Beneficial effects of melatonin in juvenile rats with heart failure

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Research article

Keywords: melatonin, heart failure, pyroptosis, calcium homeostasis

Posted Date: December 2nd, 2019

DOI: https://doi.org/10.21203/rs.2.17578/v1

Abstract

Background: Adult rodent studies showed that melatonin is very effective in treatment of ameliorating cardiovascular disease. Melatonin is also able to reduce cardiac hypertrophy and heart failure (HF) in juvenile rodents. Here, we study the effect of melatonin on the cardiac function of juvenile rats with HF.

Methods: Juvenile rats with HF were induced by abdominal aortic constriction (AAC). Sham-operated rats were established in parallel. Five weeks after the surgery, rats with ventricular dysfunction were randomly divided into two groups: the treatment group was injected with melatonin (10 mg/kg/d, intraperitoneal injection), and the HF group was injected with placebo. Simultaneously, placebo was administered to the sham group.

Results: After administration for 4 weeks, the treated rats did not exhibit a decline in cardiac function as assessed by echocardiography analysis. Moreover, the increase in expression of ANP, BNP, caspase-1, IL-1β, bax, CaMKII, PLN, and RyR2 was markedly blunted by melatonin, while the decrease in expression of bcl-2 was improved in the melatonin treated rats.

Conclusions: Our findings support a protective role of melatonin in cardiomyocytes, at least in part via reducing cardiac pyroptosis, apoptosis and remaining calcium homeostasis.

Background

Heart failure (HF) is a leading cause of hospitalization in children with cardiovascular diseases, and its associated morbidity and mortality are a great burden for the healthcare system. There is significant difference in HF between children and adults in terms of characteristics, aetiology, and drug clearance[1]. Therefore, additional investigation is needed to better understand the pediatric cardiac diseases for effective evaluation and management of HF in children.

Abdominal aortic constriction (AAC) of rats has been commonly employed as a HF pathological model and has been extensively used in the research of cardiomyocytes hypertrophy, ventricular remodeling and cardiac dysfunction through increased left ventricular (LV) afterload[2]. Congenital heart disease, such as coarctation of the aorta with increased LV afterload, also may lead hypertrophy or HF in children. Apoptosis is a regulated cell death process controlled by genes essential for maintaining normal cardiomyocyte homeostasis and is implicated in the development of HF. The morphological characteristic features of apoptosis include chromatin condensation, nuclear fragmentation, cell shrinking, membrane blebbing and apoptotic bodies formation. Apoptosis is triggered by a set of cysteine-dependent aspartate-specific proteases (caspase) including caspases−3, −6 and −7[3]. The main apoptosis pathway is regulated by members of the B-cell-lymphoma protein 2 (Bcl−2) family including pro-apoptotic members, like Bax, Bak and Bok and anti-apoptotic proteins, like Bcl−2, Bcl-W and Bcl-XL. The balance between these pro- and anti-apoptotic proteins is vital for the survival of cells[4, 5]. Inflammation associated cell death referred to as pyroptosis, which is different from anti-inflammatory

apoptosis, was first described in macrophages following a *Salmonella* infection[6]. As a pyroptosis executioner caspase, active caspase–1 is known to promote the secretion of inflammatory cytokines, such as interleukin (IL)–1 and IL–18[7]. However, our understanding of pyroptosis has yet to be complete. Furthermore, there is emerging evidence that alterations in calcium homeostasis through upregulation of calmodulin-dependent protein kinase II (CaMKII)[8], phospholamban (PLN)[9] and ryanodine receptor 2 (RyR2)[10] might play a crucial role in the development of cardiac remodeling and HF.

Melatonin (N-acetyl–5-methoxytryptamine) is a secretory product of human pineal gland with the peak of secretion from 11 pm to 6 am. The major function of melatonin is control of diurnal rhythm and anti-reproduction, however, its influence on the cardiovascular system is little known. The heart protection effect of melatonin is via two manners: receptor-mediated and receptor-independent[11]. The former refers to it is mediated by two classic melatonin membrane receptors MT1 and MT2[12]. The latter refers to it functions as a potent free radical scavenger and anti-oxidant agent [13]. Additionally, melatonin is also able to decrease hypertension[14], protect ischemic/reperfused heart[15], and attenuate atherosclerosis development[16]. While cardiomyocyte hypertrophy starts off as a compensatory response, it eventually becomes pathological and could lead to HF. Furthermore, the effect of melatonin on nitric oxide (NO) availability, hemodynamic overload, lipid profile and free radicals may also affect cardiomyocyte hypertrophy[17].

