Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

BBA - Molecular Basis of Disease 1866 (2020) 165914

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadis

Melatonin down-regulates steroidal hormones, thymocyte apoptosis and inflammatory cytokines in middle-aged *T. cruzi* infected rats

Vânia Brazão*, Fabricia Helena Santello, Rafaela Pravato Colato, Andressa Duarte, Amanda Goulart, Pedro Alexandre Sampaio, Viviani Nardini, Carlos Arterio Sorgi, Lúcia Helena Faccioli, José Clóvis do Prado Jr

College of Pharmaceutical Sciences of Ribeirão Preto (FCFRP), University of São Paulo, Ribeirão Preto, SP, Brazil

ARTICLE INFO

Keywords:
Melatonin
Aging
Trypanosoma cruzi
Steroidal hormones
Cytokines
Adrenal

ABSTRACT

Chagas disease, triggered by the flagellate protozoan *Trypanosoma cruzi* (*T. cruzi*) plays a potentially threat to historically non-endemic areas. Considerable evidence established that the immuno-endocrine balance could deeply influence the experimental *T. cruzi* progression inside the host's body. A high-resolution multiple reaction monitoring approach (MRM^{HR}) was used to study the influence of melatonin on adrenal and plasma steroidal hormones profile of *T. cruzi* infected *Wistar* rats. Young (5 weeks) and middle-aged (18 months) male *Wistar* rats received melatonin (5 mg/Kg, orally) during the acute Chagas disease. Corticosterone, 11-dehydrocorticosterone (11-DHC), cortisol, cortisone, aldosterone, progesterone and melatonin concentration were evaluated. Interleukin-1 alpha and β (IL-1α and β), IL-6 and transforming growth factor beta (TGF-β) were also analyzed. Our results revealed an increased production of corticosterone, cortisone, cortisol and aldosterone in middle-aged control animals, thus confirming the aging effects on the steroidal hormone profile. Serum melatonin levels were reduced with age and predominantly higher in young and middle-aged infected rats. Melatonin treatment reduced the corticosterone, 11-DHC, cortisol, cortisone, aldosterone and progesterone in response to *T. cruzi* infection. Decreased IL-1 α and β concentrations were also found in melatonin treated middle-aged infected animals. Melatonin treated middle-aged control rats displayed reduced concentrations of TGF-β. Melatonin levels were significantly higher in all middle-aged rats treated animals. Reduced percentages of early and late thymocyte apoptosis was found for young and middle-aged melatonin supplemented rats. Finally, our results show a link between the therapeutic and biological effects of melatonin controlling steroidal hormones pathways as well as inflammatory mediators.

1. Introduction

Chagas disease is an anthropozoonosis from the Central and South American continent, caused by the protozoan flagellate *Trypanosoma cruzi* (*T. cruzi*) and considered a neglected parasitic illness which has spread from its original boundaries to historically non-endemic areas due to widespread migration [1,2]. Around 6–7 million subjects are infected with *T. cruzi* worldwide. According to the Bulletin of the World Health Organization the increased frequency of *T. cruzi* infected people in the United States, Europe and the Western Pacific Region is evidenced [2]. Chagas disease has become an important threat being considered an extremely debilitating illness, normally linked to poverty, affecting populations with low visibility and exerts a considerable impact on morbidity and mortality with more than 10,000 deaths annually in Latin America [3] Chagas disease has increased incidence in immunocompromised people and elderly population [4].

It is well established that immune cells share receptors for hormones and cytokines through the action of systemic and local regulatory mechanisms. When one of these systems is disturbed by pathogen invasion, the physiological profile of these interactions changes, triggering the release of proinflammatory cytokines and hormones. The immuno-endocrine balance can deeply influence the experimental *T. cruzi* progression inside the host's body [5].

Some available data also reveal that a deregulation of the hypothalamic-pituitary-adrenal (HPA) axis during the acute *T. cruzi* infection induces an immunosuppression that is related to endocrine changes involving a circuit with regulatory properties in which cytokines and hormones produced by the HPA axis play essential role [6].

* Corresponding author. Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto FCFRP-USP, Universidade de São Paulo, Avenida do Café s/n°, 14040-903 Ribeirão Preto, SP, Brazil.
E-mail address: vaniabrazao@gmail.com (V. Brazão).

https://doi.org/10.1016/j.bbadis.2020.165914
Received 17 February 2020; Received in revised form 19 July 2020; Accepted 3 August 2020
Available online 06 August 2020

This immuno-endocrine cross-talk begins when HPA axis stimulation promotes the secretion of pituitary adrenocorticotropic hormone (ACTH) and adrenocortical glucocorticoids (GCs), triggered by the release pro-inflammatory cytokines like IL-6 or IL-1β into the circulation [7,8]. Others data show that glucocorticoids drive changes in the mitochondrial biogenesis [9] interfering with the stress response. Although active glucocorticoids in the circulation are primarily derived from the adrenal gland as part of the HPA axis, they can also be produced from their inactive substrate 11-dehydrocorticosterone (11-DHC) by the enzyme 11β-HSD1 [10] as well in other sites such as thymus, brain and intestinal tract [11]. Additionally, elevated corticosterone levels contribute to the aging process and age-related diseases [12].

Melatonin (*N*-acetyl-5-methoxytryptamine) is a pleiotropic [13] signaling substance with vital role in adjusting the circadian rhythmicity [14,15] and mitochondrial homeostasis [16]. The finding of melatonin synthesis in mitochondria [16] where it likely functions as an effective antioxidant [13,16,17], anti-apoptotic [18], anti-aging [19], oncostatic [20], immunomodulatory [21–23], free radical scavenger [24] and anti-inflammatory, demonstrate that the melatonergic pathways are present in different species and types of cell, such as immune cells out of pinealocytes [25–27]. Confirming this relationship, the presence of MT1 and MT2 melatonin receptors and its necessary biosynthetic machinery for producing this indoleamine in mitochondria has been described [25].

Several works have shown that besides the coordination of several different genes and hormones, melatonin is the main hormone in the regulation of our sleep-wake cycles. Although many conflicting results have been yielded, advanced age is related increased oxidative stress, respiratory functional decline, susceptibility to apoptosis [28] as well as a worse prognosis during the late phase of Chagas disease [29]. As long as age travels, a decay in pineal melatonin concentrations happens [30], triggering alterations in mitochondrial dynamics and immune cells phenotype [31] which leads to an unbalanced healthy homeostasis in aged people [30,32,33], as well as fastening the aging process [34].

The purpose of this study was to investigate the effects of melatonin on the regulation of steroid hormones and cytokine signaling during the development of acute *T. cruzi* infection. These findings have significant functional relevance, since an immunoendocrine imbalance occurs during both aging and Chagas disease. Then, we focused on the quantification of melatonin by Elisa and circulating steroids (corticosterone, 11-dehydrocorticosterone (11-DHC), cortisol, cortisone, aldosterone and progesterone) at tissue and systemic level by using a high-resolution multiple reaction monitoring (MRM^{HR}) - based mass spectrometry approach. The cytokine repertoire, including IL-1α and IL-1β, IL-6 and TGF-β were also evaluated during the development of acute *T. cruzi* infection.

2. Material and methods

2.1. Reagents

The molecular standards cortisol, cortisone, corticosterone, progesterone, aldosterone, cortisone-d8, corticosterone-d4 and formic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). 11-Dehydrocorticosterone (11-DHC) from Steraloids Inc. (Newport, RI, USA). Methanol (MeOH), acetonitrile (ACN), both HPLC grade, and Milli-Q water system were available from Merck (Kenilworth, NJ, USA). For solid-phase extraction, a Waters Extraction Manifold (Milford, MA, USA) was used, and HyperSep C18 cartridges (500 mg sorbent, 2.8 mL) provided by Thermo Fisher Scientific (Bellefonte, PA, USA). RPMI 1640 medium, anti-corticosterone antibody and trypan staining were purchased from Sigma (Sigma-Aldrich, St Louis, MO, USA). The commercial enzyme-linked immunosorbent assay (ELISA) kit for IL-1β (Catalog #RLB00), IL-6 (Catalog # R6000B) were obtained from R&D Systems (Minneapolis, MN, USA). TGF-β (Catalog # MB100B) levels were analyzed using a commercial ELISA Kit (BioLegend, San Diego, CA, USA). Melatonin (Catalog # RE54021) levels were analyzed using a commercial ELISA Kit (IBL, Hamburg, Germany). Fetal bovine serum (FBS) was purchased from Gibco (GIBCO-Life Technologies, Baltimore, MD, USA).

2.2. Animals

All animal assays were designed only after due approbation of the Ethics Committee (number: 15.1.886.609) on Animal Use of the University of São Paulo, *Campus* of Ribeirão Preto, complying with the guiding principles of the National Council for the Control of Animal Experimentation (CONCEA-Brazil). Male *Wistar* rats, 5 weeks (n = 20) to 18 months (n = 20) old, weighing between 100–150 g and 500–600 g respectively, were kept in an accredited animal facility at the College of Pharmaceutical Sciences of Ribeirão Preto (FCFRP). Animals were placed in a regulated environment temperature, between 22 and 24 °C and maintained on a 12 h light-dark cycle, with *ad libitum* access to commercial rodent diet and water.

2.3. Experimental infection, treatment and euthanasia

Male *Wistar* rats were infected with the Y strain of *T. cruzi* (1×10^5 blood trypomastigotes/animal, intraperitoneally). Melatonin treatment (5 mg/kg/day; orally; suspended in 0.1 mL of polyethylene glycol 400 solution) started concomitantly with parasite infection and it was daily maintained until the day of the experiments (9 days post-infection (dpi). Animals were randomly divided into eight groups: young control (no melatonin treated - YC), young melatonin treated (YMC), young *T. cruzi* infected (YI), young *T. cruzi* infected melatonin treated (YMI), middle-aged control (MC), middle-aged melatonin treated (MMC), middle-aged *T. cruzi* infected, (MI) and middle-aged *T. cruzi* infected melatonin treated (MMI). After a short time animals were humanely anaesthetized with tribromoethanol 2.5% by administration of 0.1 mL/10 g of body weight and decapitated (at 9th dpi) for blood, serum and tissue collection for the performance of experimental protocols.

2.4. Tandem mass spectrometry (LC-MS/MS)

Corticosterone and 11DHC from plasma and adrenal were quantified by mass spectrometry (MS) as previously described (Peti et al., 2018). Tissues were homogenized (Mixer Homogenizer, Labortechnik, Wasserburg, Bavaria, Germany) in methanol:water (1:1 v/v), centrifuged, and the supernatant was recovered. Supernatants and plasma were purified as previously described (Galvão et al., 2016). Samples were analyzed using the mass spectrometer TripleTOF 5600$^+$(Sciex, Foster, CA, USA) coupled with the liquid chromatography system Nexera (Shimadzu Corp., Kyoto, Japan). Data were processed using PeakView and MultiQuant software.

2.5. Cytokine assays and melatonin levels

Serum samples were used for the detection IL-1β, IL-6, TGF-β and melatonin levels using two-site sandwich ELISA with monoclonal antibody. Standard curves were used for evaluating cytokine concentrations (pg/mL) read at 450 nm and quantified by using an automated microplate ELISA reader (BIOTEK SYNERGY H1M). MILLIPLEX assay kit (Cat. # RECYTMAG-65K Millipore) and MAGPIX Multiplexing System (MilliporeSigma) was used for the detection of IL-1α.

2.6. Statistical analysis

Graph Pad Prism version 5.0 was used to analyze all data (GraphPad Software, Inc., San Diego, CA, USA; one-way ANOVA with Bonferroni's post test) based on variance difference significance among groups. Significance was assured for $p < 0.05$ (mean ± standard error of the mean (SEM).

