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Protective effects of tropisetron on diabetes-induced cardiomyopathy and apoptosis in the left ventricle of rats

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Abstract

Tropisetron, an antagonist of 5-HT3 receptor (5-HT3R), has exhibited a number of beneficial effects on the treatment of several diseases; however, its effect on diabetic cardiomyopathy, which is an important causal factor in morbidity and mortality among diabetic patients, remains to be fully elucidated. Therefore, this study was designed to investigate the effect of tropisetron on diabetes-induced cardiomyopathy and the molecular mediators possibly involved. Twenty-four male wistar rats were assigned into three groups, control, diabetic, and tropisetron–treated diabetic groups. After 14 days of treatment, the results revealed a significant increase in calcium/calmodulin-dependent protein kinaseIIδ (CaMKIIδ) total and isoforms δ2 and δ3 of CaMKIIδ, as well as myosin heavy chain (MHC)-β. Furthermore, it decreased MHC-α gene expression among the diabetic group compared to the control group. Western blot analysis showed a significant increase in Bax and cleaved caspase 3 protein levels and a significant decrease in Bcl-2 contents in heart tissue of diabetic rats in comparison to the control rats. Moreover, the levels of Tumor necrosis factor alpha (TNF-α), ICAM-1, and CaMKII in heart tissue of diabetic rats were significantly higher than those in the control rats. Significant amelioration of alteration in the genes expression and protein changes along with restoration of the elevated levels of TNF-α, ICAM-1, and CaMKII were found in the tropisetron–treated diabetic group compared to the diabetic group. Collectively, our study provided strong evidence that 5-HT3 antagonist tropisetron was capable of attenuating the development of experimental diabetic cardiomyopathy associated with the reduction of intramyocardial MHCs and CaMKIIδ gene expression, inflammation and cardiac hypertrophy.

Keywords: diabetes, apoptosis, MHCs, CaMKIIδ, tropisetron

1. Introduction

Diabetes Mellitus is a complex endocrine disorder resulting from a combination of genetic, environmental and immunological factors. Diabetic cardiomyopathy is the main type of cardiovascular disease that is manifested as a structural and functional alteration, and it is considered to be independent of macrovascular / microvascular disorders, including coronary artery disease and hypertension (1, 2). Abnormal cellular changes, such as cardiac hypertrophy, inflammation, fibrosis, and apoptosis are presented as important features of diabetic cardiomyopathy leading to diastolic and systolic dysfunction, and finally resulting in heart failure (3). The pathophysiology of diabetic cardiomyopathy is complex and multifunctional, and little is known about the mechanism of cardiac damage associated with diabetic situation (4). However, the role of hyperglycemia, hyperlipidemia, oxidative stress and inflammation in diabetes has been proven and reported as pathogenic parameters in various cardiovascular anomalies including cardiomyopathy (5, 6). Considering the above mentioned issue, lowering blood sugar and blood lipids, antioxidant and anti-inflammatory drugs, and appropriate physical activity can be useful in preventing and reducing diabetic complications including cardiomyopathy (7, 8). Although, according to previous studies, different components have been used to reduce the complications of diabetes, researchers are looking for new pharmaceutical and non-pharmaceutical substances in this case. The activation of the sympathetic nerve of the heart is a very important factor in the development of cardiac hypertrophy and ultimately heart failure, and it induces apoptosis, increases oxidative stress, and so on, through the activation of renin-angiotensin system (9-11). Therefore, the use of sympathetic nervous system antagonists is a valuable treatment for hypertrophy and cardiac failure. Serotonin is a neurohormone that has many physiological and pathophysiologic effects. It contains seven receptors including 5HT1-7, all of which are G-Protein receptors except for the 5-HT3, which is a ligand-gated ion channel. This receptor plays an important role in activating

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the sympathetic nervous system (12, 13). Cardiovascular responses enhanced by activation of the sympathetic nervous system reflex led to cardiac hypertrophy and ultimately cardiac failure (14). Activation of 5-HT3 receptors stimulates the reflexes of the sympathetic nerves, and also, it releases noradrenaline from the adrenal gland and the sympathetic nerve end, which in turn causes heart damages (15, 16).