In this study, we reported that pharmacologic administration of melatonin to juvenile rats ameliorated cardiac damage in an increased afterload model. Taken together, melatonin might play a protective role against HF, at least in juvenile rats.

Methods

Animals

Juvenile Sprague-Dawley (SD) rats (male, age 21~28 days, body weight 50~80 g) used were purchased from the Animal Experiment Center of Chongqing Medical University (Chongqing, China). The animals were housed with food and water free access and a 12h:12 h light-dark cycle. All animal studies were approved by the Ethics Committee of the Children's Hospital of Chongqing Medical University.

Surgical procedures

Anesthesia was induced by 3% isoflurane (RWD Life Science, China) with an inhalation device and maintained with 3% isoflurane during surgery. HF was induced by AAC as described previously [18].

Drug preparation

After the surgery, rats that survived for 5 weeks with signs of decline in cardio function were randomly divided to three groups (n = 8 per group): 1) melatonin group (10 mg/kg, i.p.; once every evening 23:00~24:00), 2) vehicle group (0.5 % alcohol), and 3) control group (sham-ligated rats received vehicle), and treated for 4 weeks[20]..

Doppler Echocardiogram Studies

Rats were anesthetized using 10% chloral hydrate, and echocardiography was then examined using ultrasound (GE, US) with a 12.5 MHz linear array ultrasound transducer. The LV was evaluated based on the parasternal short- and long-axis views at a 120 Hz frame rate. The end-diastole and end-systole mean when LV occupies the largest and smallest area, respectively. The following parameters were measured: LV internal diastolic diameter (LVIDd), LV internal dimension systole (LVIDs), LV end-diastolic volume (LVEDV), LV end-systolic volume (LVESV), LV fractional shortening (LVFS) and LV ejection fraction (LVEF).

Blood sample collection

Blood samples were harvested from the heart of anesthetized rats in the evening during 23:00~24:00 immediately after administration of melatonin or placebo, and stored for 15 min at room temperature to allow clotting. Then, the blood samples were centrifuged 3000 rpm for 10 min, and the supernatants (serum) were collected and stored in a –80C freezer prior to testing.

Measurement of serum levels of melatonin, IL–18 and IL–1ß using ELISA

The serum levels of melatonin, IL–18 and IL–1β in juvenile rats with HF and in sham rats were measured using melatonin, IL–18 and IL–1β ELISA kit (WESTANG BIO-TECH, China; Assay sensitivity is 2 ng/L, 0.5 ng/L and 0.1 ng/L, respectively) following the manufacturer's instructions.

Tissue collection and storage

The animals were sacrificed by cervical dislocation while thoroughly anesthetized. The hearts were removed immediately after, and the left ventricles were dissected and placed in liquid nitrogen before being stored at –80C until use.

RT-PCR

Total RNA was extracted from rat hearts using an RNA isolation kit (Bioteke Corporation, Beijing, China). A reverse transcriptase kit was applied to synthesize cDNA, which was amplified by real time PCR. Data were collected and normalized to GAPDH. The mRNA expressions were analyzed using the △△CT method.

Western blot

The extracted proteins were separated by SDS-PAGE and transferred to a PVDF membrane. The membranes were then blocked with 5% skim milk in Tris-buffered saline–0.1% Tween 20 (TBST) at room temperature for 1 h, and incubated with primary antibodies (1:1000 dilution) at 4Covernight. After three washes with TBST, the membranes were incubated with HRP-conjugated secondary antibody (1:5000 dilution, Lianke, China) at room temperature for 1 h, and then were developed using an ECL assay kit (KeyGEN BioTECH, China).

Statistical analysis

Statistical analyses were carried out using SPSS software (version 19.0), and the differences between two groups (AAC vs. Sham) were analyzed using Student's t test. The differences among the three treatment groups (AAC vs. AAC+melatonin vs. Sham) were analyzed using one-way ANOVA, and the LSD method was used to estimate pairwise comparisons. Data are presented as the mean ± standard deviation, and $P < 0.05$ was considered to be statistically significant.

Results

In order to explore the cardiac protective effect of melatonin, the rats were randomly assigned to one of three experimental groups: AAC surgery followed by 4 weeks of treatment with 10 mg/kg melatonin every night, AAC surgery followed by vehicle treatment, or sham operation followed by placebo treatment. At the end of the course of treatment, all groups were subject to critical evaluation of the effects of these manipulations.

