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Original Article

Moderate exercise mitigates cardiac dysfunction and injury induced by cyclosporine A through activation of the PGI_2 / PPAR-γ signaling pathway

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Abstract

Background and purpose: The present study investigated the role of the prostaglandin I_2/peroxisome proliferator activator receptor (PGI_2/PPAR) signaling pathway in cardiac cell proliferation, apoptosis, and systemic hemodynamic variables under cyclosporine A (CsA) exposure alone or combined with moderate exercises.
Experimental approach: Twenty-four male Wistar rats were classified into three groups, namely, control, CsA, and CsA + exercise.
Findings/Results: After 42 days of treatment, the findings showed a significant enhancement in the expression of the β-MHC gene, enhancement in protein expression of Bax and caspase-3, and a significant decline in the protein expression of Bcl-2 expression, as well as increased proliferation intensity in the heart tissue of the CsA group compared to the control group. Systolic pressure, pulse pressure, mean arterial pressure (MAP), QT and QRS duration, and T wave amplitude, as well as QTc amount in the CsA group, showed a significant increase compared to the control group. PPAR-γ and PGI_2 showed no significant changes compared to the control group. Moderate exercise along with CsA significantly enhanced the protein expression of PPAR-γ and PGI_2 and declined protein expression of Bax, and caspase-3 compared to those in the CsA group. In the CsA + exercise group, systolic pressure, MAP, and T_{wave} showed a significant decrease compared to the CsA group. Moderate exercises along CsA improved heart cell proliferation intensity and significantly reduced β-MHC gene expression compared to the CsA group.
Conclusions and implications: The results showed moderate exercise alleviated CsA-induced heart tissue apoptosis and proliferation with the corresponding activation of the PGI_2/PPAR-γ pathway.

Keywords: Cyclosporine; Exercise; Heart; PGI_2/PPAR signaling pathway; Proliferation.

INTRODUCTION

Cyclosporine A (CsA) is considered the backbone of immunosuppressive drugs and its application revolutionized the management of allotransplants and improved tissue survival in the early stages of transplantation (1). Despite this vital role of CsA in preventing transplanted organ rejection and increasing its survival, CsA chronic application is associated with hazardous side effects in various organs particularly in cardiac that limit its clinical administration (2,3). Pathological left ventricular abnormalities, including cardiomyocyte apoptosis (4-7), hypertension (8,9), and differentiation (6) have been reported by previous studies. In addition, enhanced systolic and diastolic blood pressure, and mean arterial pressure following exposure to CsA were also previously reported (8,10).

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Furthermore, CsA has already been reported to cause changes in myocardial size, shape, and organization (11). Moreover, some studies considered apoptosis to be responsible for the pathogenesis of myocardial changes (12). Although previous studies have identified different aspects of CsA-induced cardiac anomalies, to our knowledge, no evidence has been provided about the particular effect of CsA on the heart's structure and function as a molecular mediator. Nonetheless, studies have shown that peroxisome proliferator-activated receptors (PPARs) contribute to cell proliferation, differentiation, and apoptosis (13). PPAR belongs to the superfamily of ligand-activated transcription factor nuclear receptors and includes three isoforms, such as PPAR-α, PPAR-β/δ, and PPAR-γ (13). PPARs govern the expression of key molecules involved in the regulation of complex pathways of mammalian cells' metabolism, as a tumor suppressor gene (13). PPAR-γ is the most widely investigated subtype among three PPAR subtypes to date, performing significant maintenance through all various species that it is cloned (14). There is evidence that PPAR-γ regulates the expression of several genes associated with carcinogenesis (14). PPAR-γ ligands induce stunting growth apoptosis and differentiation in a variety of transformed cells. The PPAR-γ ligand has multiple prostanoids, including prostaglandin (PGI_2) (14). It has been documented that a single bout of exercise, as well as an endurance training program, increased the concentration of the PGI_2 metabolite, in urine, blood, or the interstitial fluid of muscles (15). The expression of the myosin heavy chain isoforms gene (MHCs) is another major mediating factor that has a considerable effect on cardiac functions. The isoforms α and β-MHC are documented as significant molecules involved in the contractile function as well as the energy expenditure in the heart. Shifting gene expression of these isoforms has been identified as a harmful molecular shift in heart failure and has been detected in pathological conditions such as hypertrophy induced by volume overload, diabetes, and hormonal changes (15,16). The isoforms α and β-MHC play a fundamental role in the gene expression transition in the initiation and development of heart abnormalities and heart failure (16). Hence, the current research intended to assess the possible adverse impacts of chronic CsA ingestion on the heart tissue of male rats, in terms of functional, histological, and molecular levels 42 days after treatment. In the current study, digital wave analyses were used for evaluating hemodynamic variables. In addition, it tried to investigate whether moderate aerobic exercises can sedate the structural alternations of the heart and molecular changes abnormalities and its corresponding molecular mediators (PGI_2/PPAR-γ pathway and MHC isoforms gene expression alteration) induced by chronic CsA exposure in rats' hearts.