In the current study, the fundamental role of CaMKIIδ, MHCs and inflammation in initiation and development of cardiomyopathy and heart failure tempted us to examine the following issues: we examined the effect of tropisetron on diabetic heart hypertrophic indexes including left ventricular weight, L.V.W / B.W and L.V.W / H.W. The second aim was to find out whether tropisetron mitigated the gene expression alteration of CaMKIIδ isoforms and MHCs in the diabetic heart. Investigating the effects of tropisetron on pro and anti-aopototic protein expression were our third aim in the current study. Finally, alleviating effects of tropisetron on heart tissue TNF-α, ICAM-1 and CaMK amounts were also investigated in the current study.

2. Materials and Methods

All procedures followed during this examination conformed to the ‹National Health and Medical research Care published by the National Institutes of Health (NIH publication, no.85–23, revised 1985), and the experimental protocol approved by the Animal Care Committee of Urmia University of Medical Sciences. Twenty-four male wistar rats (body weight: 240 ± 20 g) were maintained under standard laboratory conditions, having ad libitum access to standard laboratory chow and water. After one-week of acclimatization, rats were randomly divided into three experimental groups (n = 8 in each group): control, diabetic, and tropisetron–treated diabetic groups. Diabetes was induced by intraperitoneal injection of 55 mg/kg B.W, streptozotocin (STZ; Sigma, St Louis, Mo, USA) diluted in 0.1 mol/citrate buffer pH 4.5. The control rats received an equal volume of citrate buffer. The diabetic condition was determined 72 h later by measuring tail vein blood glucose using glocometer (Boehringer Mannheim Indianapolis); rats with a blood glucose concentration higher than 300 mg/dl were considered diabetic. Rats in the tropisetron–treated diabetic group received tropisetron with a dose of 3mg/kg body weight (Cayman Chemical Co, USA) saluted in normal saline (20% W/V) intraperitoneally once a day, for 14 days. The control rats received an equal volume of normal saline. After 14 days of treatment, the rats were anesthetized by ketamine (10%, 80 mg/kg B.W, IP) and xylazine (2%, 10 mg/kg B.W IP).

Following test termination, the animals were weighted out and anesthetized by ketamine (60 mg/kg) and xylazine (6mg/kg). The hearts were dissected out and freed from adipose and connective tissues for later measurements. Then we excised left ventricular wall (with septum) from the heart and weighed. Due to total RNA isolation, 100 mg of ventricular tissue was homogenized and immersed in one ml RiboxEX (GeneALL, Seoul, Korea) and kept at -80°C, up to the time of RNA isolation. The other parts of the tissues were rinsed and dried for biochemical analysis. In continue, we added the extraction buffer (10% wt / vol) containing a 50 mM phosphate buffer (pH 7.4) and subsequently homogenized in homogenizer namely Ultra Turrax (T10B, IKA, Germany). Next, the products were centrifuged at 10,000 × g at 4°C for 20 minutes. The collecting supernatant was stored at -80°C, up to the time of measurment. The other part of each left ventricular tissue was kept in the deep freeze for apoptotic protein detection by western blotting method.

2.1. Biochemical examinations

The TNF-α, ICAM-1, and CaMKII contents in the heart tissue were measured conducting the quantitative sandwich enzyme immunoassay method using a commercial rat TNF-α, ICAM-1(Shanghaicrystal Day, Biotech, China), and CaMKII (ZellBio, Germany) kit, following the protocol provided by the manufacturer.