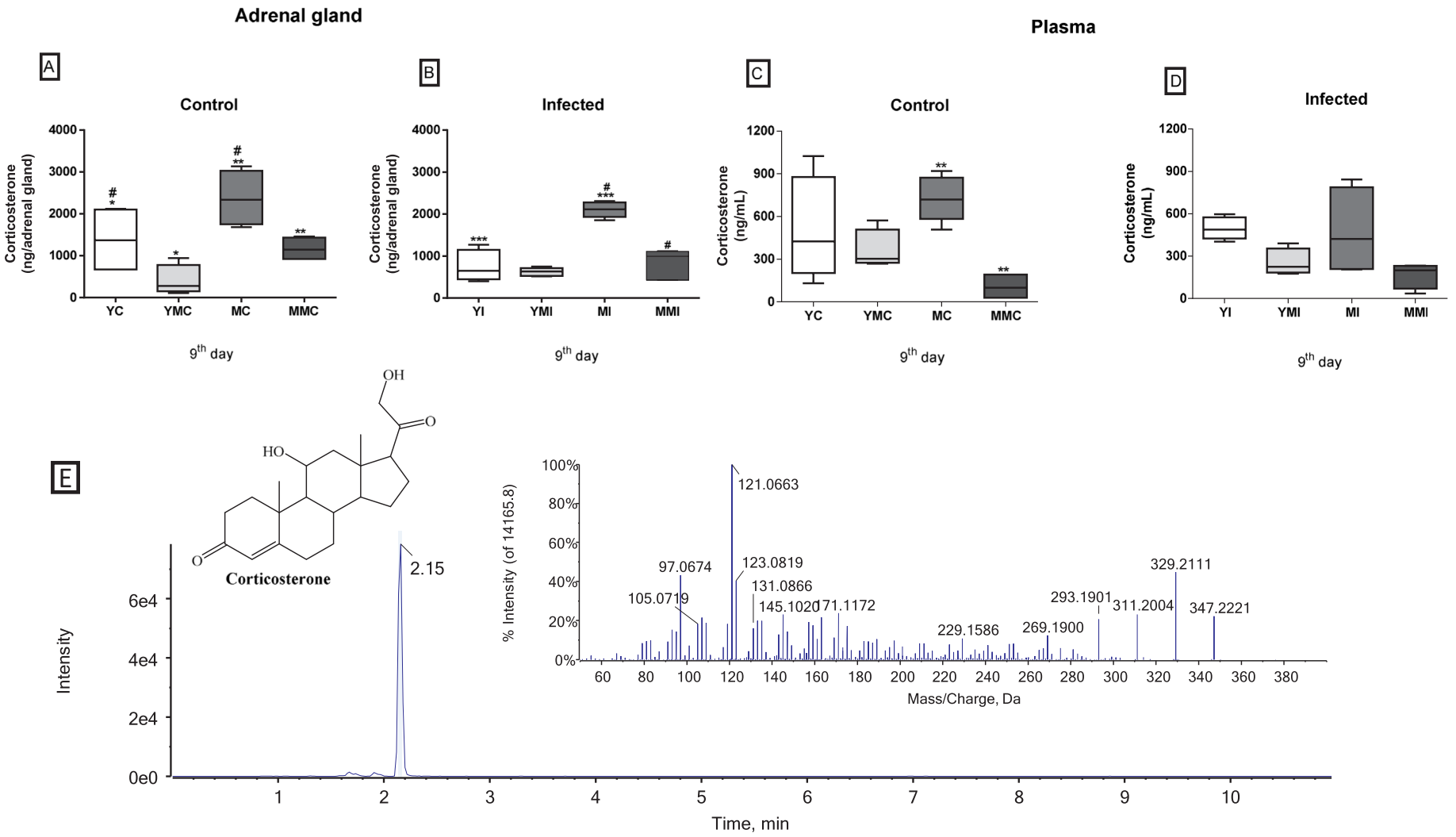

Fig. 1. Corticosterone quantification in adrenal tissue (ng/adrenal gland) and plasma (ng/mL) samples evaluated by MRM^{HR} method through liquid chromatographic tandem mass spectrometry (HPLC-MS/MS), from young and middle-aged *Wistar* rats, on the 9th day post-infection of experimental *T. cruzi* infection, for the following groups: young control (YC), young melatonin control (YMC), young infected (YI), young melatonin infected (YMI), middle-aged control (MC), middle-aged melatonin control (MMC), middle-aged infected (MI) and middle-aged melatonin infected (MMI). (A-B)- Adrenal tissue, (C-D)-Plasma samples, (E)-representative chromatogram retention time and CAD spectra for corticosterone. Results are shown as the means ± SEM of n = 5 to 6 rats. One-way ANOVA followed by Bonferroni's multiple comparison test were used to compare groups (*$P < 0.05$).

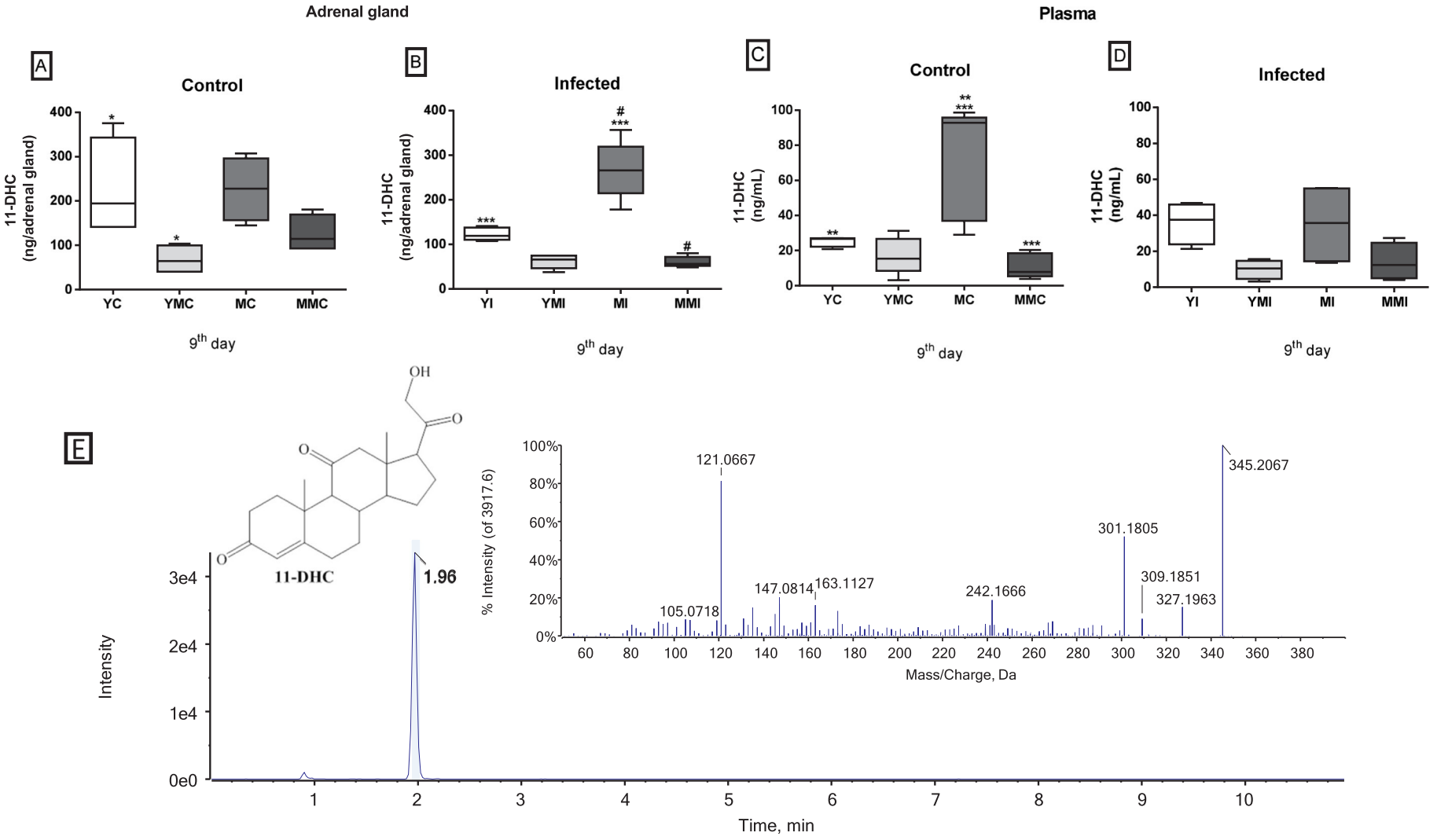

Fig. 2. 11-DHC quantification in adrenal tissue (ng/adrenal gland) and plasma (ng/mL) samples evaluated by MRM^{HR} method through liquid chromatographic tandem mass spectrometry (HPLC-MS/MS), from young and middle-aged *Wistar* rats, on the 9th day post-infection of experimental *T. cruzi* infection, for the following groups: young control (YC), young melatonin control (YMC), young infected (YI), young melatonin infected (YMI), middle-aged control (MC), middle-aged melatonin control (MMC), middle-aged infected (MI) and middle-aged melatonin infected (MMI). (A-B)- Adrenal tissue, (C-D)-Plasma samples, (E)-representative chromatogram retention time and CAD spectra for 11-DHC. Results are shown as the means ± SEM of n = 5 to 6 rats. One-way ANOVA followed by Bonferroni's multiple comparison test were used to compare groups (*$P < 0.05$).

