International Journal of Biochemistry Research & Review
4(1): 66-79, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Cross Talk between Oxidative Stress, PPAR- γ and RAS System in Diabetic Rat Heart Exposed to Ischemia / Reperfusion Injury

Maha M. Sabry[1], Nagwa K. Roshdy[2*], Laila A. Rashed[2], Hany E. ElSebaee[1], Mary A. Youssef[1] and Amal F. Tawadrous[1]

[1]Physiology and Medical Biochemistry & Molecular Biology Departments, Faculty of Medicine, Cairo University, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Author MMS designed the study and wrote the protocol, author NKR supervised the experiment and managed the analyses of the study, author HEE supervised the experiment and wrote the first draft of the manuscript. Authors LAR managed the practical work and authors MAY and AFT managed the practical work and the literature search and performed the statistical analysis. All authors read and approved the final manuscript.

Original Research Article

Received 17th July 2013
Accepted 10th October 2013
Published 20th November 2013

ABSTRACT

Introduction: The present study aimed at clarifying the cross talk between peroxisome proliferator-activated receptor-gamma (PPAR- **γ)**, cardiac reactive Oxygen species (ROS) and Renin-Angiotensin System (RAS).
Methods: A total of 90 male albino rats were used. The rats were divided into: *Group 1:* Control group, *Group 2:* Type 2 diabetic rats, *Group 3:* PPARγ agonist protected type 2 diabetic rats. *Group 4*: Antioxidant protected type2 diabetic rats, *Group 5:* Metformin treated type 2 diabetic rats. Blood samples were collected for measurement of FBS and fasting insulin. Half the number of each group was sacrificed and the heart excised and perfused, from the rest of the group small piece from the heart was taken for estimation of malondialdehyde (MDA), Angiotensin 2 Receptor (AT2R) and Angiotensin Converting Enzyme 2 (ACE2) gene expression.
Results: Treatment with pioglitazone and Vitamin E significantly lowered blood glucose,

**Corresponding author: Email: nagwaroshdy@yahoo.com;*

insulin levels and Homeostasis Model Assessment Insulin Resistance (HOMA IR). However, values did not return to control values. Pioglitazone and Vitamin E improved myocardial performance and percentage recovery following ischemia reperfusion. The cardioprotective effect was more pronounced in the pioglitazone group. This positively correlated with decreased MDA levels and increased AT2R and ACE2 expression in cardiac tissue.

Conclusion: Pioglitazone and Vitamin E in type 2 DM significantly offered cardio-protection through improving the diabetic condition and / or decreasing MDA levels.

Keywords: Type 2 Diabetes mellitus; cardiac ischemia reperfusion; PPAR γ; oxidative stress; ACE2; AT2R expression.

1. INTRODUCTION

Diabetes mellitus (DM) leads to high cardiovascular morbidity and mortality as a result of functional and morphological damage in diabetic hearts.

Most of the diabetes-related cardiovascular complications can be largely attributed to overproduction of reactive oxygen species such as superoxide and hydrogen peroxide, and/or depletion of antioxidant defense mechanisms [1].

Antioxidant therapy has shown promise in prevention of development of diabetic heart complications but the results of intervention studies with classic antioxidants such as vitamin E, were shown to be elusive and unsuccessful [2]**.** On this matter, it has recently been suggested that antioxidant therapy with vitamin E or other antioxidants is limited to scavenging already-formed oxidants and may therefore be considered a more "symptomatic" rather than a causal treatment for vascular oxidative stress. Hyperglycemia-induced oxidative stress is accompanied by increased production of advanced glycosylation end products (AGEs), upregulation of the receptor for AGEs (RAGE) [3] and downregulation of PPAR **γ** system [4]**.**

AGE binds to its cell surface receptor, RAGE, resulting in the activation of postreceptor signaling, generation of intracellular reactive oxygen species, and alteration of gene expression [5]

Reactive oxygen species have been indicated as one of the earliest and most important components of tissue injury after reperfusion of ischemic organ. Free radical production is continuous during ischemia, while during reperfusion it is primarily confined to the early stage when fresh oxygen is supplied to the ischemic region [6]**.**

PPAR-**γ** system plays a critical role in oxidative stress that seems to be implicated in the development of insulin resistance and diabetic complications. Moreover, current evidence points to a cardioprotective role for PPAR**-γ** ligands in ischemia reperfusion animal models, but the functional role of PPAR-γ in diabetic hearts is not fully understood [7].

On the other hand, it has been recently suggested that angiotensin signaling may be regulated at multiple levels by PPAR-**γ** ligands, significantly reducing the expression of AT1 receptor and repressing signal transduction through this receptor to suppress oxidative stress. However, the

relation between PPAR-γ, MDA levels, AT2 receptor and ACE2 expression in diabetic hearts needs to be clarified [8].