Table 1. Sequences of primers used to evaluate expression of GAPDH, CaMKIIδtotal, and CaMKIIδ1, CaMKIIδ2, as well as MHC ispforms

Target Gene	Primer sequence	Product size
$CaMKII\delta_{total}$(forward)	5′-TGG CAA ACT AAA GAG GGA GC-3′	**199**
$CaMKII\delta_{total}$(reverse)	5′-CCA AAA TCC CAA TGA GAA GCC C-3′	
$CaMKII\delta_2$(forward)	5′-AAC CGG ATG GGG TAA AGG AG-3′	**230**
$CaMKII\delta_2$(reverse)	5′-CAA TGC TTC GGG TTC AAA GG-3′	
$CaMKII\delta_3$(forward)	5′-CGG ATG GGG TAA AGA AAA GG-3′	**164**
$CaMKII\delta_3$(reverse)	5′-CTC GAA GTC CCC ATT GTT GA-3′	
MHC-α(forward)	5'-AGA GTG ACA GGA TGA CGG CG-3'	**213**
MHC-α(reverse)	5'- TCT TGC CGT TTT CAG TTT CG-3'	
MHC-β(forward)	5'- CCA GAC AGA GGA AGA CAG GAA-3'	**280**
MHC-β(reversed)	5'- CAT CCT TAG GGT TGG GTA GCA-3'	
GAPDH (forward)	5′-AGA CAG CCG CAT CTT CTT GT-3′	**207**
GAPDH (reverse)	5′-CTT GCC GTG GGT AGA GTC AT-3′	

2.2. Quantitative real time PCR and RT-PCR

Total RNA was extracted using an extraction kit (Gene All, South Korea), according to the manufacturer's protocol. The real-time quantification of the target genes was carried out as described previously in our earlier study (17, 18). Reverse transcriptase (RT) was presented using Hyperscript™ Reverse Transcriptase (Gene All, South Korea). RT-PCR was performed with cDNA synthesis using an amplification reagent kit (Ampliqon, Denmark) by the XP-Cycler instrument (TCXPD, Bioer, and USA). The relative expression of CaMKIIδtotal and isoforms δ2 and δ3 of CaMKIIδ, as well as MHC-β and α isoforms mRNA were normalized to the amount of GAPDH in the same cDNA sample using the standard curve method. Gene Bank (http://blast.ncbi.nlm.gov/Blast.cgi) was used for planning the primer sequences (forward and reverse) of the target genes confirming with the Gene Runner software (Table 1). The amount of PCR yields was normalized to GAPDH as a housekeeping gene. We used the $2^{-(\Delta\Delta Ct)}$. Results were presented as the fold-difference to the relevant controls.

2.3. Western blotting

Bax, Bcl2, and cleaved caspase-3 proteins levels in the left ventricular tissue were determined by Western immunoblotting. Briefly, the tissue was washed once with phosphate buffer and lysed with lysis buffer containing 500 µL Tris-Hcl, 0.003gr EDTA, 0.08gr NaCl, 0.025gr Sodium Deoxycholate, 0.01gr SDS, 1 tablet Protease inhibitor cocktail, and 10 µl Triton (Np40) 1%. After a 30 min incubation on ice, the total cell lysates was centrifuged at 15,000 g for 20 min at 4º C, and the supernatant was collected and stored at 80º C until analysis. The protein concentration of the supernatant was measured by the Bradford assay kit (Bradford 1976). Equal amounts of proteins were loaded in each well after being mixed with a 2X sample loading buffer. The proteins were separated in 10% SDS-gels and then transferred to polyvinylidene fluride (PVDF) membrane. The membrane was incubated with a primary and an HRD-labled secondary antibody. After one hour of incubation in the shaker, the membranes were bathed in wash buffer and were washed for at least 3×5 min. Then, the membranes were incubated with the enhanced chemiluminescence (ECL, Amersham) reagents in a dark room. This was followed by exposure of the membrane to an X-ray film and visualization of the chemiluminescence of the binding by means of a visualizing machine. The intensity of the bands was determined using the Image J software (IJ 1.46r version, NIH, USA) and normalized to the bands of the internal control (beta-actin).

2.4. Statistical analysis

Normal distribution of data within each group was examined conducting the Kolmogorov–Smirnov test. The statistical differences between the groups were tested conducting a one-way ANOVA and then the Tukey's post hoc test. The data obtained from each test are presented as the mean ± S.E.M, and $p < 0.05$ is set as statistically significant.