MATERIALS AND METHODS

Animals

Twenty-four male Wistar rats were purchased from the animal house of Urmia University of Medical Sciences. All animal experiments were conducted after confirmation of the first local ethics committee on animal experiments at the Mohaghegh Ardabili University in Iran, based on the principles of laboratory animal care (Ethic No. IR.UMA.REC.1400.032).

Chemical and kits

CsA was purchased from Sandimmune®, New Jersey, USA. A special kit for total RNA extraction was purchased from Gene All South Korea, Cat no 305-101. A universal cDNA synthesis kit for synthesizing the complementary DNA was prepared from Ampliqon Co, Denmark. The chemiluminescence detection kit was purchased from ECL; Thermo Scientific, Illinois, USA. The anti-proliferating cell nuclear antibody (PCNA) antibody was purchased from Dako Denmark A/S, Denmark. The protease inhibitor cocktail kit was prepared from Sigma-Aldrich S8820, USA.

Study design

Twenty-four male Wistar rats (220 ± 20 g) were randomly categorized into three groups (n = 8) including control, CsA, and CsA-exercise groups. Rats in the CsA group were

treated with CsA (30 mg/kg body weight) solute in tap water once daily *via* intragastric gavage for six weeks (17). Rats in the training group were acquainted with how to work on the treadmill and also the training protocol for a week according to Table 1. The severity of rats' exercise was controlled using a treadmill following their running speed and observing the principle of gradual increase of the load according to Table 1 (18). After 42 days of treatment, the animals were weighed and then anesthetized with a mixture of ketamine (10%, 80 mg/kg BW, ip) and xylazine (2%, 10 mg/kg BW, ip) for further processing.

Blood pressure and electrocardiography

Systemic hemodynamic variables and electrocardiogram were determined by the digital waveform contour analyzing method directly recording from the carotid artery using a physiograph (NARCO, Bio-system, USA). The detail of the procedure was previously described in our recently published article (19). Data or recorded digital volume pulses were investigated by Pawerlab Software (ADInstruments, Australia).

Tissue sampling

After blood pressure recording (only in PN90 pups), the thoracic cavity was opened and the heart was removed. Then, the left ventricle and septal muscle bands were excised from the heart, and part of the left ventricular tissue was fixed in 10% formalin embedded in paraffin and sectioned at 4.5 µm for histological examination. The other part of the left ventricle tissue was kept at -70 °C.

Primer design and real-time polymerase chain reaction

A special kit was used to extract the total RNA following the protocol published by the manufacturer. A spectrophotometer at the absorbance of 260-280 nm was used to confirm the concentration of RNA; then, it was assessed using a combination of tris-acetic-ethylenediaminetetraacetic acid (EDTA) (TAE)-agarose gel electrophoresis. A universal complementary DNA (cDNA) synthesis kit was used to synthesize the cDNA from the total RNA extracts, which was performed according to the manufacturer's instructions. The gene runner software was used to investigate the specific primers (shown in Table 2) of the target genes and the hypoxanthine guanine phosphoribosyl transferase gene, as a housekeeping gene (20). The $2^{-(\Delta\Delta Ct)}$ technique was used to assess the relative quantitative expressions of the genes. The data were expressed as the fold difference to the reference gene.

Table 1. Exercise protocol in the training groups.

Week	Speed (m/min)	Training duration (min)
Acquaintance	5-8	5-10
First	9	15
Second	12	20
Third	15	25
Fourth	18	30
Fifth	18	30
Sixth	18	30

Table 2. Primer sequences for each of the genes in the studied groups.