As shown in Table 2 and Fig. 1A, the ventricular functions were deteriorated 5 weeks after AAC, as evidenced by the increase in LVIDs, LVESV, and decrease in LVEF and LVFS ($P = 0.002$, $P = 0.029$, $P<0.001$, and $P<0.001$, respectively) of the rats in the AAC group when compared with those in the Sham group. 2 of 10 rats with AAC in the placebo groups died before the endpoint due to congestive HF. After 4 weeks of administration, the serum levels of melatonin decreased in AAC -surgery rats treated with placebo when compared with sham rats treated with vehicle, while a smaller decrease was observed in the melatonin-treated rats ($P = 0.0146$) (Fig. 1E). As shown in Table 3 and Fig. 1B, treatment with melatonin attenuated ACC-induced ventricular dysfunction, as evidenced by improvements in LVIDd, LVIDs, LVEDV, LVESV, LVEF and LVFS ($P = 0.012$, $P<0.001$, $P = 0.01$, $P = 0.005$, $P<0.001$, $P<0.001$, respectively); no significant differences were observed in LVIDd, LVIDs, LVEDV and LVESV between the ACC-surgery rats treated with melatonin and the sham-operated, vehicle-treated controls. As shown in Fig. 1D, the ratio of left ventricle to body weight increased significantly in vehicle-treated HF rats, indicating ventricular hypertrophy. However, this increase was much less obvious in the melatonin-treated rats ($P = 0.001$). Moreover, the gene levels of ANP and BNP, two markers of HF, in the AAC group were significantly higher than in the sham group. These increases in ANP and BNP were also much smaller in the melatonin-treated rats relative to the control rats (all $P<0.001$) (Fig. 2).

To further investigate the effects of melatonin on apoptosis of cardiomyocytes, we performed ELISA, RT-PCR and Western blot analyses to determine whether melatonin affects the levels of caspase–1, IL–18, IL–1β, caspase–3, bax and Bcl–2. As shown in Fig. 3 and 4, levels of caspase–1, IL–1β and bax increased while bcl–2 decreased in heart tissue of vehicle-treated AAC rats compared with controls (all $P<0.001$). These changes were also much less apparent in the melatonin-treated rats relative to the controls group. Interestingly, levels of caspase–3 and IL–18 did not differ among the three groups (all $P>0.05$).

We also detected relative Ca^{2+} handling proteins in cardiomyocytes (Fig. 5 and 6) The levels of phospho-CaMKII, oxidized-CaMKII, total PLN and RyR2 increased while phospho-PLN decreased in heart tissue of

vehicle-treated AAC rats compared with the controls (all $P<0.001$). The changes of phospho-CaMKII, oxidized-CaMKII, phospho-PLN, total PLN, RyR2 and phospho-PLN were less noticeable in the melatonin-treated AAC rats relative to controls. These results indicate that melatonin restores Ca^{2+} homeostasis in HF.

Discussion

HF is a serious pathological symptom in pediatric patients that manifests as cardiac output fails to meet the needs of the body. HF can lead to high rates of hospitalization and mortality in children. Clinical symptoms of HF in older children are similar to those in adults, while clinical manifestations in neonates and infants are atypical. Though extensive research on HF adults has been conducted and evidence-based guidelines have been developed, there is a lack of knowledge on cardiac pathologies in children[19]. Especially, the molecular mechanism of cardiac myocyte's response to HF, myocardial cell necrosis, apoptosis, calcium disturbance, ventricular remodeling and hypertrophy has not been thoroughly understood.

Previous studies showed that increased oxidative stress resulted from reactive oxygen species (ROS) contributes to development and deterioration of HF[20]. Overactive ROS can damage intracellular proteins and lipids, which are important in various signaling cascade. Previous study demonstrated that ROS level was elevated in patients with chronic HF[21]. Similar results were observed in vivo and in vitro, which implies a central role of ROS in HF[22]. ROS also leads to the abnormality in calcium homeostasis in the myocardium[23]. Recently, an oxidation site on CaMKII was discovered, which plays a role in the activation of CaMKII, an important regulatory protein in the calcium circle[24]. In addition, it had been demonstrated that HF is an inflammatory process involving an increase in inflammatory factors, for examples, TNF-α, IL−6, and IL−1β, which result in cellular damage and contractile dysfunction[25].

Plenty of studies have shown that melatonin and its metabolites can serve as broad-spectrum antioxidants and free radical scavengers[26–28]. They can potentially regulate the process of apoptosis and inflammation in cardiovascular diseases. A clinical study also reported that melatonin treatment can lower blood pressure in healthy women[29]. Others have also demonstrated the cardio protective effect of melatonin against ischemic heart disease[30]. Additional research has shown that melatonin was associated with reverse remodeling in adult failing hearts after cardiac resynchronization therapy[31]. Based on these findings, we set out to explore the potential protective role of melatonin on juvenile heart dysfunction, which could be a potential therapy for children with HF.