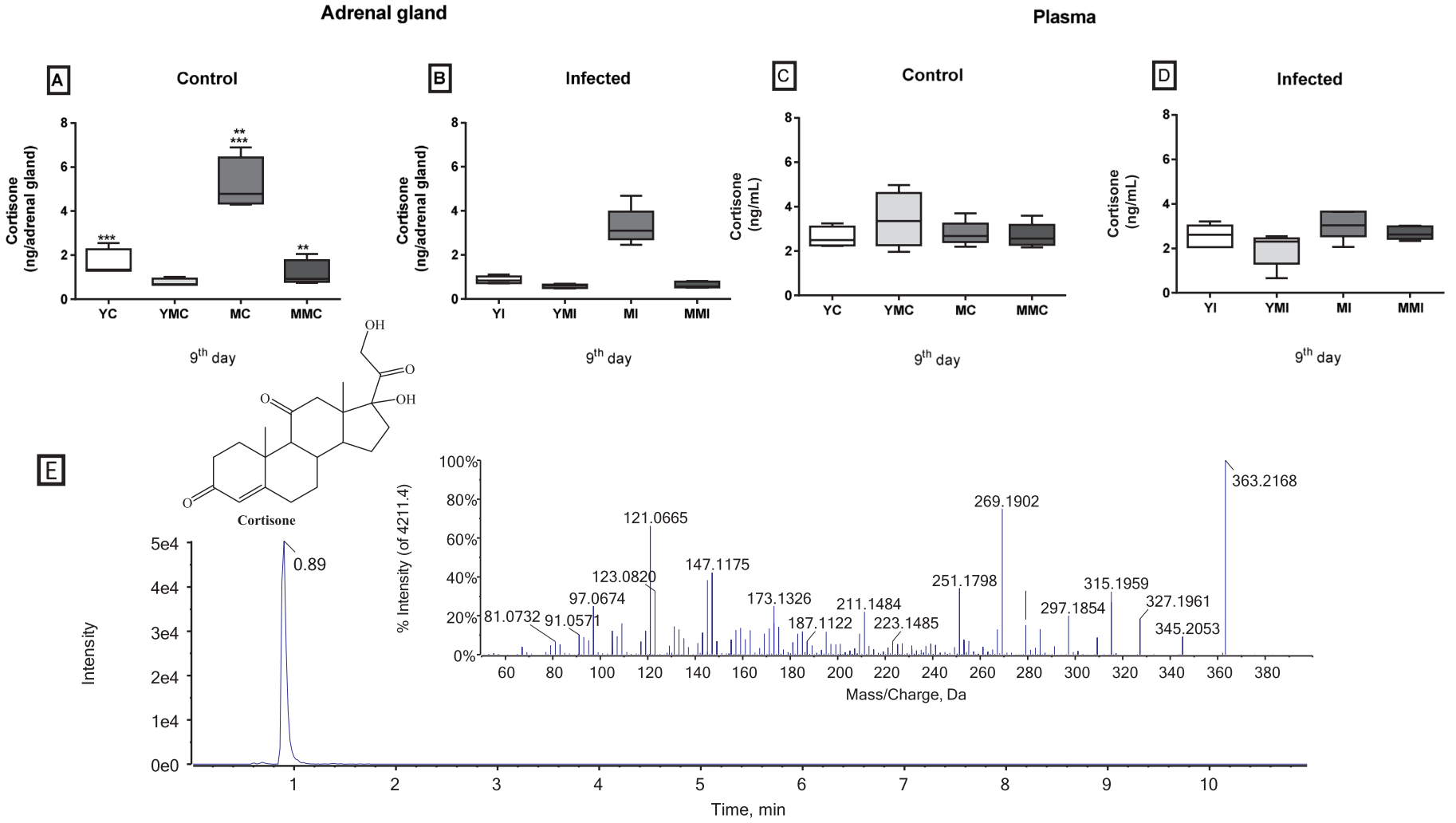

Fig. 3. Cortisone quantification in adrenal tissue (ng/adrenal gland) and plasma (ng/mL) samples evaluated by MRM^{HR} method through liquid chromatographic tandem mass spectrometry (HPLC-MS/MS), from young and middle-aged *Wistar* rats, on the 9th day post-infection of experimental *T. cruzi* infection, for the following groups: young control (YC), young melatonin control (YMC), young infected (YI), young melatonin infected (YMI), middle-aged control (MC), middle-aged melatonin control (MMC), middle-aged infected (MI) and middle-aged melatonin infected (MMI). (A-B)- Adrenal tissue, (C-D)-Plasma samples, (E)-representative chromatogram retention time and CAD spectra for cortisone. Results are shown as the means ± SEM of n = 5 to 6 rats. One-way ANOVA followed by Bonferroni's multiple comparison test were used to compare groups (*$P < 0.05$).

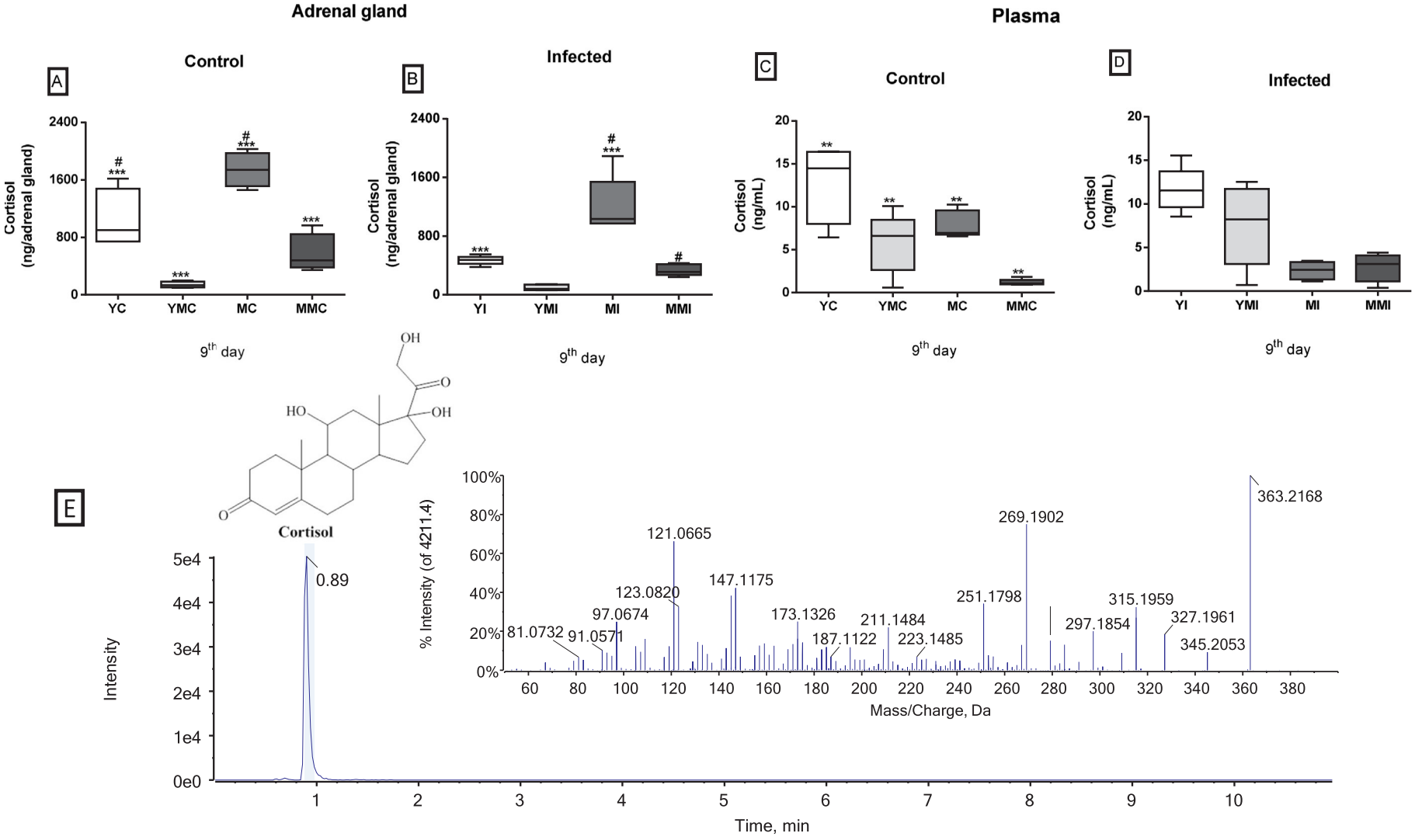

Fig. 4. Cortisol quantification in adrenal tissue (ng/adrenal gland) and plasma (ng/mL) samples evaluated by MRM^{HR} method through liquid chromatographic tandem mass spectrometry (HPLC-MS/MS), from young and middle-aged *Wistar* rats, on the 9th day post-infection of experimental *T. cruzi* infection, for the following groups: young control (YC), young melatonin control (YMC), young infected (YI), young melatonin infected (YMI), middle-aged control (MC), middle-aged melatonin control (MMC), middle-aged infected (MI) and middle-aged melatonin infected (MMI). (A-B)- Adrenal tissue, (C-D)-Plasma samples, (E)-representative chromatogram retention time and CAD spectra for cortisol. Results are shown as the means ± SEM of n = 5 to 6 rats. One-way ANOVA followed by Bonferroni's multiple comparison test were used to compare groups (*$P < 0.05$).

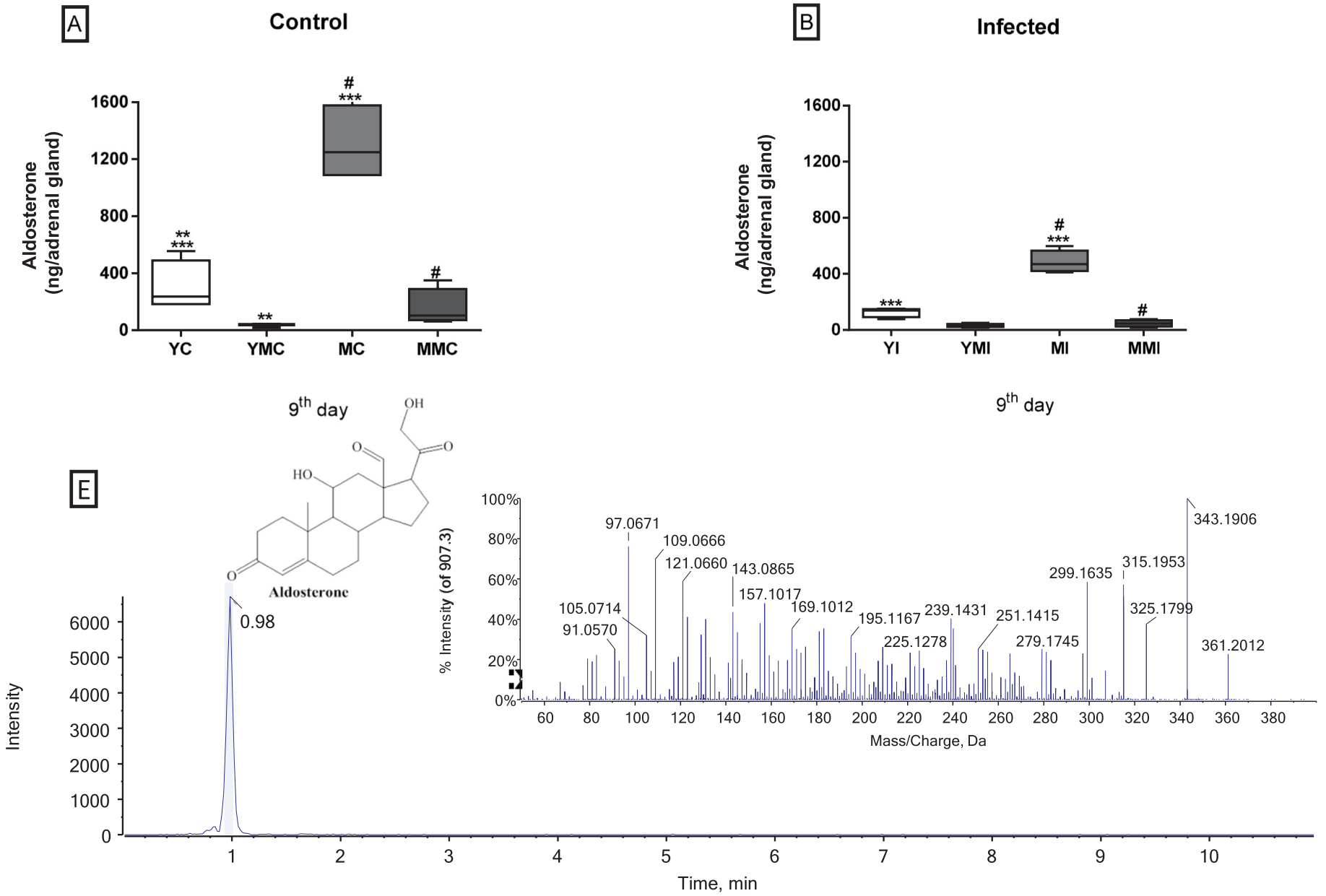

Fig. 5. Aldosterone quantification in adrenal tissue (ng/adrenal gland) and plasma (ng/mL) samples evaluated by MRM^{HR} method through liquid chromatographic tandem mass spectrometry (HPLC-MS/MS), from young and middle-aged *Wistar* rats, on the 9th day post-infection of experimental *T. cruzi* infection, for the following groups: young control (YC), young melatonin control (YMC), young infected (YI), young melatonin infected (YMI), middle-aged control (MC), middle-aged melatonin control (MMC), middle-aged infected (MI) and middle-aged melatonin infected (MMI). (A-B)- Adrenal tissue, (C-D)-Plasma samples, (E)-representative chromatogram retention time and CAD spectra for aldosterone. Results are shown as the means ± SEM of n = 5 to 6 rats. One-way ANOVA followed by Bonferroni's multiple comparison test were used to compare groups (*$P < 0.05$).