3. Results

3.1. Effects of tropisetron on diabetes-induced cardiac hypertrophy

There was no difference found between heart weights of animals in the control, diabetic and tropisetron–treated diabetic groups when the hearts were harvested fourteen days after the treatment (table-2). Compared with the animals in the control group, the left ventricular weight (L.V.W), L.V.W / body weight (B.W) and L.V.W / heart weight(H.W) of animals in diabetic group were significantly higher ($p < 0.05$). In tropisetron–treated diabetic group, L.V.W, L.V.W / B.W and L.V.W / H.W were significantly lower than those in the diabetic group ($p < 0.05$). (Table-2).

Table 2. Left ventricular hypertrophic indexes, ICAM-1, CaMK, and TNF-α amounts in left ventricular tissue of the control, diabetes and diabetes+tropisetron groups

	Control	Diabetes	Diab+Tropisetron
L.V.W/B.W	1.51±0.06	2.55±0.25*	**2.14±0.07***
L.V.W/H.W	0.549±0.012	0.8±0.026*	**0.66±0.02*†**
H.W(gr)	0.8±0.017	0.68±0.12	**0.68±0.08**
L.V.W(gr)	0.43±0.018	0.57±0.05*	**0.46±0.025†**
ICAM-1(pg/ml)	0.99±0.144	1.55±0.11*	**0.77±0.11†**
CaMK(pg/ml)	95.23±4.6	177.17±2.89*	**151.7±3.97*†**
TNF-α(pg/ml)	38.47±2.59	72.87±3.2*	**53.69±4.45*†**

Values expressed as mean± S.E.M. *p<0.05, Significant difference versus the control group. †p< 0.05, Significant difference versus the diabetes group. Left ventricular weight (L.V.W), Body weight (B.W), heart weight (H.W)

3.2. Effect of diabetes and tropisetron treatment on left ventricular TNF-α, ICAM-1, and CaMKII

Results of the current study showed that STZ-induced diabetes resulted in a significant increase in left ventricular content of TNF-α, ICAM-1, and CaMKII in the diabetic group compared to the normal control group (table-2) ($p < 0.05$). Moreover, Ttropisetron treatment significantly attenuated the increased heart tissue level of these enzymes in tropisetron –treated diabetic group compared to the diabetic group ($p < 0.05$).

3.3. Effect of diabetes and tropisetron treatment on the left ventricular genes expression

STZ-induced diabetes significantly increased the expression of CaMKIIδtotal and isoforms δ2 and δ3 of CaMKIIδ related genes (mRNA) in the left ventricular of the diabetic group, when compared with the control group ($p < 0.004$). Tropisetron treatment in diabetic animals reduced the CaMKIIδ isoform related genes expressions markedly, compared to the diabetic group ($p < 0.004$). Furthermore, there were no significant differences between the tropisetron–

treated diabetic and the control rats. The MHC-α mRNA gene expression showed a significant decrease in the diabetic group compared to the control group. There were no significant differences found between the tropisetron-treated diabetic group and the control group in terms of MHC–α mRNA expression. In the diabetic group, a significant increase in the expression of MHC-β mRNA in the left ventricular of the rats was found when compared with the control group ($p < 0.05$).

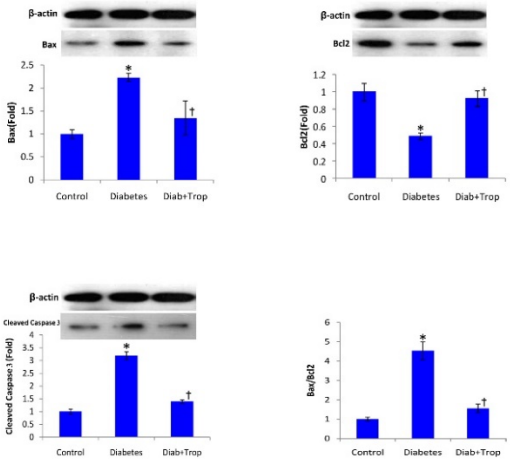

Fig. 1. Effect of tropisetron administered daily to diabetic rats for 14 days on bax, Bcl-2 and caspase-3 proteins expression in myocardium. The upper panel shows protein bands from a typical record and β-actin as housekeeping controls. Data are mean ±S.E.M. of 8 rats per group. *p< 0.05 vs. control group. †p < 0.05 vs. diabetic group