We hypothesized that melatonin administration ameliorates HF in juvenile rats. Melatonin or placebo treatments were given to AAC groups for five weeks after surgery at first sign of cardio-dysfunction, while the sham group was given placebo. Naser Farhadi and his colleagues demonstrated that melatonin oral administration to adult Wistar rats did not change the serum melatonin levels [32].In our study, the serum melatonin levels in the melatonin group (10 mg/kg/day) also didn't show significant difference compared

with the AAC group. Yeleswaram K and his colleagues found that after an intraperitoneal administration of 10 mg/kg melatonin to rats, the bioavailability of melatonin was 74.0%, and the apparent elimination half-life of melatonin following an intravenous dose of 5 mg/kg was 19.8 minutes in rats[33]. The pharmacokinetics of melatonin maybe induce short half-life and melatonin entering organizations soon, so it might the reason why there was no difference in serum melatonin concentration between treated and untreated rats. The levels of melatonin in the Sham group are significantly increased than those in the AAC group indicating that melatonin still play a role in the HF. And we further found that administration of melatonin played a protective role in maintaining proper cardiac function with the increase in LVEF and LVFS, and improving the ventricular remodeling with the decrease in LVIDd, LVIDs, LVEDV and LVESV form the transthoracic echocardiography in rats nine weeks after AAC. Furthermore, the gene expressions of ANP and BNP were decreased by melatonin, suggesting the positive role of melatonin on cardiac function. We found that the expression of caspase–1 was significantly increased in AAC rats while IL–18 was unchanged. Interestingly, both IL–1β gene and serum level of IL–1β were significantly enhanced in AAC rats. It has been demonstrated previously that the expression of IL–1β is very low in healthy cells, while in some pathological processes, active caspase–1 can promote conversion of pro-IL–18 and –1β into cleaved and active IL–1β and induce an inflammatory necrosis process called pyroptosis[7]. In another cardiovascular disease study, caspase–1 was increased during pathological processes, such as hypoxia[34] or doxorubicin treatment[35], however, the levels of IL–1β and –18 were not significantly higher. The differences in age of rats, establishment of pathological model or time points of the studies in these previous studies might be responsible for the discrepancies in results. Moreover, as the apoptosis indices, bax increased while bcl–2 decreased in AAC rates. In addition, the changes in caspase–1, IL–1β, bcl–2 and bax were weakened by melatonin administration. Taken together, these findings indicated a protective effect of melatonin on apoptosis or pyroptosis.

Calcium homeostasis plays a critical role in regulating myocardial contraction and development of HF. In our study, the phosphorylation and oxidation of CaMKII were significantly increased in dysfunctioning myocardium when compared with controls. It is known that over-activated CaMKII could lead to disruption of calcium homeostasis, which could induce possible malignant arrhythmias[36] and cause hypertrophy or remodeling in HF[37]. Additionally, the RyR2 expression in AAC rats was significantly higher than in sham rats. During the myocardial contractile period, a small amount of Ca^{2+} enters cardiomyocytes and triggers the release of a large amount of Ca^{2} from the sarcoplasmic reticulum through RyR2. SERCA is involved in uptake, during the diastolic period. Dysfunctional RyR2 response to abnormal Ca^{2+} leak can deteriorate arrhythmia[38]. In addition, PLN phosphorylation decreases with cardio dysfunction in juvenile rats compared with controls. PLN de-phosphorylation induces active monomeric PLN, which can serve as an inhibitor of SERCA2a, causing low affinity of SERCA2a to Ca^{2+} and accelerating the progress of HF[9]. Melatonin reversed the over-activation or over-expression of CaMKII, RyR2 and PLN, implying a role of melatonin in maintaining calcium homeostasis in HF. Ahmet and his colleagues also found that melatonin protected against ischemic HF in adult rats through

improving cardiac Na^+,K^+-ATPase and SERCA activities in HF, which further supported the beneficial role of melatonin in calcium homeostasis[39].

We observed significantly increased inflammation and apoptosis markers in hearts from juvenile animals after AAC, while the proteins involved in Ca^{2+} regulation were dysregulated. These alterations may be due to the accumulation of ROS, through damages proteins, lipids, and DNA, which contribute to the occurrence of HF[40]. Administration of melatonin reduced these changes. This may result from its anti-oxidant and free radical scavenging activities, while the melatonin receptor with an anti-adrenergic activity mediated by NO, guanylyl cyclase and protein kinase C (PKC) might also be involved. [41]. Melatonin may be potential agents for HF in juveniles.