Gene	Sequence (5´-3´)	Annealing temperature (°C)
α-MHC	Forward: AGAGTGACAGGATGACGGAT Reverse: TCTTGCCGTTTTCAGTTTCG	62.2
β-MHC	Forward: GCCAAGAGCCGTGACATTG Reverse: GGCTTCACAGGCATCCTTAG	63.5
HPRT	Forward: CTCCTCAGACCGCTTTTCCC Reverse: TAATCACGACGCTGGGACTG	65

MHC, Myosin heavy chain; HPRT, hypoxanthine guanine phosphoribosyl transferase

Western blot

The western immunoblotting was conducted according to the previously mentioned protocol (21). In short, homogenization of the cardiac tissue was performed in the RIPA lysis buffer and the protease inhibitor cocktail to extract the proteins. The total protein content of the heart was measured using the bovine serum albumin (BSA) method. Afterward, dilatation of protein samples was performed in loading buffer and heated at 95 ℃ for 5 min and separated by electrophoresis on appropriate sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for each protein at 120 V. Next, all proteins were transferred to a polyvinylidene fluoride (PVDF) membrane at 100 V for 1-2 h. Then, 5% non-fat milk buffer was used (overnight) to incubate the PVDF membranes to block the endogenous peroxidases. The membrane was then rinsed with buffered tris saline (pH 7.2) and incubated for 2 h at 4 ℃ with anti-PCNA and anti-antibodies. After removing unbound antibodies by rinsing buffer, the membranes were incubated with the horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. Afterward, an enhanced chemiluminescence detection kit was used to visualize the blots. Eventually, an Arash Teb Pishro (ATP) enhanced laser densitometer (Tehran, Iran) was used to analyze protein intensities. The antibodies dilution for Bax, Bcl-2, caspase-3, PGI2, and PPAR were 1:1000, 1:1000, 1:1000, 1:1000, and 1:1500, respectively.

Immunohistochemical staining for the PCNA protein

The tissue sections were de-paraffinized in xylene (2 changes) and rehydrated by declining degrees of ethanol (90%, 80%, 70%, 50%) for immunohistochemical staining. After rehydration, 10 mM sodium citrate buffer was used to perform the antigen retrieval process was performed in (pH 7.2). Also, a peroxidase-blocking solution (0.03% hydrogen peroxide-containing sodium acid; for 5 min) was used to block the endogenous peroxidase. Afterward, the slides were incubated overnight using the monoclonal anti-PCNA antibody as a primary antibody (at 4 °C). A sufficient amount of streptavidin-HRP for 20 min was used to incubate the sections. Positive reaction sites for the target proteins were visualized by incubating diaminobenzidine sections. Hematoxylin staining dye was employed to counterstain the slides before permanent mounting. A light microscope (Olympus, BH2, Japan), equipped with a SONY onboard camera (Zeiss, Cyber-Shot, Japan), was used to examine the slides. Adobe Photoshop CS10 (Adobe System Inc., Mountain View, CA, USA) was used to compile the captured figures. The software analyses for pixel-based intensity were evaluated using image pro-insight software (version 9.00).

Data analysis

The data analysis was displayed using SPSS (version 23.0; IBM Corp, Chicago, USA). One-way ANOVA and Tukey's post hoc tests were used to make inter-group comparisons. The data were presented as mean ± SEM. *P*-values < 0.05 indicated significant differences.

RESULTS

Hemodynamic variable changes

Figure 1 shows the basic characteristics of the systemic hemodynamic variable obtained by contour analysis of the digital volume pulse among the different groups of studies. No significant differences were observed between the data obtained from various heart rate assessment groups. Systolic blood pressure and pulse pressure revealed a significant enhancement in the CsA-treated group compared to controls ($P < 0.03$). In the exercise group, systole pressure and pulse pressure were significantly lower than that in the CsA-Exe group ($P < 0.029$). Diastole pressure, mean arterial, and dicrotic pressure showed no significant differences among the study groups.

Electrocardiographic variable alteration

Electrocardiographic variable changes are shown in Fig. 2. The QT and QRS complex duration showed a significant enhancement in the CsA and CsA-Exe groups compared to the control groups. As can be seen in Fig. 2, in the CsA-Exe group, the duration of QT and QRS showed no significant changes compared to the CsA group.