MHC-β mRNA expression had no significant differences between the tropisetron–treated diabetic group and the control. Interestingly, the ratio of MHC-β mRNA/ MHC-α mRNA expression demonstrated a significant increase in the diabetic group compared to the control group ($p < 0.05$). Tropisetron decreased the ratio of MHC-β mRNA/ MHC-α mRNA expression in diabetic animals in comparison with diabetic group ($p < 0.05$). Finally, no significant differences have been established between the tropisetron–treated diabetic group and the control group in terms of MHC-β mRNA/ MHC-α mRNA ratio.

3.4. Effect of diabetes and tropisetron treatment on left ventricular bax, Bcl-2 and caspase-3 activation

To estimate the effect of diabetes and tropisetron treatment on bax, Bcl-2, Bax/Bcl2 ratio, and caspase-3 activation, western blot analysis was performed in left ventricular tissue obtained from different groups of study after 14 days of treatment. As shown in figure 1, Bax protein expression increased significantly and Bcl-2 expression decreased in heart tissue of diabetic rats compared to the control rats ($p < 0.05$). The ratio of Bax / Bcl-2 also showed a significant increase among diabetic rats compared to the control rats ($p < 0.05$). Tropisetron administration to diabetic rats significantly reduced diabetes-induced, increased Bax and Bax/Bcl-2 ratio, and significantly increased diabetes-induced decreased Bcl-2 compared to the diabetic group. As an important mediator of apoptosis, in the current study, we examined the enzymatic activity of the caspase-3 with respect to protein cleaved caspase-3 conducting a western blot analysis. As shown in fig.1, the results revealed that diabetes led to a significant increase in cleaved caspase-3 expression in the heart tissue compared to the control group ($p < 0.05$). Tropisetron treatment significantly decreased cleaved caspase-3 expression in heart tissue of tropisetron–treated diabetic group compared to the diabetic group.

Table 3. Effect of diabetes and tropisetron +diabetes on changes of heart tissue gene expression of CaMKIIδ, β -MHC and α -MHC after 14 days treatment

	Control	Diabetes	Diab+Trop
CaMKIIδ$_{total}$ (fold)	1.49±0.26	5.88±0.53*	1.11±0.13†
CaMKIIδ$_2$ (fold)	1.68±0.23	7.35±0.47*	1.72±0.19†
CaMKIIδ$_3$ (fold)	2.15±0.1	8.45±0.74*	1.83±0.12†
MHC-α(fold)	1.27±0.15	0.54±0.3*	1.36±0.49†
MHC-β(fold)	3.73±0.34	7.59±0.6*	3.55±0.08†
β -MHC/ α -MHC	2.95±0.18	14.05±0.4*	2.61±0.7†

Values expressed as mean± S.E.M. *p<0.05, Significant difference versus the control group. †p< 0.05, Significant difference versus the diabetes group

4. Discussion

Our recent works and several others indicated that the overexpression of the predominant cardiac isoforms of CaMK including CaMKIIδ1 and CaMKIIδ2 is one of the hallmarks of molecular alteration that induces myocardial hypertrophy and heart failure (18-21). In addition, the association between CaMKIIδ isoforms expression alteration and shifts in cardiac function has been reported in pathologic conditions such as dilated cardiomyopathy, myocardial infarction, arrhythmia, and heart failure upon injuries such as pressure overload and ischemia-reperfusion (22-24). Furthermore, studies have also demonstrated that CaMKIIδ-knockout preserved the heart in several situation including ischemia-reperfusion damage, interstitial fibrosis after pressure overload and β-adrenergic stimulation, indicating strong documents for CaMKIIδ maladaptive functions in cardiac pathogenesis (25, 26). Some recent reports are in line with our study indicating a direct relationship between CaMKIIδ, hyperglycemia and type 1 diabetes-induced cardiomyopathy (4, 27). Another important achievement gained by enhancing CaMKIIδ activities in type 2 diabetes and its induced consequent alteration of contraction and relaxation prior to the development of heart failure have been yielded by Daniels et al (28). Based on their findings, Daniels et al., concluded that metabolism alteration in diabetic heart activated CaMKIIδ and altered heart function at the myocytes level, leading to the development of cardiac