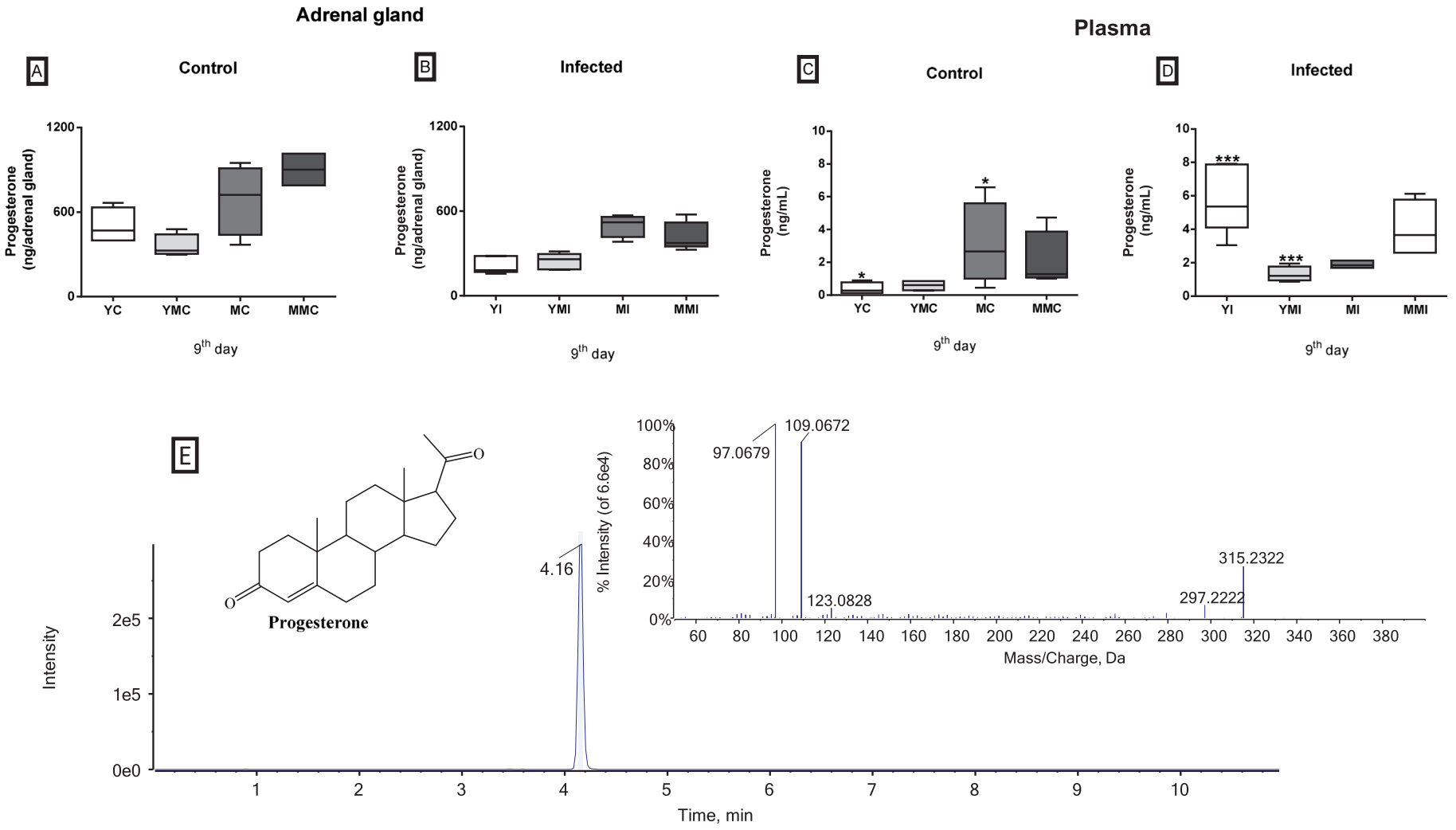

Fig. 6. Progesterone quantification in adrenal tissue (ng/adrenal gland) and plasma (ng/mL) samples evaluated by MRM^{HR} method through liquid chromatographic tandem mass spectrometry (HPLC-MS/MS), from young and middle-aged *Wistar* rats, on the 9th day post-infection of experimental *T. cruzi* infection, for the following groups: young control (YC), young melatonin control (YMC), young infected (YI), young melatonin infected (YMI), middle-aged control (MC), middle-aged melatonin control (MMC), middle-aged infected (MI) and middle-aged melatonin infected (MMI). (A-B)- Adrenal tissue, (C-D)-Plasma samples, (E)-representative chromatogram retention time and CAD spectra for progesterone. Results are shown as the means ± SEM of n = 5 to 6 rats. One-way ANOVA followed by Bonferroni's multiple comparison test were used to compare groups (*$P < 0.05$).

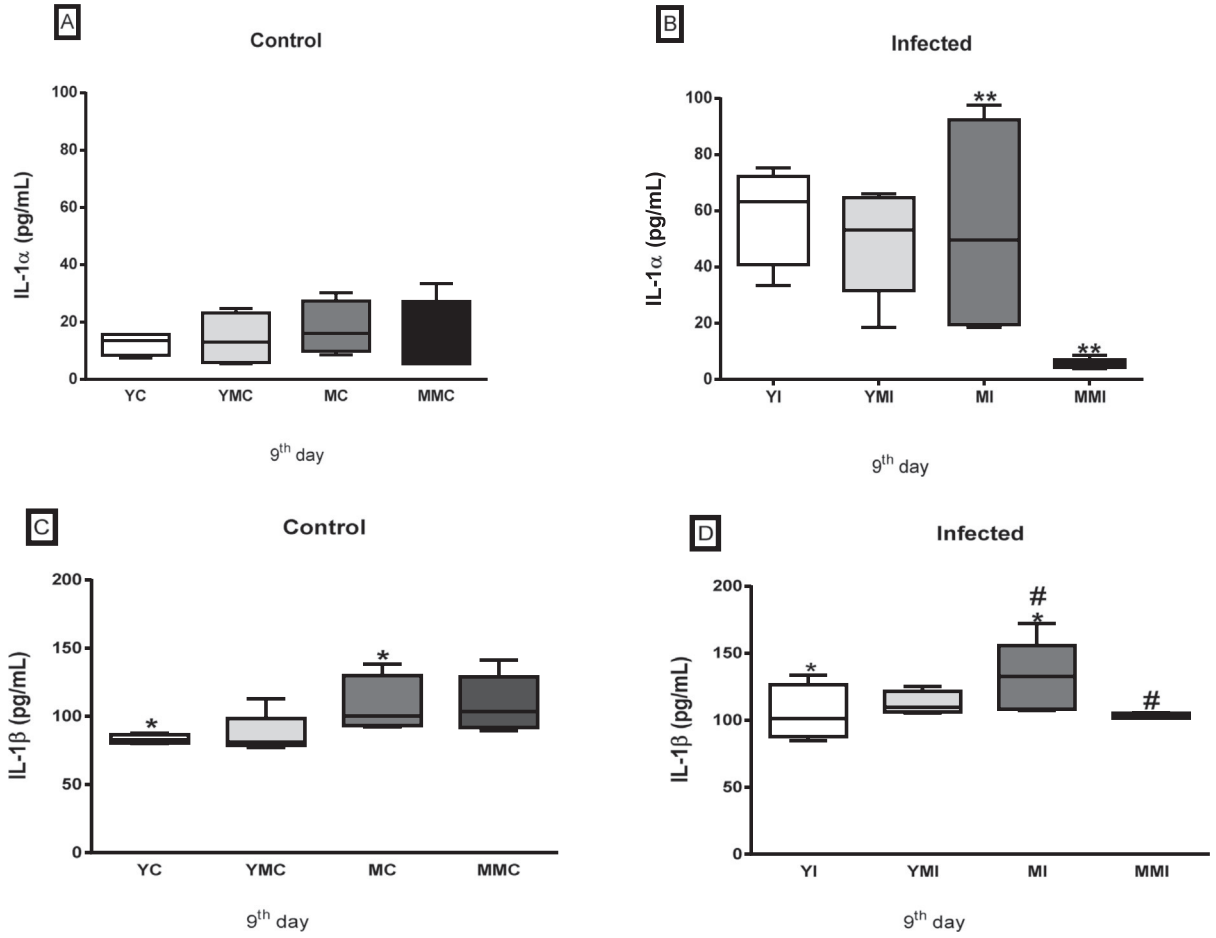

Fig. 7. IL-1α levels (pg/mL), were measured by MILLIPLEX assay (A-B) and IL-1β levels (pg/mL), measured by ELISA (C-D), after melatonin treatment, from young and middle-aged *Wistar* rats, on the 9th day post-infection of experimental *T. cruzi* infection, for the following groups: young control (YC), young melatonin control (YMC), young infected (YI), young melatonin infected (YMI), middle-aged control (MC), middle-aged melatonin control (MMC), middle-aged infected (MI) and middle-aged melatonin infected (MMI). (A) Control groups and (B) Infected groups. Results are shown as the means ± SEM of n = 5 to 6 rats. One-way ANOVA followed by Bonferroni's multiple comparison test was used to compare groups (*$P < 0.05$).

3. Results

Studies in rats have demonstrated that during *T. cruzi* infection proinflammatory mediators such as IL-1α and IL-6 trigger the stimulation of the HPA axis [6]. Only one paper describe the relationship of melatonin and steroidal hormones [35], and for experimental Chagas disease nothing is known concerning to the role of this indoleamine upon the systemic and tissue levels of steroidal hormones such as 11-DHC, cortisol, cortisone, aldosterone and progesterone.

The MRM^{HR} method is one of accurate method of analysis through liquid chromatographic tandem mass spectrometry for evaluation of low-concentration metabolites in biological samples. This method was applied in the adrenal tissue and plasma samples of rats and allows the quantification of lower steroid hormones concentrations simultaneously with precision and accuracy.

To get some insight into the ability of melatonin to modulate the hormonal response of *T. cruzi* infected rats, we evaluated the production of corticosterone (Fig. 1), 11-DHC (Fig. 2), cortisone (Fig. 3), cortisol (Fig. 4) and aldosterone (Fig. 5) and progesterone (Fig. 6) in young and middle-aged animals. Treatment with melatonin reduced the basal levels of these hormones in all middle-aged melatonin treated rats, infected or not, as compared to their untreated groups. However, the plasma levels of corticosterone (Fig. 1D), 11-DHC (Fig. 2D), cortisone (Fig. 3D), cortisol (Fig. 4D) did not significantly decline among all infected melatonin-treated animals. On the other hand, reduced concentrations of progesterone were induced by melatonin, for juvenile and infected subjects (Fig. 6D).

Our results confirm that the aging affects the steroidal hormone profile, since an increased production of corticosterone (Fig. 1A), 11-DHC (Fig. 2C), cortisone (Fig. 3A), cortisol (Fig. 4A), aldosterone (Fig. 5A) and progesterone (Fig. 6C) was observed in middle-aged control animals as compared to young ones. Corticosterone (Fig. 1C), 11-DHC (Fig. 2C), cortisone (Fig. 3C) and cortisol (Fig. 4C) concentrations were significantly lower in plasma from uninfected middle-aged melatonin treated animals. *T. cruzi* infection down-regulated the adrenal production of cortisone (Fig. 3A–B), cortisol (Fig. 4A–B) and aldosterone (Fig. 5A–B) in middle-aged infected rats, as compared to their respective uninfected counterparts.