Conclusions

In conclusion, we investigated the protective effect of melatonin administration on juvenile rats with AAC through improving ventricular remolding, promoting anti-inflammatory response and restoring the normal calcium cycle. Furthermore, we show that melatonin could reverse the increase in caspase–1 in juveniles with failing hearts although the specific mechanisms remain unclear. In order to assess any side effect of prolonged prescription of melatonin, long-term evaluation of full-body effect is required.

Abbreviations

HF: Juvenile rats with heart failure

AAC: abdominal aortic constriction

LV: left ventricular

Caspase: cysteine-dependent aspartate-specific proteases

Bcl–2: B-cell-lymphoma protein 2

IL: interleukin

CaMKII: calmodulin-dependent protein kinase II

PLN: phospholamban

RyR2: ryanodine receptor 2

NO: nitric oxide

SD: Sprague-Dawley

LVIDs: LV internal dimension systole

LVIDd: LV internal diastolic diameter

LVESV: LV end-systolic volume

LVEDV: LV end-diastolic volume

LVEF: LV ejection fraction

LVFS: LV fractional shortening

ROS: reactive oxygen species

PKC: protein kinase C

Declarations

Ethics approval and consent to participate: All animal studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, and efforts were made to minimize suffering. The Ethics Committee of the Children's Hospital of Chongqing Medical University (Permit Number: SYXK2007–0016) approved all experiments.

Consent for publication: Not applicable.

Availability of data and material: The datasets generated and analysed during the current study are not publicly available due to the Children's Hospital of Chongqing Medical University regulations, but are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

Funding: No funding was received.

Authors' contributions: Yao Wu made the study design and wrote the first draft of the manuscript; Li Luo completed the analysis and interpretation of data; Wu Yao and Fengchuan Jing built the animal model; Feifei Si and Kunfeng Jiang detected the echo of rats; Siqi Feng and Ya Su performed the q-PCR and western blot analysis; Qijian Yi decided to submit the paper for publication. All authors had seen and approved the submission of this version of the manuscript and takes full responsibility for the manuscript.

Acknowledgements: None.

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Tables

Table 1. RT-PCR primers of ANP, BNP, caspase-1, IL-18, IL-1β, caspase-3, PLN, CaMKII, RyR2 and GAPDH

Target	Forward Q-PCR Primer	Reverse Q-PCR Primer
ANP	5-GCTCCCAGGCCATATTGGAG-3′	5-CTTCATCGGTCTGCTCGCTC-3
BNP	5-GCTGCTGGAGCTGATAAGAGA-3′	5-CGCCGATCCGGTCTATCTTC-3′
Caspase-1	5-TATGGAAAAGGCACGAGACC-3′	5-TGATGGACCTGACTGAAGCTC-3′
IL18	5-AGACCACTTTGGCAGACTTCA-3′	5-TGGGATTCGTTGGCTGTT-3′
IL1β	5-AAAAATGCCTCGTGCTGTCT-3′	5-TCGTTGCTTGTCTCTCCTTG-3′
Caspase-3	5-TAACCGGGTGCGGTAGAGTA-3′	5-GCTGGACTGCGGTATTGAGA-3′
PLN	5-GCAGCAGACATATCAAGATGAGAC-3′	5-CCTTACTCGCTCGGCTATCA-3′
CaMKII	5-GGACCCCGAACGATGAAAGT-3′	5-GAACCCTCACGTACACCTGG-3′
RyR2	5-ATGGACTGTTCTCCGCTGTT-3′	5-AGGGTATCTCATTGGTGGTGA-3′
GAPDH	5-CAGTGCCAGCCTCGTCTCAT-3′	5- AGGGGCCATCCACAGTCTTC -3′

Table 2. Ventricular diameter, ventricular volume and heart function in juvenile rats 5 weeks after AAC( )

Group	n	LVIDd(cm)	LVIDs(cm)	LVEDV(ml)	LVESV(ml)	LVEF(%)	LVFS(%)
ACC	18	0.46±0.1	0.24±0.06**	0.27±0.16	0.05±0.04*	83.82±13.61***	47.57±6.04***
Sham	8	0.37±0.1	0.14±0.04	0.16±0.1	0.01±0.01	93.25±2.73	61.11±5.79
t		2.016	3.472	1.769	2.324	4.22	5.339
P		0.055	0.002	0.09	0.029	<0.001	<0.001

*: *P*<0.05, **: *P*<0.01, ***: *P*<0.001, vs Sham group.