The aim of this study is to:

1. Test PPAR-**γ** agonist and antioxidant therapy on the development of type 2 DM in rats.
2. Test the possibility of improving the myocardial function and reducing the susceptibility to ischemia reperfusion injury through reducing MDA, one of the biomarkers of the oxidative stress.
3. Estimate the cardiac ACE2 and AT2R gene expression in relation to PPAR- **γ** system.
4. Compare the possible cardio-protective effects of PPAR-**γ** agonist, antioxidant therapy and metformin in type 2 diabetic hearts

2. MATERIALS AND METHODS

2.1 Experimental Animals

A total of 90 male albino rats were used in this study weighing 160-180 grams. These rats were treated in accordance with the guidelines approved by the Animal Use Committee of Cairo University. The animals were housed in animal house of faculty of Medicine, Cairo University, in wire mesh cages at room temperature, with 12 hour light dark cycle. They were fed the commercial rat chew diet and had free access to water.

These rats were divided into the following groups:

Group 1: Control group

Consists of 18 normal rats.

Group 2: Type 2 diabetic rats.

Type 2 diabetic rats (n=18).

Group 3: PPARγ agonist protected type2 diabetic rats group

Rats were treated with oral pioglitazone 20 mg/kg/day for the whole time of the experiment (n=18).

Group 4: Antioxidant protected type2 diabetic rats group

Rats were treated with oral vit E 500 mg/kg/day for the whole time of the experiment (n=18).

Group 5: Metformin treated type2 diabetic rats group

Treatment was initiated at 350 mg/kg/ day and was gradually increased to a dose of 650 mg/kg/ day for the whole time of the experiment (n=18).

Half the number of each group was sacrificed and the heart excised and perfused according to the Langendorff technique, from the rest of the group small piece from the heart was taken for estimation of AT2R and ACE2 expression and malondialdehyde (MDA).

2.2 Experimental Protocol

2.2.1 Induction of type 2 diabetes

Beginning on day 0, rats were fed either normal rodent chow (12% calories as fat)(group 1) or high fat diet (60% calories as fat)(group 2) for two weeks. On day 14, rats on the high fat diet (HFD) were injected with a single low dose of streptozotocin (STZ, 35 mg/kg i.p., in 0.01M citrate buffer pH 4.3) to induce type 2 diabetes [9]. Consumption of high fat diet leads to insulin resistance and is considered to be a major predisposing factor for type 2 diabetes [10]. Subsequent to STZ treatment, rats had free access to food and water and were continued on their respective diets for the duration of study.

2.2.2 Characterization of type 2 diabetes model

On day 20, in the HFD + STZ group 2, type 2 diabetes was confirmed by measuring fasting plasma glucose and insulin levels. Blood sampled from the retro orbital plexus under diethyl ether anesthesia was used to measure plasma glucose and insulin concentration.

2.2.3 Heart perfusion

Was performed according to the Langendorff technique with Krebs-Heinseleit medium at a hydrostatic pressure of 55 cm H_2O and bubbled with a mixture of 95% O_2 and 5% CO_2 [11].

2.2.4 Measurements of myocardial function

Mechanical performances of the left ventricle of the heart was determined by the systolic pressure, the diastolic pressure, the heart rate and the peak rate of maximum left ventricular pressure rise (dp/dt) which is considered as a good index of contractility. These mechanical performances were monitored during pre-ischemic; ischemic and post-ischemic reperfusion phases by a balloon inserted into the left ventricle and connected to a polygraph apparatus (San-ei instrument LTD Nee, Tokyo, Japan). The developed pressure was calculated (systolic pressure – Diastolic pressure).

2.2.5 Morphological measurements

At the end of the perfusion, hearts were removed from the perfusion apparatus, excess water was absorbed on tissue paper, and the heart mass was weighed.

2.2.6 Measurement of fasting blood insulin

Insulin concentrations were measured in previously frozen and thawed serum samples by enzyme immunoassay using the Rat Insulin ELISA kits.

2.2.7 Measurement of fasting blood glucose

The blood glucose was assayed by the method adopted by Trinder P (1969) [12]. The test materials for this method were supplied as kits by “Diamond Diagnostics”.