The next step, the pro-inflammatory cytokines involved in the HPA regulation: IL-1α, IL-1β, IL-6 and TGF- β were evaluated. A significant increase in IL-1α (Fig. 7A and B) concentrations was observed for infected middle-aged and young rats, when compared with the control ones. As expected, the immunosenescence process was linked to elevated levels of IL-1 β (Fig. 7C and D) and IL-6 (Fig. 8A and B) as found for all middle-aged rats as compared to juvenile ones. We further examine if melatonin's actions were related with alterations in the kinetics of cytokine output during the early *T. cruzi* infection. Our results demonstrated reduced IL-1α and IL-1β production, a key cytokine that has been entangled with chagasic cardiac hypertrophy, for middle-aged infected melatonin supplemented rats (Fig. 7B and D). Melatonin treated middle-aged rats displayed lower levels of IL-6, excepting the young ones (Fig. 8A and B). Reduced concentrations of TGF-β were

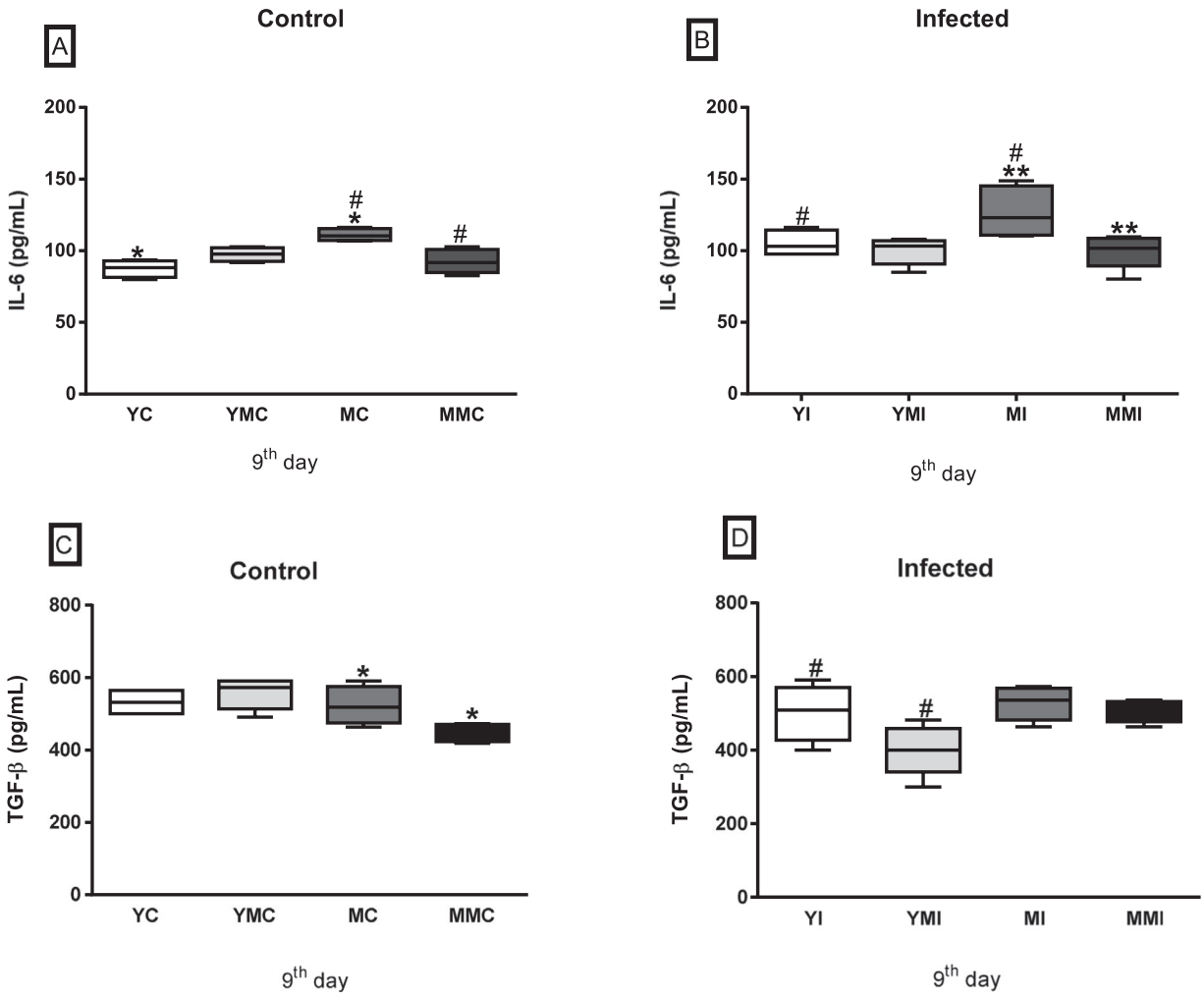

Fig. 8. IL-6 levels (A-B) and TGF-β (C-D) (pg/mL) were measured by ELISA, after melatonin treatment, from young and middle-aged *Wistar* rats, on the 9th day post-infection of experimental *T. cruzi* infection, for the following groups: young control (YC), young melatonin control (YMC), young infected (YI), young melatonin infected (YMI), middle-aged control (MC), middle-aged melatonin control (MMC), middle-aged infected (MI) and middle-aged melatonin infected (MMI). (A) Control groups and (B) Infected groups. Results are shown as the means ± SEM of n = 5 to 6 rats. One-way ANOVA followed by Bonferroni's multiple comparison test was used to compare groups (*$P < 0.05$ and **$P < 0.01$).

triggered for young infected rats when compared to the untreated ones (Fig. 8D). Melatonin treated middle-aged control groups displayed reduced concentrations of TGF-β (Fig. 8C).

Since, an important decay in endogenous melatonin synthesis occurs with aging, systemic levels of this hormone were quantified in young and middle-aged groups. Serum melatonin levels were reduced with age and predominantly higher in young and middle-aged infected rats compared to control animals (Fig. 9). Melatonin levels were significantly higher in all middle-aged rats (control or infected) supplemented with melatonin, as compared to untreated ones.

The anti-apoptotic abilities of melatonin were evidenced in our study. Early and late thymocyte apoptosis from all middle-aged melatonin-treated animals were statistically reduced as compared to non-supplemented ones respectively (Fig. 10A and B). However, as shown in Fig. 10, the percentages of early (A) and late thymocyte apoptosis (B) did not significantly decline among all young melatonin-treated animals. Significantly higher proportions of both early (A) and late apoptotic thymocytes (B) were displayed for all middle-aged animals, as compared to young counterparts (Fig. 9) triggered by the aging process.

The percentage of viable thymocytes in all young and middle-aged melatonin treated infected rats, was statistically enhanced when compared to untreated counterparts (Fig. 10C). Oppositely, a significant drop in the proportion of viable thymocytes in middle-aged control animals was observed as compared to young groups (Fig. 10).

4. Discussion

A bulk of evidence has shown that altered steroidal hormone production might be related to a number of stress-induced disorders such as major depression, stress/trauma, hypertension, immune suppression and septic shock [36–38]. Although the exact underlying complex mechanisms of the Chagas disease pathology are still unveiled, the coexistence of endocrine and immunological disturbances occurs during the early *T. cruzi* infection although the mechanisms are not well established [3,39]. Then, herein our aim was the quantification of the steroidal hormones in a sensitive and specific way, using the MRMHR method, and explores the impact of melatonin supplementation on cytokine production and steroidal hormones fractions during the early *T. cruzi* infection.

Aging process is associated with functional changes in the nervous, endocrine and immune systems [40]. Mitochondrial dysfunction also appears to contribute to progression of the aging process leading to defects in the steroidogenesis. As we age, morphological and functional changes of the adrenal gland occur leading to alterations in hormonal output and decline in adrenal androgen synthesis, although increased

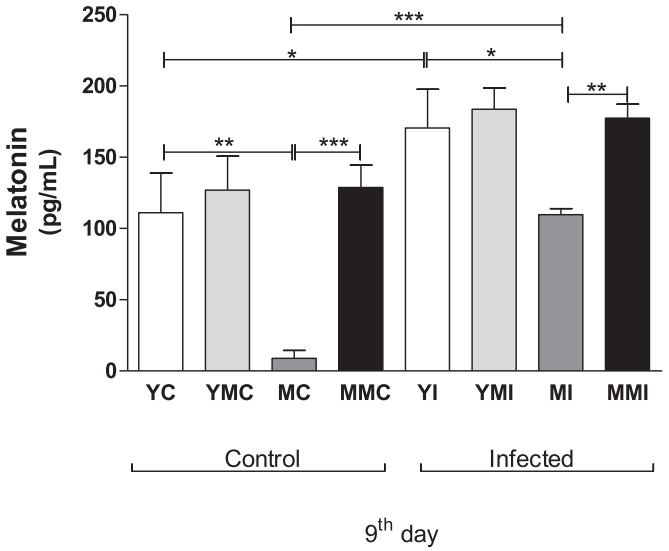

Fig. 9. Melatonin (pg/mL) levels were measured by ELISA, after melatonin treatment, from young and middle-aged *Wistar* rats, on the 9th day post-infection of experimental *T. cruzi* infection, for the following groups: young control (YC), young melatonin control (YMC), young infected (YI), young melatonin infected (YMI), middle-aged control (MC), middle-aged melatonin control (MMC), middle-aged infected (MI) and middle-aged melatonin infected (MMI). Results are shown as the means ± SEM of n = 5 to 6 rats. One-way ANOVA followed by Bonferroni's multiple comparison test was used to compare groups (*$P < 0.05$).

expression and activity of enzymes related to glucocorticoid synthesis occurs, promoting an immunoendocrine imbalance, with adrenal hypertrophy and cortisol hypersecretion [41] While the adrenal cortisol concentration increases with the aging process, a reduced melatonin release by pineal gland is evident, being cortisol considered by some authors as an antagonist mediator for pineal melatonin release. In line with this, our data show that serum melatonin levels were reduced in middle aged animals as compared to young ones.

Since in rodents stress responses are controlled by corticosterone production. Some researchers have described enhanced plasmatic cortisol concentrations and hypertrofic adrenal glands in mice, after a stress episode. Other papers have considered cortisol as a key for stress activation in both mice [42] and rats [43]. Furthermore, cortisol displays enhanced glucocorticoid potency when compared to corticosterone [44].

Interestingly, independent corticosterone secretion was observed in rat testis out of the HPA axis bustle from progesterone [45]. This helps to explain how the higher age-related corticosterone concentrations, may be due to the elevated availability of progesterone, which can be correlated with the individual concentrations of progesterone and corticosterone. Lymphoid organs are able to produce locally corticosterone through the action of the inactive metabolite, from 11-DHC to 11β-HSD1, being this last compound normally elevated in aged animals [46]. Furthermore, physiological investigations have also shown aldosterone autonomous secretion in age-related subjects leading to enhanced risk of developing cardiovascular diseases [47]. Interestingly, this pattern of steroidal composition during the aging process parallels our findings. We found that the adrenal production of corticosterone, cortisone, cortisol and aldosterone were markedly increased in middle-aged control animals as compared to young ones. Furthermore, increased plasma concentrations of 11-DHC and progesterone were induced by aging process, as observed for middle-aged rats as compare to young counterparts.