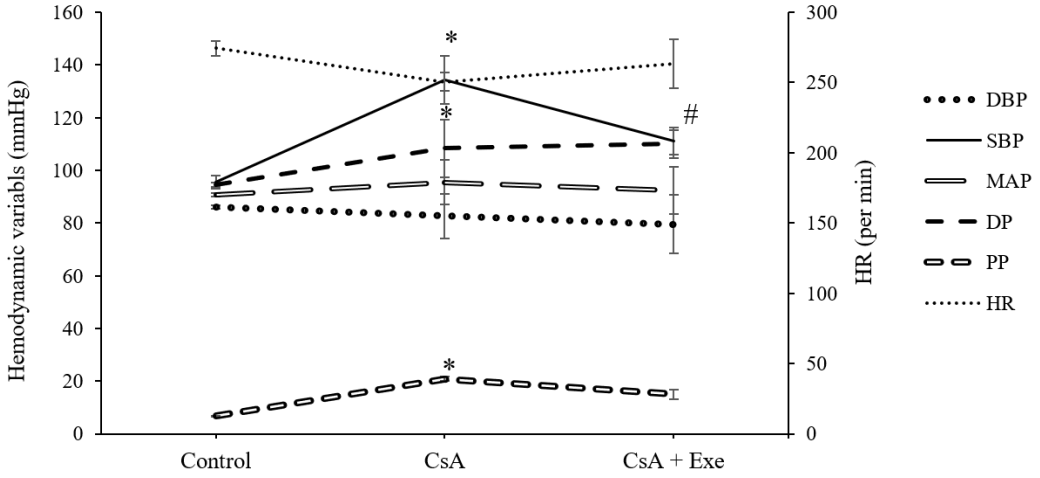

Fig. 1. The effect of chronic administration of CsA (30 mg/kg/day for 6 weeks, gavage) and CsA along with moderate exercise on the HR, SBP, DBP, MAP, PP, and DP in male rats (n = 8). Systemic hemodynamic variables were determined by the digital waveform contour analyzing method directly recording from the carotid artery. Data are given as mean ± SEM. *$P < 0.05$ Indicates significant differences compared to the control and $^{\#}P < 0.05$ versus the CsA group. CsA, Cyclosporine A; Exe, exercise; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; DP, dicrotic pressure.

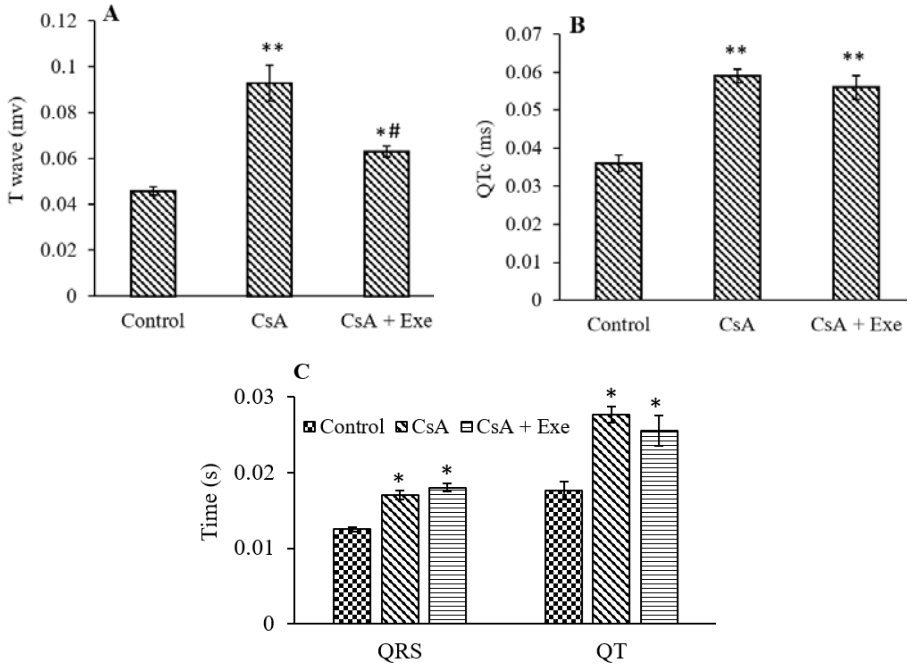

Fig. 2. Comparisons of electrocardiogram parameters including (A) T wave, (B) OTc, and (C) QRS and QT between the CsA administration group (30 mg/kg/day for 6 weeks, gavage) and CsA along with moderate exercise group in the male rats (n = 8). Data are presented as mean ± SEM. *$P < 0.05$ and **$P < 0.01$ indicates significant differences compared to the control and $^{\#}P < 0.05$ versus the CsA group. CsA, Cyclosporine A; Exe, exercise.

The T wave amplitude increased significantly in the CsA group compared to the control. In the CsA-Exe group, T wave amplitude revealed a significant decrease in comparison with the CsA group. However, it was still significantly more than the controls. The QTc of CsA and CsA-Exe rats were significantly higher than controls.