Table 3. Ventricular diameter, ventricular volume and heart function in juvenile rats with or without melatonin administration ( )

Group	n	LVIDd(cm)	LVIDs(cm)	LVEDV(ml)	LVESV(ml)	LVEF(%)	LVFS(%)
AAC	8	0.67±0.09	0.44±0.10	0.70±0.26	0.24±0.18	67.05±13.61	33.53±8.83
AAC+ melatonin	8	0.55±0.08*	0.30±0.05**	0.41±0.17*	0.08±0.03	81.77±0.9	44.93±0.88*
Sham	8	0.53±0.08**	0.23±0.06***	0.38±0.14**	0.04±0.02*	91.39±4.07**##	58.47±7.18**##
F		5.688	17.589	5.934	7.049	15.214	24.662
P		0.012	<0.001	0.01	0.005	<0.001	<0.001

*: *P*<0.05, **: *P*<0.01, ***: *P*<0.001, vs AAC group; ##: *P*<0.01, vs AAC+melatonin group.

Figures

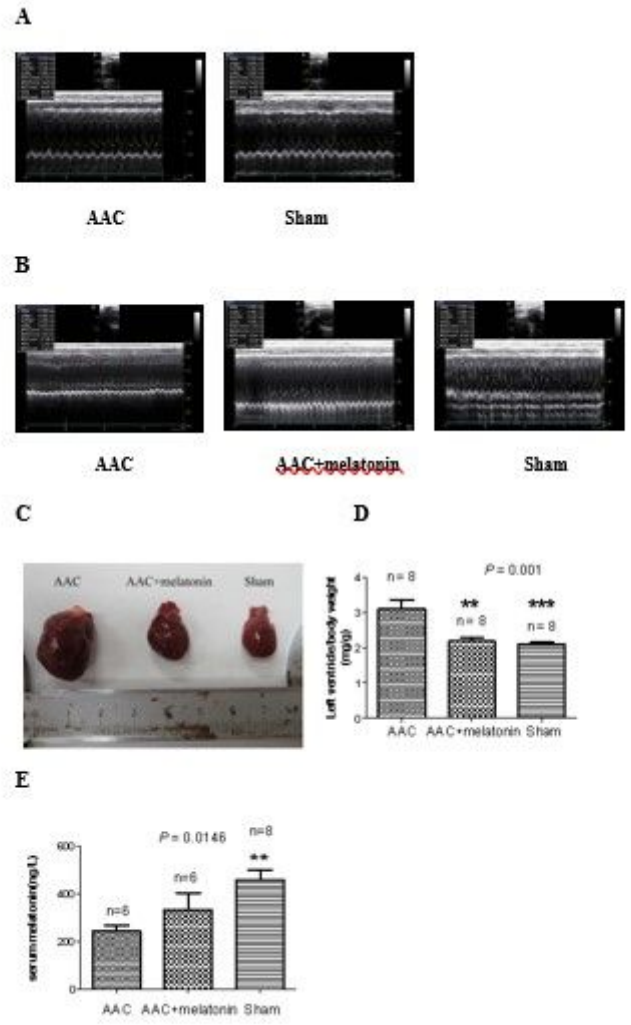

Figure 1

A. Representative M-mode images of transthoracic echocardiography in juvenile rats 5 weeks after AAC. B. Representative M-mode images of transthoracic echocardiography in different groups 4 weeks after melatonin administration. C. Gross anatomy of hearts in rats of the three groups. D. Effects of chronic increased pressure overload on left ventricle to body weight ratio in juvenile rats. E. Levels of serum melatonin in three groups of rats. **: P < 0.01, ***: P < 0.001 versus AAC group.

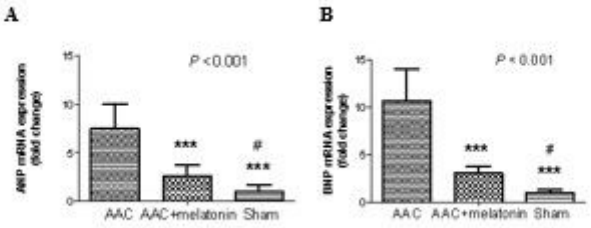

Figure 2

A and B. Relative ANP and BNP mRNA expression levels in AAC–treated juvenile rat ventricular cardiomyocytes (real-time PCR). A significant induction of ANP and BNP was observed in rats with AAC, whereas melatonin-treated rat ventricular cardiomyocytes slight upregulated ANP and BNP in response to AAC. (***P <0.001 vs AAC, #P < 0.05 vs AAC+melatonin, all n=8).