An increase in the secretion of corticosterone induced by *T. cruzi* drive a systemic inflammation which is closely correlated with hypertrophic adrenal glands, specially the fasciculate zone, and thymic alterations in infected animals [48]. Yet, Villar et al. have reported that patients with cardiac forms of chronic Chagas disease have higher adrenal expression of several steroidogenic enzymes, including cytochrome P450, family 11, subfamily A, polypeptide 1 (CYP11A1), CYP11B1, 11β-hydroxysteroid dehydrogenase type 1 (HSD1), and steroidogenic acute regulatory protein [48]. Chumbinho et al. (2012) also described that the mortality rate in *T. cruzi* infected mice was under the direct influence of aldosterone. According to the same authors, aldosterone blockage with spironolactone (an aldosterone antagonist) attenuates the parasite load and reduces the severity of Chagas cardiomyopathy [49]. Currently is also known that a prolonged aldosterone treatment (between 4 and 5 weeks) triggers enhanced oxidative stress production and accounts for a proinflammatory phenotype. Although the exact role of melatonin on aldosterone production has remained controversial, we found that melatonin treatment decreased the adrenal aldosterone production in all treated rats. Our findings demonstrate that the adrenal steroid production (cortisone, cortisol and aldosterone) in middle-aged and infected rats was worsened with a significant reduction of these adrenal hormones as compared to their respective uninfected counterparts. Interestingly, *T. cruzi* infection *per si* triggered significant increased systemic levels of melatonin in both young and middle-aged infected animals as compared to uninfected ones. In infected animals the immune system orchestrates a cellular and humoral response and all these cells display receptors for this indoleamine, being able to produce this hormone. Then, we propose that melatonin enhanced levels has a wide range of inducers, such as seasonal period, immune response, infection and animal species.

An adequate melatonin supplementation, even if started on late life, has been investigated for their potential to reversibly counteract the age-related impairment of thymopoiesis and immune dysfunctions in old animals [50]. Cortisol secretion is inhibited by melatonin's action [51], indicating that there is an upside down relationship between circadian cortisol and melatonin cycles [52]. Yet, these inhibitory effects were reversed when cells were co-treated with the MT1/MT2 antagonist luzindole, suggesting the presence of functional melatonin receptors in the adrenal cortex [51]. However, nothing is known about endogenous 11-DHC levels and melatonin treatment. Our findings demonstrated that the exogenous administration of melatonin exerts an inhibitory effect on the adrenal secretion of corticosterone, 11-DHC, cortisone and cortisol in elderly *T. cruzi* infected rats. Longer melatonin schedules in *T. cruzi*-infected rats trigger enhanced serum concentrations of this indoleamine, specially in middle-aged groups.

It is well explained that the exposure to glucocorticoids and hydroxyl radical induce thymocyte apoptosis, and melatonin treatment reverts this process [53,54]. Sainz et al. described that melatonin treatment triggered a reduction in pro-apoptotic markers in the cortex of the murine thymus [55]. A plethora of *in vivo* and *in vitro* experiments conclusively demonstrate that melatonin can protect primary lymphoid organs against apoptosis through a mechanism that is dependent of its anti-apoptotic properties [54] and its ability to modulate cell proliferation [50]. Another potential theory to explain melatonin's anti-apoptotic effects is its role in the down regulation on the glucocorticoid receptor in thymocytes [53] as well as the antioxidant properties of this indoleamine. Our experiments support the previously reported, since we found that early and late apoptotic thymocytes were reduced in *T. cruzi* middle-aged melatonin supplemented groups.

Glucocorticoids have been reported as the central mediators of thymocyte apoptosis during *T. cruzi* infection, worsening thymocyte depletion probably due to enhanced glucocorticoid levels [3,6]. Furthermore, some papers describe that the age-related deregulation in pinealocytes and thymocytes is linked with enhanced mortality percentages triggered by distinct pathologies such as infectious diseases, cancer, and autoimmunity, as well as reduced vaccine response in the elderly [5,56–58]. Over a lifetime, increased apoptosis in various cell types occurs. For animal models, thymocyte apoptosis has also been described for older mice [59,60] and the most significant changes are

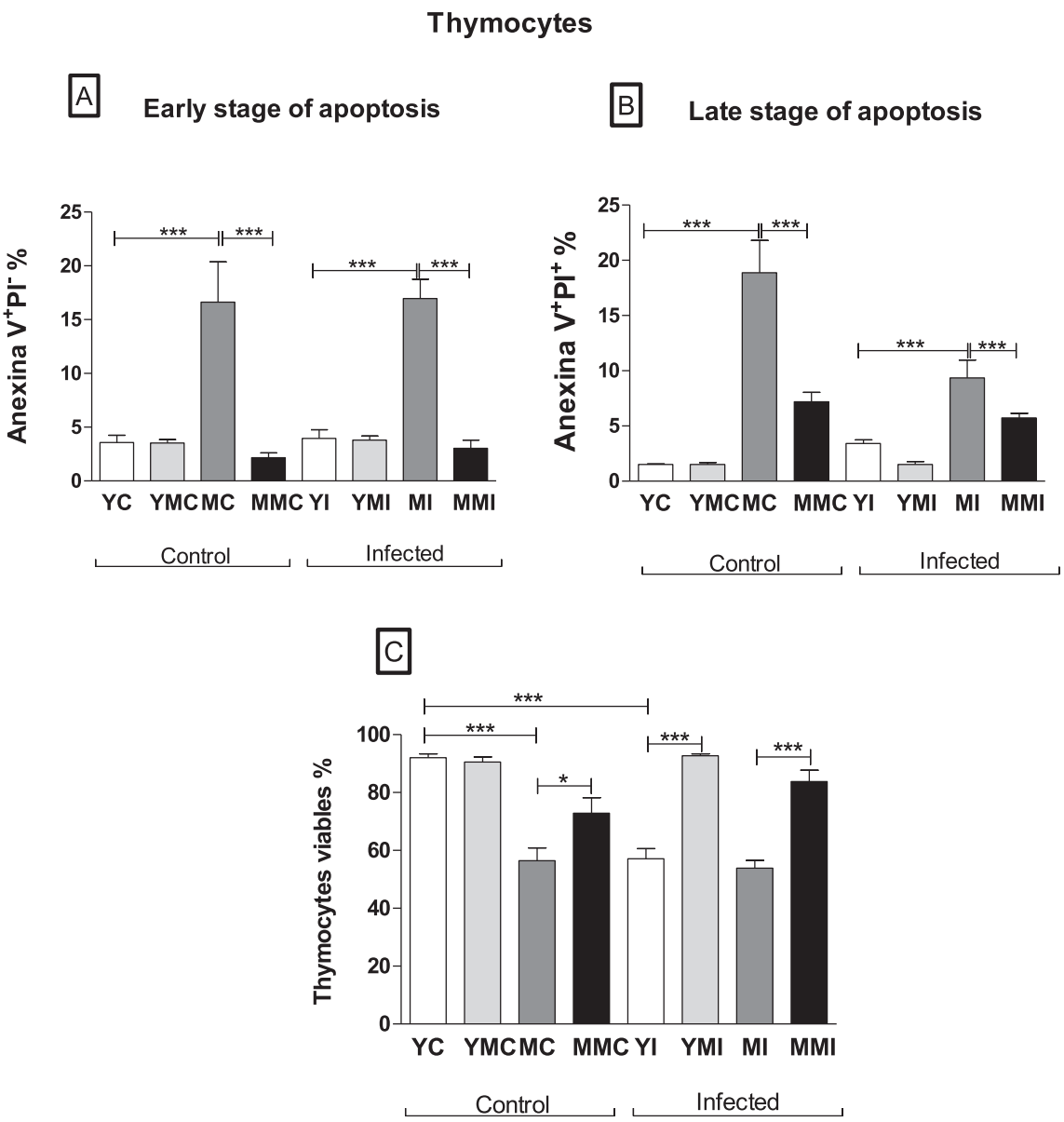

Fig. 10. Thymocyte apoptosis was measured by double staining with FITC-labeled annexin V and PI in young and middle-aged *Wistar* rats, on the 9th day post-infection of experimental *T. cruzi* infection, for the following groups: young control (YC), young melatonin control (YMC), young infected (YI), young melatonin infected (YMI), middle-aged control (MC), middle-aged melatonin control (MMC), middle-aged infected (MI) and middle-aged melatonin infected (MMI). Data are expressed as: (A) percentages (%) of early apoptotic cells (annexin V+/PI−), (B) late apoptotic cells (annexin V+/PI+) (%), (C) cell viability of thymocytes (%). Results are shown as the means ± SEM of n = 5 to 6 rats. One-way ANOVA followed by Bonferroni's multiple comparison test was used to compare groups (*$P < 0.05$).

found in the thymic cortex [61]. In line with this, for all middle-aged animals, we found higher proportions of both early and late apoptotic thymocytes, as compared to young counterparts. Furthermore, reduced rates of thymocyte viability for infected animals and for middle-aged control groups was evidenced.

Overproduction of systemic cytokines and pro-inflammatory markers, such as IL-1β and IL-6 [62–65] are a common phenomenon during the aged-related changes and may be linked to metabolic and pathophysiological changes that occur during different acute inflammatory diseases [66]. Upon mitochondrial dysfunction, several pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α are produced by endothelial cells, consequently up-regulating intercellular adhesion molecule-1 (ICAM-1) expression which attracts monocyte activation and adhesion [67] For a well orchestrated stress response, mitochondria play an essential role to provide energy being totally integrated to the HPA axis. Studies carried out in the naturally found old male senescence-accelerated murine model have confirmed elevated IL-1β, IL-6 and TNF-α levels in aged animals. Additionally, a hyper response of the HPA axis, through the activity of a wide range of immune cell mediators, including IL-1β and IL-6 is also observed during acute Chagas' disease [68]. A close connection between *T. brucei* infection in humans and the higher production of IL-6 has been already described [69,70]. Enhanced IL-6 gene expression has been observed after *in vitro T. cruzi* infection of ACTH-producing cells [71].

Notably, experimental assays have found a relationship between IL-6 and the induction of heighten concentrations of glucocorticoid and ACTH [72,73]. Dinkel et al. (2003) reported that chronically higher glucocorticoid concentrations in rats are correlated with increased IL-1β and TNF-α mRNA expressions. Interestingly, it has been postulated that the higher expression of nuclear factor-κB (NF-κB), mitogen-activated kinase (MAPK) as well as pro-inflammatory cytokines could be a consequence of the enhanced corticosterone concentrations, which lead

to sustain the inflammatory state [74]. Another study demonstrates that chronic chagasic patients have a series of important metabolic and hormonal abnormalities directly correlated with the increased IL-6 concentrations [75]. In line with these earlier studies, we also demonstrated the regulatory properties of IL-1β and IL-6 during aging and *T. cruzi* infection where a significant increased serum levels of these cytokines were found in middle-aged rats.

Melatonin has a protective ability, inhibiting long-term changes in inflammatory responses at different levels [76], thereby reducing the production of several pro-inflammatory immune mediators, including leukotrienes, prostanoids, cytokines and adhesion molecules. Furthermore, after melatonin administration in old mice, reduced concentrations of the Th-1 cytokines profile and elevated IL-10 concentrations were found [77,78]. Attaining similar kind of response in a rat model of heart stroke submitted to melatonin therapy, it was demonstrated that melatonin triggered a reduced inflammation reaction besides protecting against multi-organ injury as a result from severe heat exposure [79]. Peng et al. have evidenced that after melatonin administration, an important reduction in the output of inflammatory cells, neutrophils and IL-1β in the bronchoalveolar lymphoid tissue occur [80]. Furthermore, Shin et al. demonstrated that in lung tissue exposed to lipopolysaccharide, melatonin treatment downregulated the expression of TGF-β1 [81]. Our findings constitute the first report of reduced serum IL-1β and IL-6 levels in middle-aged *T. cruzi* infected rats under melatonin therapy. Melatonin also exerts a negative influence on TGF-β production, with reduced levels for young infected and middle-aged control rats.