Protein expression of PGI_2, PPAR-γ, Bax, Bcl-2, and caspase-3 in cardiac tissue

Protein expression of Bax, Bcl-2, caspase-3, PGI_2, and PPAR-γ in cardiac tissue was examined by western blot, and the findings are provided in Figs. 3 and 4. CsA exposure caused a significant increase in Bax and cleaved caspase-3 protein expression and a decline in Bcl-2 expression in the cardiac tissue of rats compared with the respective control groups. Bax and cleaved caspase-3 protein expression revealed no significant difference between CsA-Exe and controls (Fig. 3). In the CsA-Exe group, a significant enhancement in the expression of Bcl-2 was observed in the left ventricle of rats compared to the CsA group. However, in the CsA-Exe group, the expression of Bcl-2 was still significantly lower compared to the control group (Fig. 3). The Bax/Bcl-2 ratio revealed also a significant enhancement in the CsA group in the heart of male rats compared to controls. Moderate exercise along with CsA significantly lowered the Bax/Bcl-2 ratio compared to the CsA (Fig. 3). However, the Bax/Bcl-2 ratio in the CsA-Exe group is still significantly higher than the control group. Western blot analysis indicated that PPAR-γ and PGI_2 protein expression revealed no significant changes in the CsA group compared to control, whereas they significantly enhanced in the CsA-Exe group compared to the CsA and controls (Fig. 4).

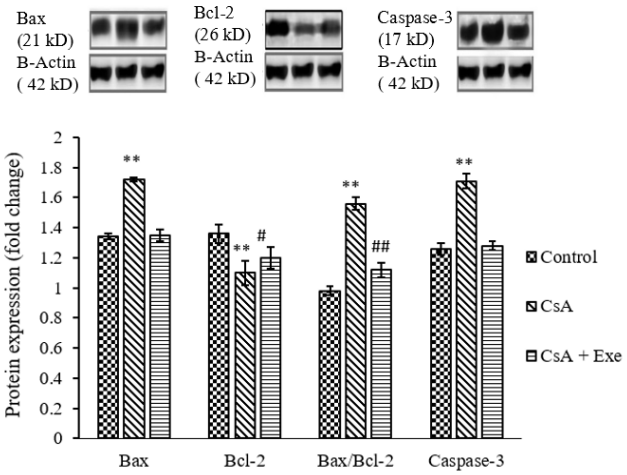

Fig. 3. The influence of CsA exposure and CsA along with moderate exercise on the protein levels of Bax, Bcl-2, Bax/Bcl-2 ratio, and caspase-3 in the male rats' heart tissue. CsA induced apoptosis in rats cardiomyocytes. The expression of Bax, Bcl-2, and cleaved caspase-3 was examined by western blot. Data are presented as mean ± SEM. $^{**}P < 0.01$ Indicates significant differences compared to the control and $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ versus the CsA group. CsA, Cyclosporine A; Exe, exercise.

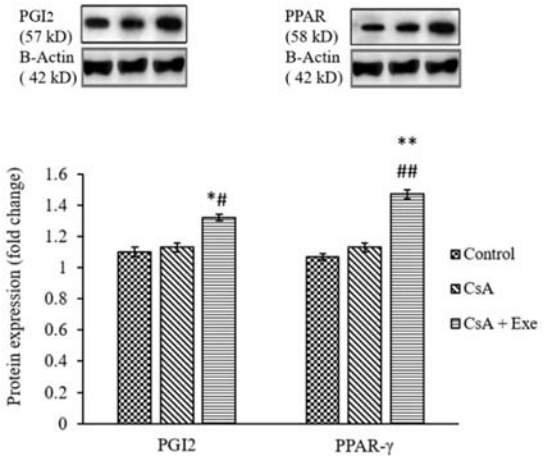

Fig. 4. The influence of CsA administration and CsA along with moderate exercise on the PGI_2 and PPAR-γ protein expression levels, detected by western blot, in the heart tissue of male rats (n = 8). Data are expressed as mean ± SEM. $^{*}P < 0.05$ and $^{**}P < 0.01$ indicate significant differences compared to the control and $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ versus the CsA group. CsA, Cyclosporine A; Exe, exercise; PGI_2, prostaglandin I_2; PPAR-γ, peroxisome proliferator activator receptor.

mRNA expression of MHC isoforms (α and β) in the cardiac ventricle

Administration of CSA significantly increased the expression of β-MHC mRNA in the left ventricular of the rats when compared with the control group. There was no significant difference in the expression of β-MHC mRNA between the Csa-Exe group and the control groups. The expression of α-MHC mRNA showed no significant changes among the various groups (Fig. 5).

Expression of the β-MHC mRNA/ α-MHC mRNA ratio revealed a significant enhancement in the CsA group in comparison with the control group. In the CsA-Exe group, the ratio between β-MHC mRNA expression and α-MHC mRNA expression revealed a significant decline relative to the CsA group.