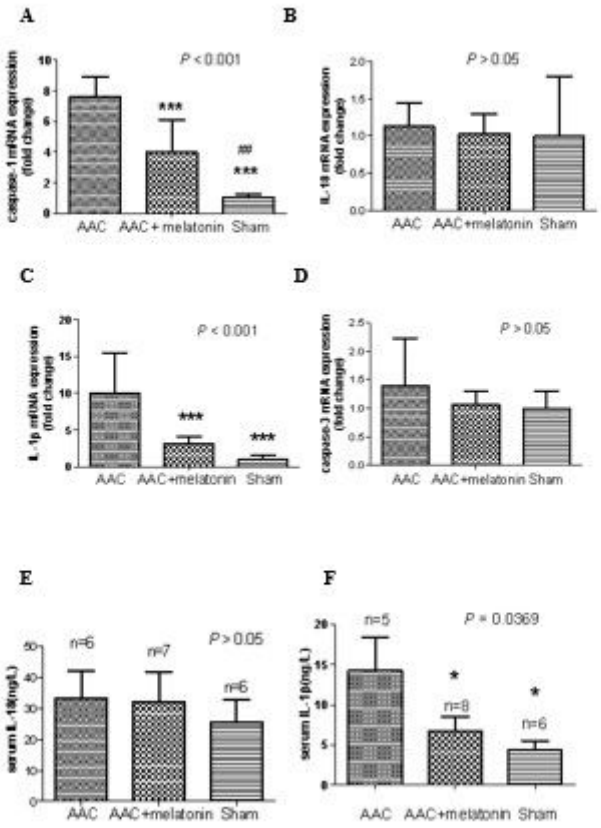

Figure 3

A, B, C and D. Relative mRNA expression levels of caspase-1, IL-18, IL-1ß and caspase-3 in AAC–treated juvenile rat ventricular cardiomyocytes (real-time PCR). A significant induction of caspase-1 and IL-1β was observed in rats with AAC, whereas melatonin-treated rat ventricular cardiomyocytes showed a slight upregulation of caspase-1 gene in response to AAC. But no significant differences in IL-18 and caspase-3 genes were detected among the three groups. (***P<0.001, vs AAC group; ##: P<0.01, vs AAC+melatonin group. all n=8). E and F. Respective serum IL-18 and IL-1ß levels in three groups of rats. (*P<0.05, vs AAC group).

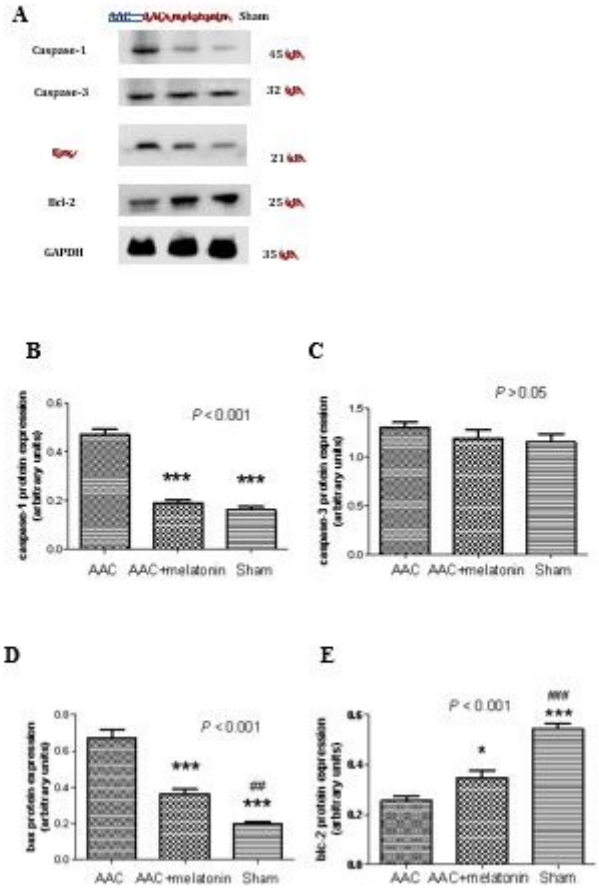

Figure 4

Relative caspase-1, caspase-3, bax and bcl-2 protein expression levels in AAC–treated juvenile rat ventricular cardiomyocytes (western-blot). The increased expression of caspase-1 and bax in dysfunctional cardiomyocytes in response to increased pressure overload was reversed by melatonin. The decreased expression of bcl-2 in dysfunctional cardiomyocytes due to increased pressure overload was converted by melatonin. Western blot analyses (A) and quantification of caspase-1 (B), caspase-3 (C), bax (D) and bcl-2 (E) in juvenile rat ventricular cardiomyocytes. (*: P<0.05, ***: P<0.001 vs AAC group; ##:P<0.01, ###:P<0.001 vs AAC+melatonin group, all n=8)