In summary, for the first time our compelling data clearly showed the age-related changes in adrenal and plasma steroidal hormones profiling as well cytokine production in *Wistar* rats confirming the efficiency of melatonin intervention in the regulation and signaling hormonal pathways and inflammatory mediators.

CRediT authorship contribution statement

We hereby certify that it is an original publication and the manuscript has not been previously submitted or published elsewhere. VB, FS and RC: participated in the conception and design of the study. AD, AG, PS and VN: performed the analysis. CS, LF, JP and VB: helped in drafting of the manuscript. All authors have made substantial contributions and final approval of the conceptions, drafting, and final version.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Cristiana Gonçalez for technical support and Ms. Fabiana Rossetto de Morais of Faculty of Pharmaceutical Sciences of Ribeirão Preto (FCFRP), University of São Paulo for the FACS data analysis. This study was supported by fellowships from FAPESP 2015/09806-3.

References

[1] E.A. Bocchi, G. Guimarães, F. Tarasoutshi, G. Spina, S. Mangini, F. Bacal, Cardiomyopathy, adult valve disease and heart failure in South America, Heart. 95 (3) (2009) 181–189, https://doi.org/10.1136/hrt.2008.151225.

[2] E.A. Bocchi, R.B. Bestetti, M.I. Scanavacca, E. Cunha Neto, V.S. Issa, Chronic Chagas heart disease management: from etiology to cardiomyopathy treatment, J. Am. Coll. Cardiol. 70 (12) (2017) 1510–1524, https://doi.org/10.1016/j.jacc.2017.08.004.

[3] E. Roggero, A. Perez, M. Tamae-Kakazu, et al., Differential susceptibility to acute Trypanosoma cruzi infection in BALB/c and C57BL/6 mice is not associated with a distinct parasite load but cytokine abnormalities, Clin. Exp. Immunol. 128 (3) (2002) 421–428.

[4] A.A. Felizardo, D.V.B. Marques, I.S. Caldas, R.V. Gonçalves, R.D. Novaes, Could age and aging change the host response to systemic parasitic infections? A systematic review of preclinical evidence, Exp. Gerontol. 104 (2018) 17–27, https://doi.org/10.1016/j.exger.2018.01.022.

[5] R. Rezzani, L. Nardo, G. Favero, M. Peroni, L.F. Rodella, Thymus and aging: morphological, radiological, and functional overview, Age (Dordr.) 36 (1) (2014) 313–351, https://doi.org/10.1007/s11357-013-9564-5.

[6] E. Roggero, A.R. Pérez, M. Tamae-Kakazu, et al., Endogenous glucocorticoids cause thymus atrophy but are protective during acute Trypanosoma cruzi infection, J. Endocrinol. 190 (2) (2006) 495–503, https://doi.org/10.1677/joe.1.06642.

[7] A.V. Turnbull, C.L. Rivier, Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action, Physiol. Rev. 79 (1) (1999) 1–71, https://doi.org/10.1152/physrev.1999.79.1.1.

[8] H.O. Besedovsky, A. del Rey, Immune-neuro-endocrine interactions: facts and hypotheses, Endocr. Rev. 17 (1) (1996) 64–102, https://doi.org/10.1210/edrv-17-1-64.

[9] M. Picard, R.-P. Juster, B.S. McEwen, Mitochondrial allostatic load puts the “gluc” back in glucocorticoids, Nat. Rev. Endocrinol. 10 (5) (2014) 303–310, https://doi.org/10.1038/nrendo.2014.22.

[10] E. Harno, A. White, Will treating diabetes with 11β-HSD1 inhibitors affect the HPA axis? Trends Endocrinol. Metab. 21 (10) (2010) 619–627, https://doi.org/10.1016/j.tem.2010.06.004.

[11] M.D. Taves, C.E. Gomez-Sanchez, K.K. Soma, Extra-adrenal glucocorticoids and mineralocorticoids: evidence for local synthesis, regulation, and function, Am. J. Physiol. Endocrinol. Metab. 301 (1) (2011) E11–E24, https://doi.org/10.1152/ajpendo.00100.2011.

[12] E. Zambrano, L.A. Reyes-Castro, P.W. Nathanielsz, Aging, glucocorticoids and developmental programming, Age (Dordr.) 37 (3) (2015) 9774, https://doi.org/10.1007/s11357-015-9774-0.

[13] R.J. Reiter, J.C. Mayo, D.-X. Tan, R.M. Sainz, M. Alatorre-Jimenez, L. Qin, Melatonin as an antioxidant: under promises but over delivers, J. Pineal Res. 61 (3) (2016) 253–278, https://doi.org/10.1111/jpi.12360.

[14] R. Hardeland, J.A. Madrid, D.-X. Tan, R.J. Reiter, Melatonin, the circadian multioscillator system and health: the need for detailed analyses of peripheral melatonin signaling, J. Pineal Res. 52 (2) (2012) 139–166, https://doi.org/10.1111/j.1600-079X.2011.00934.x.

[15] R. Hardeland, Melatonin, hormone of darkness and more: occurrence, control mechanisms, actions and bioactive metabolites, Cell. Mol. Life Sci. 65 (13) (2008) 2001–2018, https://doi.org/10.1007/s00018-008-8001-x.

[16] D.-X. Tan, R.J. Reiter, Mitochondria: the birth place, battle ground and the site of melatonin metabolism in cells, Melatonin Research 2 (1) (2019) 44–66, https://doi.org/10.32794/mr11250011.

[17] L.C. Manchester, A. Coto-Montes, J.A. Boga, et al., Melatonin: an ancient molecule that makes oxygen metabolically tolerable, J. Pineal Res. 59 (4) (2015) 403–419, https://doi.org/10.1111/jpi.12267.

[18] V. Brazão, M.D.V. Filipin, F.H. Santello, et al., Immunomodulatory properties and anti-apoptotic effects of zinc and melatonin in an experimental model of chronic Chagas disease, Immunobiology. 220 (5) (2015) 626–633, https://doi.org/10.1016/j.imbio.2014.11.018.

[19] R. Hardeland, Melatonin and the theories of aging: a critical appraisal of melatonin's role in antiaging mechanisms, J. Pineal Res. 55 (4) (2013) 325–356, https://doi.org/10.1111/jpi.12090.

[20] S. Proietti, A. Cucina, G. Dobrowolny, et al., Melatonin down-regulates MDM2 gene expression and enhances p53 acetylation in MCF-7 cells, J. Pineal Res. 57 (1) (2014) 120–129, https://doi.org/10.1111/jpi.12150.

[21] R.J. Reiter, Melatonin: clinical relevance, Best Pract. Res. Clin. Endocrinol. Metab. 17 (2) (2003) 273–285.

[22] M.M. Macchi, J.N. Bruce, Human pineal physiology and functional significance of melatonin, Front. Neuroendocrinol. 25 (3–4) (2004) 177–195, https://doi.org/10.1016/j.yfrne.2004.08.001.

[23] J.R. Calvo, C. González-Yanes, M.D. Maldonado, The role of melatonin in the cells of the innate immunity: a review, J. Pineal Res. 55 (2) (2013) 103–120, https://doi.org/10.1111/jpi.12075.

[24] H.-M. Zhang, Y. Zhang, Melatonin: a well-documented antioxidant with conditional pro-oxidant actions, J. Pineal Res. 57 (2) (2014) 131–146, https://doi.org/10.1111/jpi.12162.

[25] R.J. Reiter, R. Sharma, Q. Ma, S. Rosales-Corral, D. Acuna-Castroviejo, G. Escames, Inhibition of mitochondrial pyruvate dehydrogenase kinase: a proposed mechanism by which melatonin causes cancer cells to overcome cytosolic glycolysis, reduce tumor biomass and reverse insensitivity to chemotherapy, Melatonin Research 2 (3) (2019) 105–119, https://doi.org/10.32794/mr11250033.

[26] D. Zhao, Y. Yu, Y. Shen, et al., Melatonin synthesis and function: evolutionary history in animals and plants, Front Endocrinol (Lausanne) 10 (2019) 249, https://doi.org/10.3389/fendo.2019.00249.

[27] A. Carrillo-Vico, J.M. Guerrero, P.J. Lardone, R.J. Reiter, A review of the multiple actions of melatonin on the immune system, Endocrine. 27 (2) (2005) 189–200, https://doi.org/10.1385/ENDO:27:2:189.

[28] Y.-H. Wei, S.-B. Wu, Y.-S. Ma, H.-C. Lee, Respiratory function decline and DNA mutation in mitochondria, oxidative stress and altered gene expression during aging, Chang Gung Med. J. 32 (2) (2009) 113–132.

[29] A. Rassi, A. Rassi, S.G. Rassi, Predictors of mortality in chronic Chagas disease: a systematic review of observational studies, Circulation. 115 (9) (2007) 1101–1108, https://doi.org/10.1161/CIRCULATIONAHA.106.627265.

[30] B. Poeggeler, Melatonin, aging, and age-related diseases: perspectives for prevention, intervention, and therapy, Endocrine. 27 (2) (2005) 201–212, https://doi.org/