Histological changes

Figure 6 displays nuclear cell proliferation intensity analyzed by Image-Pro Insight software. According to the results, using CsA revealed a significant enhancement in the intensity of cell proliferation than the control. Exercise with CsA exposure reduced proliferation intensity compared to the CsA group.

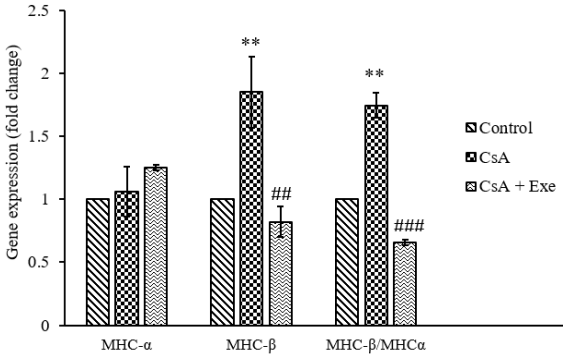

Fig. 5. Effect of CsA administration and CsA along with moderate exercise on the mRNA expression levels of MHC isoforms and MHC-β/MHC-α ratio, quantified using real-time-polymerase chain reaction, in the heart tissue of male rats (n = 8). Data represent mean ± SEM. $^{**}P < 0.01$ Indicates significant differences compared to the control and $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ versus the CsA group. CsA, Cyclosporine A; Exe, exercise; MHC, myosin heavy chain.

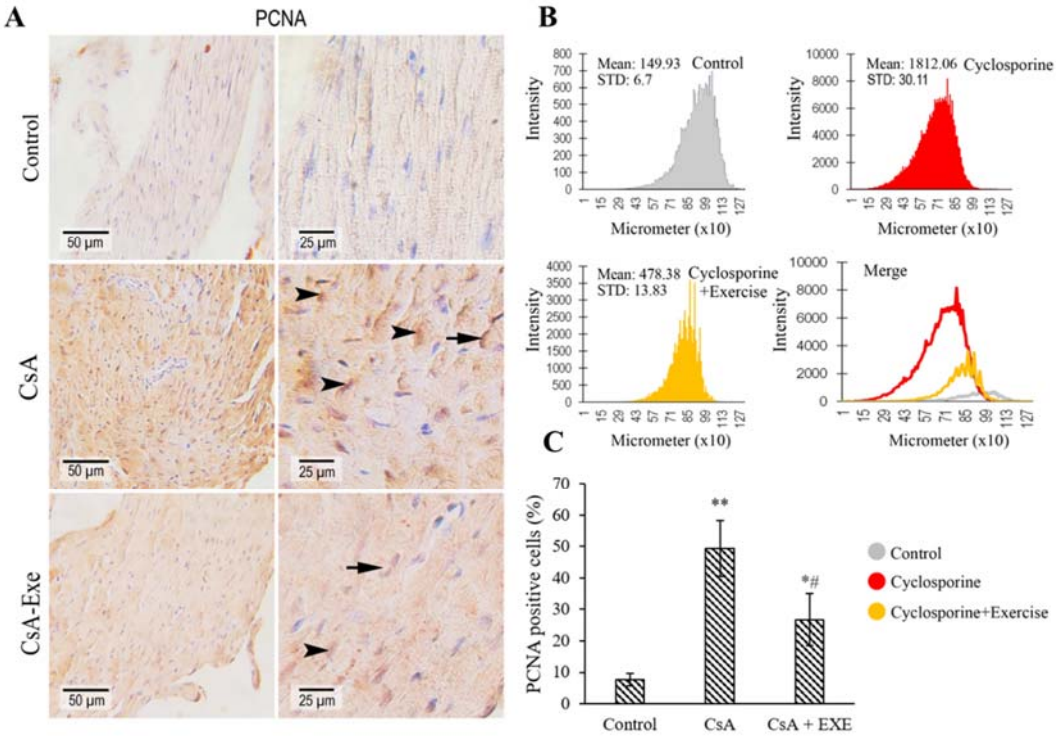

Fig. 6. (A) Immunohistochemical staining of PCNA in a section of male rats' cardiac tissues. An up-regulated expression of PCNA protein was represented with increased $PCNA^+$ cells (arrowhead) *versus* $PCNA^-$ (arrows) in the section from the cyclosporine group (25 µm). A significant reduction in the PCNA expression in the CsA + Exe group compared to the cyclosporine group (25 µm) and (B) pixel-based intensity analysis for brown reaction (representing PCNA) in 1270 µm × 1270 µm of tissue in different groups, (C) mean percentages of PCNA+ cells per 1 mm^2 of tissue in different groups; data are presented as mean ± SEM. $^{**}P < 0.01$ and $^{***}P < 0.001$ indicates significant differences compared to the control and $^{\#\#}P < 0.01$ versus the CsA group. CsA, Cyclosporine A; Exe, exercise; PCNA, anti-proliferating cell nuclear antibody.

