. Melatonin crosses the placenta [38] and the blood–brain barrier [39]; thus, maternally administered, melatonin could reach fetuses and protect them from inflammation. Our results indicate that melatonin may have a dual mechanism of protection, as it not only behaved as a tocolytic agent, but also improved fetal survival. At present, the anatomical locus for the protective effect of melatonin on fetuses has not been identified. However, it was shown that maternal administration of melatonin before and after complete umbilical cord occlusion, abolishes neuronal lipid peroxidation, prevents cerebrovascular instability, reduces astrogliosis and inflammation, and prevents cell death of sheep fetuses, suggesting that melatonin may contribute to fetal development [40], probably acting through receptor- and non-receptor-mediated mechanisms, as mentioned above.

(A)

(C)

(B)

Fig. 4. Effect of melatonin and LPS on uterine PGE_2, $PGF_{2\alpha}$, and COX-2 protein levels. Uterine strips were collected and the production of PGE_2 and $PGF_{2\alpha}$ was measured by RIA, while COX-2 levels were assessed by Western blot. Five hours after the second injection of vehicle or LPS, the production of PGE_2 (panel A), $PGF_{2\alpha}$ (panel B), and COX-2 levels (panel C) was significantly higher in mice treated with LPS in the absence of melatonin. Melatonin, which showed no effect per se, significantly decreased the effect of LPS on these parameters. Data are mean ± S.E.M., n = 8 for both PG and n = 5 for COX-2 analysis. Bars with different superscript letters denote significant differences. $P < 0.05$ by Tukey's test, a ≠ b ≠ c. Representative Western blot analysis of COX-2 level assessment (panel C).
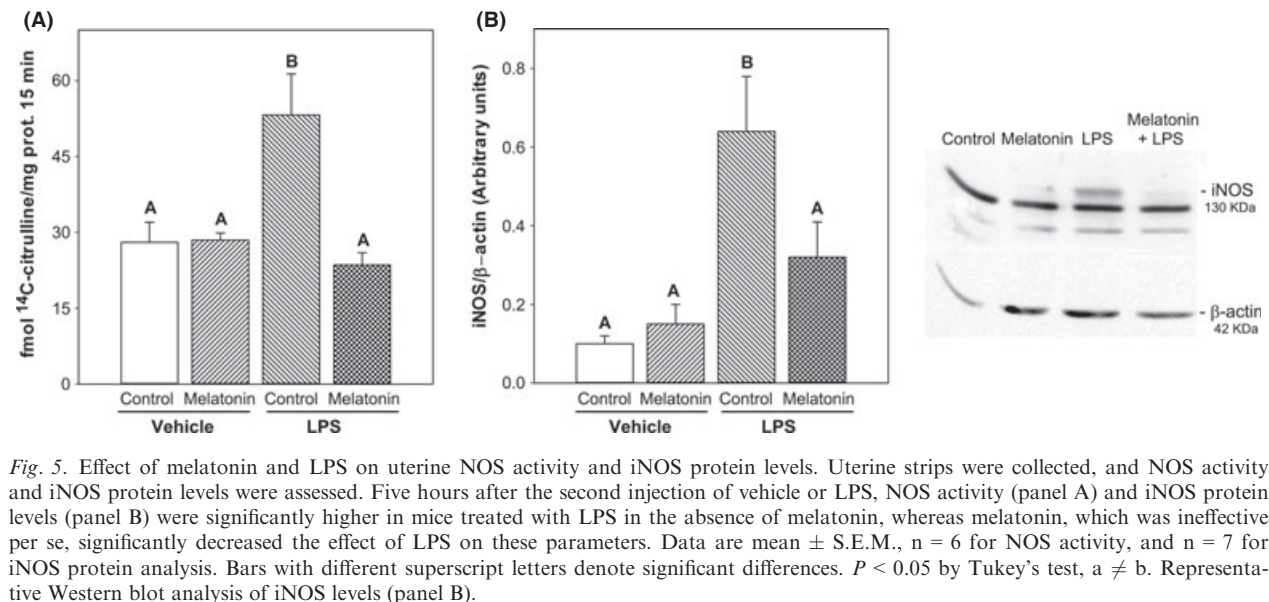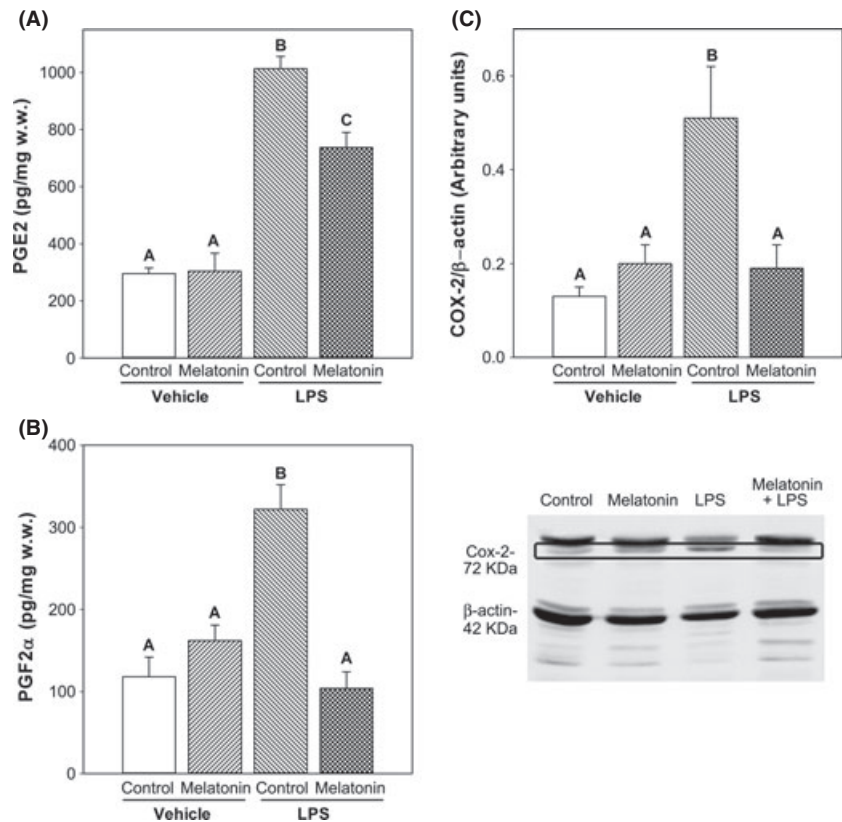
(A)