10.1385/ENDO:27:2:201.
[31] G. Anderson, R.J. Reiter, Melatonin: roles in influenza, Covid-19, and other viral infections, Rev. Med. Virol. 30 (3) (2020) e2109, , https://doi.org/10.1002/rmv.2109.
[32] Y.-H. Wu, D.F. Swaab, The human pineal gland and melatonin in aging and Alzheimer's disease, J. Pineal Res. 38 (3) (2005) 145–152, https://doi.org/10.1111/j.1600-079X.2004.00196.x.
[33] U. Mattam, A. Jagota, Differential role of melatonin in restoration of age-induced alterations in daily rhythms of expression of various clock genes in suprachiasmatic nucleus of male Wistar rats, Biogerontology. 15 (3) (2014) 257–268, https://doi.org/10.1007/s10522-014-9495-2.
[34] M. Sánchez-Hidalgo, J.M. Guerrero Montávez, M.D.P. Carrascosa-Salmoral, M.D.C. Naranjo Gutierrez, P.J. Lardone, C.A. de la Lastra Romero, Decreased MT1 and MT2 melatonin receptor expression in extrapineal tissues of the rat during physiological aging, J. Pineal Res. 46 (1) (2009) 29–35, https://doi.org/10.1111/j.1600-079X.2008.00604.x.
[35] K. Yu, S.-L. Deng, T.-C. Sun, Y.-Y. Li, Y.-X. Liu, Melatonin regulates the synthesis of steroid hormones on male reproduction: a review, Molecules 23 (2) (2018), https://doi.org/10.3390/molecules23020447.
[36] M. Quinkler, P.M. Stewart, Hypertension and the cortisol-cortisone shuttle, J. Clin. Endocrinol. Metab. 88 (6) (2003) 2384–2392, https://doi.org/10.1210/jc.2003-030138.
[37] D.A. Padgett, R. Glaser, How stress influences the immune response, Trends Immunol. 24 (8) (2003) 444–448, https://doi.org/10.1016/s1471-4906(03)00173-x.
[38] J.T. Ho, H. Al-Musalhi, M.J. Chapman, et al., Septic shock and sepsis: a comparison of total and free plasma cortisol levels, J. Clin. Endocrinol. Metab. 91 (1) (2006) 105–114, https://doi.org/10.1210/jc.2005-0265.
[39] W. Savino, Endocrine immunology of Chagas disease, Front. Horm. Res. 48 (2017) 160–175, https://doi.org/10.1159/000452914.
[40] G. Csaba, Immunity and longevity, Acta Microbiol. Immunol. Hung. 66 (1) (2019) 1–17, https://doi.org/10.1556/030.65.2018.029.
[41] A. Yiallouris, C. Tsioutis, E. Agapidaki, et al., Adrenal aging and its implications on stress responsiveness in humans, Front Endocrinol (Lausanne) 10 (2019) 54, https://doi.org/10.3389/fendo.2019.00054.
[42] V.G. Podsevatkin, S.V. Kiriukhina, D.V. Podsevatkin, S.V. Podsevatkina, D.S. Blinov, Dynamics of the behavioral response and cortisole level caused by the combined action of mexidole, diazepam, thymogen, and hyperbaric oxygenation in mice under immobilization stress conditions, Eksp Klin Farmakol. 71 (1) (2008) 22–25.
[43] Y. Cheng, D. Wang, S. Li, Z. Geng, Supplementation with micronutrients attenuating stress injuries in rats, Wei Sheng Yan Jiu 33 (2) (2004) 179–182.
[44] M.A. Magiakou, G.P. Chrousos, Corticosteroid therapy, nonendocrine disease, and corticosteroid withdrawal, Curr. Ther. Endocrinol. Metab. 6 (1997) 138–142.
[45] N. Maeda, S. Tahata, T. Yagi, et al., Assessment of testicular corticosterone biosynthesis in adult male rats, PLoS One 10 (2) (2015) e0117795, , https://doi.org/10.1371/journal.pone.0117795.
[46] R.M. Barrientos, M.G. Frank, L.R. Watkins, S.F. Maier, Aging-related changes in neuroimmune-endocrine function: implications for hippocampal-dependent cognition, Horm. Behav. 62 (3) (2012) 219–227, https://doi.org/10.1016/j.yhbeh.2012.02.010.
[47] K. Nanba, A. Vaidya, W.E. Rainey, Aging and adrenal aldosterone production, Hypertension. 71 (2) (2018) 218–223, https://doi.org/10.1161/HYPERTENSIONAHA.117.10391.
[48] S.R. Villar, M.T. Ronco, R. Fernández Bussy, et al., Tumor necrosis factor-α regulates glucocorticoid synthesis in the adrenal glands of Trypanosoma cruzi acutely-infected mice. The role of TNF-R1, PLoS ONE 8 (5) (2013) e63814, , https://doi.org/10.1371/journal.pone.0063814.
[49] L.C. Chumbinho, C.C. Pizzini, F. de Souza Oliveira, W. Batista, G.M. de Oliveira, Cardiorenal interaction during the acute phase of experimental Trypanosoma cruzi infection: the influence of aldosterone and the AT1 receptor on mortality, J. Exp. Integrat. Med. 2 (2012) 199–206.
[50] Y.-M. Tian, G.-Y. Zhang, Y.-R. Dai, Melatonin rejuvenates degenerated thymus and redresses peripheral immune functions in aged mice, Immunol. Lett. 88 (2) (2003) 101–104.
[51] C. Torres-Farfan, H.G. Richter, P. Rojas-García, et al., mt1 melatonin receptor in the primate adrenal gland: inhibition of adrenocorticotropin-stimulated cortisol production by melatonin, J. Clin. Endocrinol. Metab. 88 (1) (2003) 450–458, https://doi.org/10.1210/jc.2002-021048.
[52] R.J. Reiter, D.-X. Tan, M.-J. Jou, A. Korkmaz, L.C. Manchester, S.D. Paredes, Biogenic amines in the reduction of oxidative stress: melatonin and its metabolites, Neuro Endocrinol. Lett. 29 (4) (2008) 391–398.
[53] R.M. Sainz, J.C. Mayo, R.J. Reiter, I. Antolin, M.M. Esteban, C. Rodriguez, Melatonin regulates glucocorticoid receptor: an answer to its antiapoptotic action in thymus, FASEB J. 13 (12) (1999) 1547–1556.
[54] Y.M. Tian, P.P. Li, X.F. Jiang, G.Y. Zhang, Y.R. Dai, Rejuvenation of degenerative thymus by oral melatonin administration and the antagonistic action of melatonin against hydroxyl radical-induced apoptosis of cultured thymocytes in mice, J. Pineal Res. 31 (3) (2001) 214–221.
[55] R.M. Sainz, J.C. Mayo, R.J. Reiter, D.X. Tan, C. Rodriguez, Apoptosis in primary lymphoid organs with aging, Microsc. Res. Tech. 62 (6) (2003) 524–539, https://doi.org/10.1002/jemt.10414.
[56] G. Pawelec, R.B. Effros, C. Caruso, E. Remarque, Y. Barnett, R. Solana, T cells and aging (update february 1999), Front. Biosci. 4 (1999) D216–D269.
[57] F.T. Hakim, F.A. Flomerfelt, M. Boyiadzis, R.E. Gress, Aging, immunity and cancer, Curr. Opin. Immunol. 16 (2) (2004) 151–156, https://doi.org/10.1016/j.coi.2004.01.009.
[58] D.B. Palmer, The effect of age on thymic function, Front. Immunol. 4 (2013) 316, https://doi.org/10.3389/fimmu.2013.00316.
[59] T. Miyao, S. Floess, R. Setoguchi, et al., Plasticity of Foxp3(+) T cells reflects promiscuous Foxp3 expression in conventional T cells but not reprogramming of regulatory T cells, Immunity. 36 (2) (2012) 262–275, https://doi.org/10.1016/j.immuni.2011.12.012.
[60] N. Komatsu, K. Okamoto, S. Sawa, et al., Pathogenic conversion of Foxp3+ T cells into TH17 cells in autoimmune arthritis, Nat. Med. 20 (1) (2014) 62–68, https://doi.org/10.1038/nm.3432.
[61] R. Scollay, D.I. Godfrey, Thymic emigration: conveyor belts or lucky dips? Immunol. Today 16 (6) (1995) 268–273, https://doi.org/10.1016/0167-5699(95)80179-0 (discussion 273-274).
[62] H. Bruunsgaard, K. Andersen-Ranberg, B. Jeune, A.N. Pedersen, P. Skinhøj, B.K. Pedersen, A high plasma concentration of TNF-alpha is associated with dementia in centenarians, J. Gerontol. A Biol. Sci. Med. Sci. 54 (7) (1999) M357–M364.
[63] F. Zanni, R. Vescovini, C. Biasini, et al., Marked increase with age of type 1 cytokines within memory and effector/cytotoxic CD8+ T cells in humans: a contribution to understand the relationship between inflammation and immunosenescence, Exp. Gerontol. 38 (9) (2003) 981–987.
[64] M. Palmeri, G. Misiano, M. Malaguarnera, et al., Cytokine serum profile in a group of Sicilian nonagenarians, J. Immunoass. Immunochem. 33 (1) (2012) 82–90, https://doi.org/10.1080/15321819.2011.601781.
[65] T. Jiang, E. Cadenas, Astrocytic metabolic and inflammatory changes as a function of age, Aging Cell 13 (6) (2014) 1059–1067, https://doi.org/10.1111/acel.12268.
[66] B. Möller, P.M. Villiger, Inhibition of IL-1, IL-6, and TNF-alpha in immune-mediated inflammatory diseases, Springer Semin. Immunopathol. 27 (4) (2006) 391–408, https://doi.org/10.1007/s00281-006-0012-9.
[67] S.-J. Choi, S. Piao, H. Nagar, et al., Isocitrate dehydrogenase 2 deficiency induces endothelial inflammation via p66sh-mediated mitochondrial oxidative stress, Biochem. Biophys. Res. Commun. 503 (3) (2018) 1805–1811, https://doi.org/10.1016/j.bbrc.2018.07.117.
[68] A.M. Judd, G.B. Call, M. Barney, et al., Possible function of IL-6 and TNF as intraadrenal factors in the regulation of adrenal steroid secretion, Ann. N. Y. Acad. Sci. 917 (2000) 628–637.
[69] M. Reincke, W. Arlt, C. Heppner, F. Petzke, G.P. Chrousos, B. Allolio, Neuroendocrine dysfunction in African trypanosomiasis. The role of cytokines, Ann. N. Y. Acad. Sci. 840 (1998) 809–821.
[70] F. Petzke, C. Heppner, D. Mbulamberi, et al., Hypogonadism in Rhodesian sleeping sickness: evidence for acute and chronic dysfunction of the hypothalamic-pituitary-gonadal axis, Fertil. Steril. 65 (1) (1996) 68–75.
[71] E. Corrêa-de-Santana, M. Paez-Pereda, M. Theodoropoulou, et al., Hypothalamus-pituitary-adrenal axis during Trypanosoma cruzi acute infection in mice, J. Neuroimmunol. 173 (1–2) (2006) 12–22, https://doi.org/10.1016/j.jneuroim.2005.08.015.
[72] E. Arzt, M.P. Pereda, C.P. Castro, U. Pagotto, U. Renner, G.K. Stalla, Pathophysiological role of the cytokine network in the anterior pituitary gland, Front. Neuroendocrinol. 20 (1) (1999) 71–95, https://doi.org/10.1006/frne.1998.0176.
[73] W. Savino, The thymus is a common target organ in infectious diseases, PLoS Pathog. 2 (6) (2006) e62, , https://doi.org/10.1371/journal.ppat.0020062.
[74] C.D. Munhoz, S.F. Sorrells, J.R. Caso, C. Scavone, R.M. Sapolsky, Glucocorticoids exacerbate lipopolysaccharide-induced signaling in the frontal cortex and hippocampus in a dose-dependent manner, J. Neurosci. 30 (41) (2010) 13690–13698, https://doi.org/10.1523/JNEUROSCI.0303-09.2010.
[75] F. González, S. Villar, L. D'Attilio, et al., Dysregulated network of immune, endocrine and metabolic markers is associated to more severe human chronic Chagas cardiomyopathy, Neuroimmunomodulation. 25 (3) (2018) 119–128, https://doi.org/10.1159/000491699.
[76] J.L. Mauriz, P.S. Collado, C. Veneroso, R.J. Reiter, J. González-Gallego, A review of the molecular aspects of melatonin's anti-inflammatory actions: recent insights and new perspectives, J. Pineal Res. 54 (1) (2013) 1–14, https://doi.org/10.1111/j.1600-079X.2012.01014.x.
[77] K. Forman, E. Vara, C. García, et al., Effect of a combined treatment with growth hormone and melatonin in the cardiological aging on male SAMP8 mice, J. Gerontol. A Biol. Sci. Med. Sci. 66 (8) (2011) 823–834, https://doi.org/10.1093/gerona/glr083.
[78] S. Cuesta, R. Kireev, C. García, et al., Beneficial effect of melatonin treatment on inflammation, apoptosis and oxidative stress on pancreas of a senescence accelerated mice model, Mech. Ageing Dev. 132 (11−12) (2011) 573–582, https://doi.org/10.1016/j.mad.2011.10.005.
[79] X.-J. Lin, G.-P. Mei, J. Liu, et al., Therapeutic effects of melatonin on heatstroke-induced multiple organ dysfunction syndrome in rats, J. Pineal Res. 50 (4) (2011) 436–444, https://doi.org/10.1111/j.1600-079X.2011.00863.x.
[80] Z. Peng, W. Zhang, J. Qiao, B. He, Melatonin attenuates airway inflammation via SIRT1 dependent inhibition of NLRP3 inflammasome and IL-1β in rats with COPD, Int. Immunopharmacol. 62 (2018) 23–28, https://doi.org/10.1016/j.intimp.2018.06.033.
[81] N.-R. Shin, J.-W. Park, I.-C. Lee, et al., Melatonin suppresses fibrotic responses induced by cigarette smoke via downregulation of TGF-β1, Oncotarget 8 (56) (2017) 95692–95703, https://doi.org/10.18632/oncotarget.21680.