DISCUSSION

Giving the importance of CsA in autoimmune disease and organ transplantation, researchers are trying to reduce the side effects of CsA. For this purpose, in recent years, approaches such as the administration of antioxidants (11,22), adjuvant drugs (23,24), and regular exercise (25,26), are used to reduce the side effects of CsA, but the agreeable achievement has not yet been yielded due to its complex pathological mechanism. The results of the current study showed that administration of CsA led to some abnormalities in electrocardiogram including a widening of QRS and QT duration, and increased T-wave amplitude as well as QTc. Similar to our findings, previous reports have demonstrated that chronic CsA administration induced signs of myocardial ischemia, which was evidenced by QTc prolongation and increased T-wave amplitude (27). As well as, enhancement in QT and T peak trend intervals found in CsA-treated male rats associated with enhanced propensity to left ventricular ischemia, delayed ventricular repolarization, and arrhythmogenesis (27). In addition, the QT interval as an indicator of ventricular electrical depolarization and repolarization was significantly prolonged in rats exposed to CsA, suggesting that CsA causes a slower rate of ventricular depolarization and repolarization. In the current study, the digital waveform contour analysis was recorded directly from the carotid and analyzed. Accordingly, CsA exposure significantly increased systolic pressure, and pulse pressure, with no changes in heart rate, diastolic pressure, mean arterial pressure, as well as dicrotic pressure. Studies from different researchers reported ambiguous results about the effect of CsA administration on blood pressure variables. In contrast to our study, some researchers indicated that CsA exposure in animal and human studies led to a significant enhancement in systolic, diastolic, and mean arterial pressure (8,28). Similar to our results, a study by Kingma *et al*. showed that daily treatment of CsA for three weeks failed to significantly alter mean arterial and diastolic pressures (9,29). In another study, CsA exposure did not affect blood pressure variables (30). The controversy among different studies and our results may be due to age, gender, dosage, administration duration, and technical differences in the method of blood pressure measurement. Previous studies used tail-cuff in animal studies and brachial artery-cuff methods in human studies to obtain blood pressure-related variables (31,32), but we took advantage of the digital counter analysis of hemodynamic variables using carotid arterial cannulation. Even though we know using this method is aggressive, but obtained results are more informative and reliable than results collected by the traditional methods such as tail-cuff methods. We monitored the apoptotic signals associated with CsA, such as an increase of Bax and cleaved caspase-3, and a decrease in Bcl-2, as well as an increase in Bax/Bcl-2 ratio. These apoptosis signals present in CsA-treated rats were reversed by moderate exercise. These data are consistent with previous studies indicating that CsA, induced apoptosis in cardiomyocytes (4,6), and regulating exercise can prevent the activation of the Bax and caspase-3, which contributes to the apoptosis cascade (33). In the exercise group, along with apoptotic signal expression suppression, the endogenous PGI_2 and PPAR-γ protein expression showed a significant increase. The association between training such as regulated exercise or marathon runs and an increase in systemic PGI_2 is very well established by previous studies (15,34). Our previous study and others proved that CsA's deleterious side effect is mediated by oxidative stress and inflammatory reaction (1,35,36). Since oxidative stress and ROS generation are well-known apoptotic stimulator, on the other hand, it has been reported that PGI_2 possesses ROS scavenging potential, with acknowledging previous studies, we suppose that protection against apoptosis through the inhibition of ROS generation is one of the protective mechanism of moderate exercise that can be mediated by PGI_2 (37). Moreover, studies also reported that inhibition of ROS by PGI_2 is mainly because of the enhanced activity of catalase and superoxide dismutase enzymes; induced by augmentation of cellular PGI_2 (38). Furthermore, the growing body of evidence indicated that regular exercise can improve myocardial oxidative metabolism, ventricular function, coronary circulation (39), and immune function (26), as well as, enhance antioxidant enzyme activity (25). Another mechanism by which PGI_2 shows its anti-