dysfunction (28). In the present study, we confirmed that the expression of total and isoforms of CaMKIIδ, as well as the amount of CaMKIIδ in the heart tissue increased, following STZ-induced diabetes along high left ventricular weight, and increased L.V.W / B.W, and L.V.W / H.W ratio. Furthermore, tropisetron administration to diabetic rats restored CaMKIIδtotal and isoforms gene expression and decreased left ventricular weight, L.V.W / B.W, and L.V.W / H.W ratio. It has been recently reported that 5-HT3 receptor antagonists, such as tropisetron, protected against cardiac hypertrophy and restored the desensitization of cardiac adrenergic sensitivity in overload–induced cardiac hypertrophy in murine (29). It is well known that cardiac failure resulting from cardiac hypertrophy is a deadly condition and that sympathetic activation is an important pathological factor in promoting heart hypertrophy to heart failure (29). Put together, diabetes induced cardiomyopathy may be mediated at least in part by activation of CaMKIIδ pathway and blocking of this route by tropisetron protected against diabetes-induced cardiac hypertrophy.

In agreement with earlier studies (30, 31) in the present study, we found that STZ-induced diabetes caused an increase in expression of ventricular MHC-β and a reduction in expression of ventricular MHC-α. It has been previously reported that diabetes reduces cardiac and myocytes contractile kinetics (30). It is well established that daily ATP consumption by heart is five-fold greater than its own mass for maintenance of cellular ionic homeoatasis and rhythmic contraction (32). MHCs' consume a great part of this ATP to form strong cross-bridges with actin for production of the power stroke (32). Although, more than 93% amino acid sequence homology is between two isoforms of MHCs isoforms, the ATPase activities and actin filament rapid contractile velocity of MHC-α is two to three times more than MHC-β (33). In contrast to MHC-α, the MHC-β has a lower ATPase activity with a lower actin filament contractile velocity, and allows for a greater economy with a cross-bridge force generation (34). Due to the difference between physiologic function of α and MHC-β isoforms in stressful conditions such as diabetes and pressure-overload cardiac hypertrophy, the tendency is always to shift towards the lower activity of MHC-β for enhancing the efficiency and economy of force generation (34). Accordingly, gene expression transition from α to MHC-β isoform motivates compensatory/adaptive changes when sufficient amounts of ATP are not available, and this is because the β isoform is associated with a greater economic force generation compared to α isoforms. Here we found that compared with the control group, 5HT3 receptor antagonist restored cardiac fatal genes expression alteration to a favorite direct. Although pharmacologic strategies such as insulin therapy and lifestyle management are two factors that improve the ability of individuals to cope with diabetes and to maintain near normal glycemia, diabetes-induced cardiomyopathy and heart failure continue to be the main causes of morbidity and mortality in individuals with diabetes mellitus. Due to the basic pathological role of sympathetic activation in promoting heart hypertrophy to heart failure, current study results and those of previous studies provide evidence that antagonism of sympathetic nervous system is a valuable therapeutic method for diabetes-induced cardiac hypertrophy and heart failure (29, 35). In the current study, cardiac Bax and cleaved caspase-3 expression and Bax / Bcl-2 ratio in STZ-induced diabetes were significantly increased compared to the non-diabetic and the decreased pro-survival BCL-2 was improved by tropisetron administration. These finding are in agreement with those of a previous study showing similar alteration in pro-apoptotic and pro-survival members in rat pheochromocytoma cells exposed to high glucose and cardiac of STZ-induced diabetic rats (35, 36). As a pro-apoptotic protein Bax plays a fundamental role in the induction of apoptosis. Bcl-2, however, is known as an anti-apoptotic protein having protective properties against apoptosis pathways (37). Previous studies showed that suppression of Bax gene improved myocardial ischemia-reperfusion injury including amelioration of necrosis and apoptotic cell death in heart of the mice (38). The results of another study revealed that diabetes led to cardiac structural abnormality along decreased cardiac Bcl-2 and increased Bax, and that exercise restored cardiac structural alteration to normal conditions by improving Bcl-2 and Bax pathways (36). In the current study, tropisetron administration improved Bax, Bcl-2 and cleaved caspase-3 expression was parallel with improvement of MHCs isoforms and CaMKIIδ genes expression alteration, as well as amelioration of left ventricular weight, L.V.W / B.W, L.V.W / H.W. Our previous works and several other studies have indicated that oxidative stress plays an important role in diabetes-induced cardiomyopathy (6, 37-39). Oxidative stress-induced diabetic cardiomyopathy results from enhancement of reactive oxygen species and a decreased endogenous antioxidant capacity in myocardium (40, 41). Moreover, another important early and notable effect of oxidative stress on the heart is cardiac inflammation that is actively involved in the development of heart failure during diabetic cardiomyopathy (42). From among the various inflammatory cytokines involved in diabetic cardiomyopathy, TNF-α plays a crucial role in developing cardiac hypertrophy and heart failure through several ways including activation of mitogen-activated protein kinases (MAPK) and the extracellular signal-regulated kinase (ERK)(43). In addition, TNF-α is able to promote reactive oxygen species generation and modulate an inflammatory response by inducing production of pro-inflammatory cytokines such as interleukin (IL)-1β and its own production (44). Moreover, it has been reported that TNF-α induces myocardial hypertrophy and fibrosis by an increase in TGF-β1 signaling activation of nuclear factor –κB (NF-κB) (44, 45). Furthermore, the study by Westermann et al. pointed out that TNF- α antagonism improved the experimental cardiomyopathy corresponding to