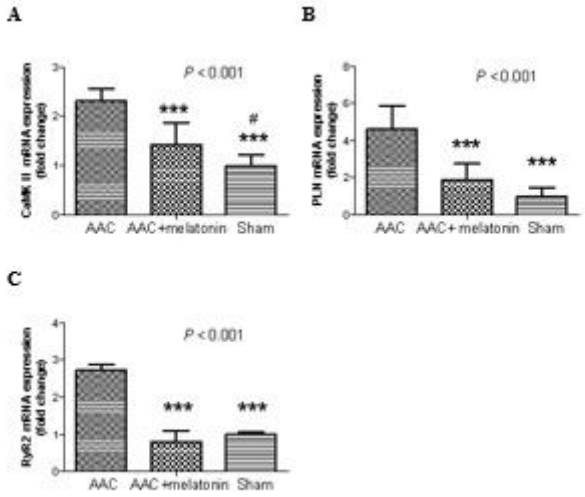

Figure 5

A, B and C. Relative CaMK, PLN and RyR2 mRNA expression levels in AAC–treated juvenile rat ventricular cardiomyocytes (real-time PCR). A significant induction of CaMK, PLN and RyR2 was observed in rats with AAC, which was reversed by melatonin. (***:P < 0.001 vs AAC group; #:P<0.05 vs AAC+melatonin group, all n=8).

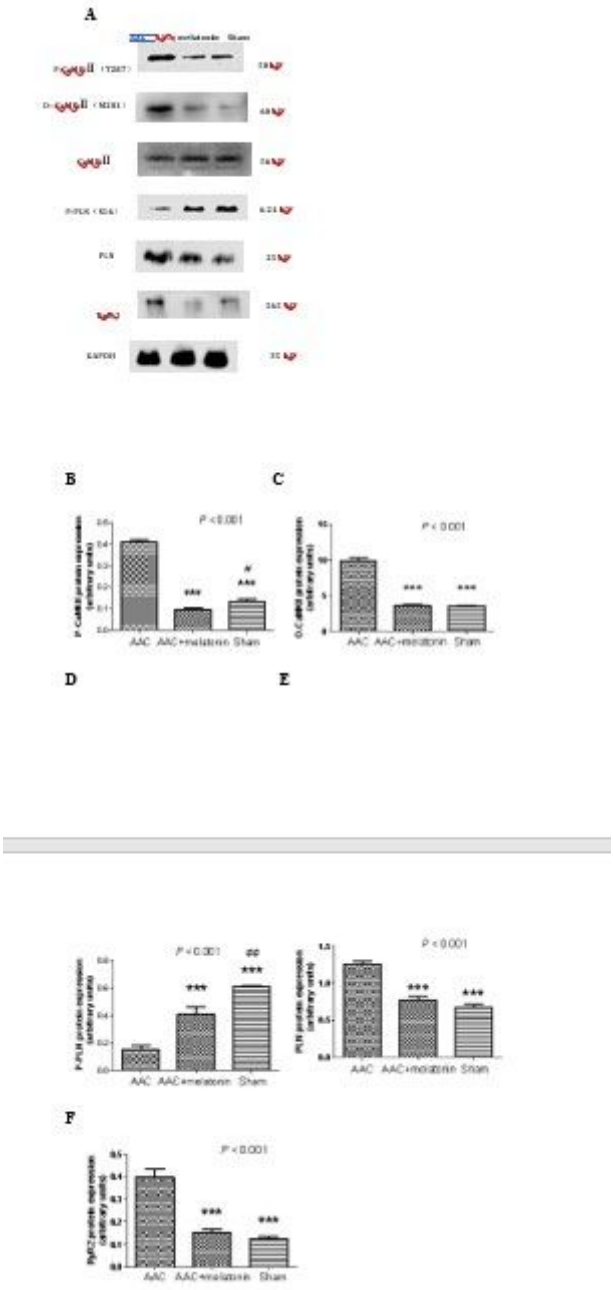

Figure 6

Relative CaMKII, PLN and RyR2 protein expression levels in AAC–treated juvenile rat ventricular cardiomyocytes (Western blot). The increased expression of P- CaMKII, O- CaMKII, PLN and RyR2 in dysfunctional cardiomyocytes due to increased pressure overload were protected by melatonin. The decreased expression of P-PLN in dysfunctional cardiomyocytes due to increased pressure overload was converted by melatonin. Western blot analyses (A) and quantification of the phosphorylation (B) and oxidation (C) of CaMKII, the phosphorylation (D) of PLN, total PLN (E) and RyR2 (F) in juvenile rat ventricular cardiomyocytes. (***: P<0.001 vs AAC group; #:P<0.05, ##:P<0.01 vs AAC+melatonin group, all n=8)

Supplementary Files

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