apoptotic effects is in part the activation of PPAR-γ as a nuclear receptor by PGI_2. Antioxidant and anti-apoptotic effects of PPAR-γ and its agonists have been reported in previous works (40). PPAR-γ can regulate apoptosis and inhibit the proliferation of endothelial cells, which is an important contributing factor to heart failure (41). Overexpression of PPAR-γ was also evidenced to inhibit proliferation, myofibroblast transformation, and extracellular matrix overproduction in cardiac fibroblast in response to chronic pressure overload myocardium and angiotensin II (42-44). Moreover, numerous studies indicated that activation of inflammatory signalling pathways and overexpression of inflammatory factors led to failed hypertrophied myocardium (45,46). It is established that the PPAR family particularly PPAR-γ possesses the potential capacity to decline inflammation caused by their trans-repressive impact on the pro-inflammatory transcriptional factors' expression (47). Furthermore, PPAR-γ has anti-fibrotic and anti-hypertrophic effects on the ventricular tissue. According to the findings, PPAR activation can suppress collagen (types I and III) expression, inhibit angiotensin-converting enzyme-induced fibrosis, and suppress endothelin-1 and nuclear factor kappa B function. However, PPAR-γ also regulates the ability to infiltrate T cells by reducing the release of fibrosis-promoting factors. Some studies have also shown that activation of PPAR-γ induces the insulin-like growth factor-1/phosphoinositide 3-kinases survival pathway under hemodynamic stress, enhances the expression of Bcl-2, and downregulates the expression of Bax protein (48-50). Interestingly, current study results showed that co-administration of moderate exercise training and CsA activates the PGI_2 / PPAR-γ and reduced significantly cell proliferation and apoptotic signal alteration in the ventricular of rats. To our knowledge, this is the first *in vivo* study to show that moderate exercise is not only a non-expensive and but also easy way to reduce CsA-induced serious side effects in heart tissue. Another point of interest in the findings of the current study is that CsA administration significantly increased ventricular β-MHC mRNA expression and β-MHC / α-MHC ratio with no change in α-MHC mRNA expression. It has been previously reported that up-regulation of β-MHC mRNA expression caused heart failure in adult rats (51,52). Furthermore, previous observations indicated that ischemic and dilated cardiomyopathies are associated with the increased expression of β-MHC (53). Similarly, increased β-MHC mRNA expression could provide an early and good predictor of ventricular dysfunction and decompensated heart failure (53). This study showed that exercise, when used in combination with CsA, reduced ventricular expression of β-MHC mRNA and β-MHC / α-MHC ratio in rats. CsA consumption impairs the cellular energy balance as well as ATP generation and availability for cells. In the case of heart tissue, heart tissue's daily ATP intake is fivefold more than its mass (54). The daily high ATP intake by heart tissue is necessary for the maintenance of cellular ionic homeostasis and rhythmic contraction (54). A great part of this ATP is consumed by MHC isoforms to form strong cross-bridges with actin for the production of the power stroke. In contrast to α-MHC, β-MHC has a lower ATPase function and lower actin filament contractile velocity (55). Hence, the transition of gene expression from α to β-MHC isoform causes compensatory/adaptive alternation in cases where the ATP level is insufficient, and it is rooted in the association between a greater economic force generation than α isoforms (56). Overall, a shift to a low ATPase function myosin isoform is defined as an enhancement in the economy of the contraction process. Nevertheless, this shift at the same moment implies that the contraction process is slowed down and chronic CsA treatment has restricted the spectrum for adaptation of contractile performance (57).

CONCLUSION

Our work launched some precious points on the mechanism underlying CsA-induced cardiotoxicity and the protective effects of moderate exercise as follows. First, exposure to CsA led to heart tissue apoptosis and proliferation concurrent with systemic hemodynamic variable alteration and abnormality in the electrocardiogram. The second point is that CsA exposure altered the expression of genes that contribute to the regulation of contractility or hypertrophy

indicated by increased β-MHC mRNA expression and increased β-MHC / α-MHC ratio. Thirdly, it was found that moderate aerobic exercise concurrent with CsA masked heart tissue proliferation and apoptosis by activation of the PGI2 / PPAR-γ signaling pathway, the pathway that was established as an anti-apoptotic and anti-proliferative pathway. The fourth point we established is that moderate aerobic exercise eliminated MHC gene expression transition, systemic hemodynamic variable, and abnormality in electrocardiogram induced by CsA. Nevertheless, future studies should investigate the details of the subjects. Those researches may disclose easy ways for developing therapies with high therapeutic benefits and low side effects of CsA.

Acknowledgments

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Conflict of interest statements

The authors declared no conflicts of interest in this study.

Authors' contributions

K. Nourmohammadi contributed to conceptualization, methodology, and software; A. Bayrami contributed to data curation, preparation and writing the original draft; R. Naderi contributed to visualization and investigation; A. Shirpoor supervised the study; H. Soraya conceived and designed the experiments. The finalized article was approved by all authors.

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