(B)

Fig. 5. Effect of melatonin and LPS on uterine NOS activity and iNOS protein levels. Uterine strips were collected, and NOS activity and iNOS protein levels were assessed. Five hours after the second injection of vehicle or LPS, NOS activity (panel A) and iNOS protein levels (panel B) were significantly higher in mice treated with LPS in the absence of melatonin, whereas melatonin, which was ineffective per se, significantly decreased the effect of LPS on these parameters. Data are mean ± S.E.M., n = 6 for NOS activity, and n = 7 for iNOS protein analysis. Bars with different superscript letters denote significant differences. $P < 0.05$ by Tukey's test, a ≠ b. Representative Western blot analysis of iNOS levels (panel B).

Although the signals that initiate preterm labor are not completely elucidated, PGs are considered one of the key mediators of preterm labor, as the concentration of biologically active PGs in the amniotic fluid, particularly PGE_2 and $PGF_{2\alpha}$, is significantly higher in women with preterm labor [41]. In addition, PGs are recognized as 'triggers' of labor, because the myometrium contracts in response to exogenous PGs in vivo and in vitro [41].

Augmentation of PGs derived from COX-2 is a crucial signal in both ripening of the cervix and stimulation of uterine contractions [42]. In a previous report, we showed that LPS increases COX-2 derived PGs level [26]. As shown herein, melatonin significantly decreased the effect of LPS on COX-2 protein levels, as well as on PGE_2 and $PGF_{2\alpha}$ content. In agreement, it was shown that melatonin decreases COX-2 activity after middle cerebral artery
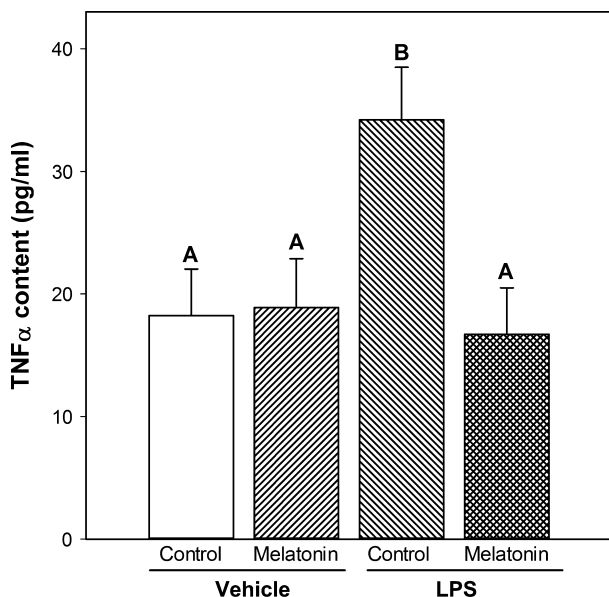
Fig. 6. Effect of melatonin and LPS on uterine TNFα levels. Uterine strips were collected at 2 hr after the second injection of vehicle or LPS, and TNFα levels were measured by ELISA. In the absence of melatonin, LPS significantly increased TNFα levels, whereas melatonin, which was ineffective per se, significantly decreased the effect of LPS. Data are mean ± S.E.M. (n = 9). Bars with different superscript letters denote significant differences. $P < 0.05$ by Tukey's test, a ≠ b.

occlusion [43], in LPS-stimulated rat astrocytoma cells [44], and in human hepatocellular carcinoma [45]. Also, melatonin prevented the increased production of NO and iNOS protein levels following LPS injection. Melatonin and its metabolites have been consistently shown to be effective inhibitors of NOS activity and potent scavengers of NO [14, 43, 46]. The results of the present study are therefore consistent with the attenuation of iNOS induction by melatonin shown in endothelial [46] and placental cells [24].

Pregnancies affected by infection are characterized by elevated levels of pro-inflammatory cytokines, particularly TNFα, in the amniotic fluid, myometrium, fetal membranes, and maternal serum [47]. Melatonin prevented the effect of LPS on TNFα levels in mouse uteri, as previously described in RAW 264.7 cells [48], fetal brain [25], macrophages, and microglia [49]. In particular, it was shown that melatonin prevents the production of TNFα induced by hypoxia/reoxygenation in the villous trophoblast [50].

NFκB is one of the most important transcription factors in transcriptional regulation of inflammatory proteins [51, 52]. Activated NFκB translocates to the nucleus, where it binds to kB binding sites in the promoter regions of target genes, and induces the transcription of pro-inflammatory signals, such as iNOS, TNFα, and COX-2, among others. As melatonin prevented the LPS-induced increase in uterine iNOS, TNFα, and COX-2/PGs levels, it seems likely that the inhibitory action of melatonin upon the effect of LPS is related to NFκB inhibition. In agreement, we have previously shown that melatonin prevents the LPS-induced increase in nuclear levels of NFκB p50 and NFκB

p65 in the hamster retina [53], and it was demonstrated that melatonin inhibits iNOS expression by inhibiting the activation of NFκB in immunostimulated murine macrophages [54] and endothelial cells [46].

As already mentioned, several lines of evidence support the inference that increased NO, PG, and cytokine production plays significant roles in preterm delivery. In fact, we have previously shown that the administration of meloxicam (a COX-2 inhibitor) [26], aminoguanidine (an iNOS inhibitor) [26], or etanercept (a competitive inhibitor of TNFα) [4] prevent inflammation-induced preterm delivery. These and previous results support the notion that the impairment of PG/COX-2 system [55], the decrease in NO levels [26], diminishing cytokine production [56] or preferably the combination of these treatments, may be a therapeutic strategy to prevent preterm labor. Therefore, melatonin could be a promising resource in the management of preterm labor, as by itself, it decreased PGs, NO, and TNFα levels in mouse uteri from animals treated with LPS. Thus, melatonin, a very safe compound for human use, may be considered as a novel tocolytic agent, with the additional capacity of protecting fetuses from alterations induced by inflammation.

Acknowledgements

Author contributions

Ana Paula Dominguez Rubio contributed to concept/design, acquisition of data, and data analysis/interpretation. Micaela Soledad Sordelli, Ana Inés Salazar, Julieta Aisemberg, and María Victoria Bariani performed the acquisition of data. Maximiliano Cella contributed to concept/design and acquisition of data. Ruth E. Rosenstein and Ana María Franchi contributed to concept/design, data analysis/interpretation, drafting of the manuscript, and approval of the article.

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