a reduction of intra-myocardial inflammation and cardiac fibrosis (46). In the current study, TNF- α and ICAM-1 levels showed an increase, and administration of tropisetron improved left ventricular hypertrophy cardiac fatal gene expression. Previous studies investigating diabetic cardiomyopathy reported an association between diabetic cardiomyopathy and cellular adhesive molecules such as ICAM-1 and vascular adhesive molecule (VCAM)-1 (46).

In conclusion, with acknowledging the results obtained by previous studies showing the structural and functional demonstration of diabetic cardiomyopathy with details; our study presents several notable findings. First, in non-treated diabetic rats, heart hypertrophy indexes including left ventricular weight, L.V.W / B.W and L.V.W / H.W were significantly higher than those in control rats. Second, we demonstrated that STZ-induced diabetes causes a higher expression of fatal gene compared to that manifested by overexpression of CaMKIIδtotal, CaMKIIδ2, and CaMKIIδ3 mRNA and a shift in the MHC isoforms expression manifested by elevation of MHC-β mRNA and the ratio of MHC-β mRNA/ MHC-α mRNA expression in the heart tissue of rats. The third point is that STZ-induced diabetes leads to an increase in TNF-α, ICAM-1, and CaMK amounts in heart tissue of diabetic animals, compared to the control ones. All these processes are known as molecular mechanisms underlying cardiomyopathy and heart failure. The fourth point which in our view is the golden point is that treatment with tropisetron decreases left ventricular weight, L.V.W / B.W and L.V.W / H.W, improves gene expression alterations and restores TNF-α, ICAM-1, and CaMK amounts compared to those in the diabetic group. Taken together, current study results indicate that tropisetron has potential therapeutic efficacy in preventing the progression of diabetic cardiomyopathic, and further experimental and / or clinical studies, including therapeutic window studies, need to be performed to reveal such effects.

Conflict of interest

The authors declare that they have no conflict of interest.

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