2.2.8 HOMA-IR

Calculated as the product of fasting insulin (microunits/ml) and fasting glucose (mMol/L) divided by 22.5. Lower index indicates greater insulin sensitivity [13].

$$\text{HOMA- IR} = \frac{\text{Fasting insulin (}\mu\text{IU/ml)} \times \text{fasting glucose (mmol/L)}}{22.5}$$

2.2.9 Semi-Quantitation of AT2R and ACE2 gene expression by reverse transcriptase polymerase chain reaction (RT-PCR)

About 30 mg of heart tissue was homogenized in RNA lysis buffer containing β-mercaptoethanol then the tissue homogenate was centrifuged at 10000 rpm for 20 min, then the clear lysate was subjected to the following:

A) RNA extraction:

1. The clear lysate solution was transferred to microcentrifuge tube by pipetting.
2. 200 µl 95% ethanol was added to the cleared lysate, mixed by pipetting 3-4 times, this mixture was transfered to spin column assembly, centrifuged at 12000-14000 rpm for 1 min.
3. The spin basket was taken from the spin column assembly, the fluid was discarded in the collection tube, and the spin basket was put into the collection tube.
4. 600 µl of RNA wash solution was added to the spin column assembly, and then centrifuged at 14000 rpm for 1 min.
5. The collection tube was empty and placed in the rack, Dnase mix was prepared by mixing 40 µl yellow core buffer, 5 µl 0.09 Mol $MgCl_2$ and 5 µl of Dnase solution was kept on ice for 1 min then about 50 µl of freshly prepared DNase solution was added directly to the membrane inside the spin basket, and incubated for 20 min at 20ºC, after the incubation, 200 µl of DNase stop solution was added to the spin basket and centrifuged at 14000 rpm for 2 min.
6. 600 µl RNA wash solution was added and centrifuged at 14000 rpm for 1 min.
7. The spin basket was transferred from the collection tube to the elution tube, and 100 µl nuclease free water was added to the membrane, centrifuged at 12000 rpm for 1 min, the spin basket was removed and discarded; the elution tube containing the purified RNA capped and stored at –70ºC.

B) RT-PCR experiments:

RT-PCR was done using the extracted RNA for detection of gene expression as follows: RNA was reverse transcribed using 12-15µl oligo (dT) 18 primer and was denaturated at 70ºC for 2 min, the denaturated RNA was placed on ice for 5 min. 6.5 µl of reverse transcription mixture was prepared containing :50 mM Tris Hcl pH 8.3. ,50 mM KCcl, 1-5 mM $MgCl_2$, 0.5 mM d NTPs, 1 unit / ml RNase inhibitor, 200 unit of Moleny murine leukemic virus reverse transcriptase (MMLV).

The mixture was added to the RNA and subjected to the following cycling condition needed for reverse transcription and synthesis of cDNA from RNA:

- 42ºC for 1 hour.

- 95ºC for 5 min to stop the reaction.

Then the PCR reaction was performed by adding PCR mix to a final volume of 100 µl, the PCR mix contain: 10 m Mol/L Tris HCl pH 8.3, 50 m Mol KCl , 1.5 mMol $MgCl_2$, 0.001% gelatin, 250 µ Mol dNTPs mix, 2.5 unit Taq polymerase, 100 mMol of each primer

- Specific primer sequence for ACE2 (gene bank number NM 001012006):

  5′ CGCTGTCACCAGACAAGAA 3′ (sense)
  5′ GCCATTATTTCGTCCAATCC 3′ (antisense)

The final PCR product was 139 bp in size.

- Specific primer sequence for AT2R (gene bank number NM 0124943):

  5′ GGA GCG AGC ACA GAA TTG AAA GC 3′ (sense)
  5′ TGC CCA GAG AGG AAG GGT TGC C ′(antisense)
  The final PCR product was 445 bp in size.

- Specific primer sequence for b-actin (used as house keeping gene) (gene bank accession number : NM 001014725.2)

  5'-TTC-TACAATGAGCTGCGTGTGGC-3' (sense)
  5'-AGAGGTCTTTACGGATGTCAACG-3'; (antisense)

The reaction mixture was then subjected to 40 cycles of:

- 95ºC for 1 min.
- 55ºC for 1min.
- 72ºC for 1min.

After the last cycle, a final extension at 72ºC for 1min. was done.

C) Agarose gel electrophoresis:

All PCR products were electrophoresed on 2% agarose gel stained with ethidium bromide and visualized by ultra-violet transilluminator.

D) Gel documentation:

The PCR products were semiquantitated using the gel documentation system (Bio Doc Analyze) supplied by Bio metra, using standard DNA with different concentration to make curve from which concentration of PCR products was detected.

2.2.10 Estimation of MDA levels in rat heart

This was done by the thiobarbituric acid (TBA) test. *In brief,* 2.5 ml of 20 mg/dl trichloracetic acid is added to 0.5 ml of tissue homogenate. The tube is left to stand for 10 min at room temperature. After centrifugation at 3500 rpm for 10 min the supernatant is washed once with

0.05 M sulfuric acid.2.5 ml of sulfuric acid and 3 ml of TBA in 2 Mol sodium sulfate are added to this precipitate and the coupling of lipid peroxide with TBA is carried out by heating in a boiling water bath for 30 min. After cooling in cold water, the resulting chromogen is extracted with 4 ml of n-butyl alcohol by vigorous shaking, separation of organic phase is facilitated by centrifugation at 3000 rpm/min for 10 min and its absorbance is determined at the wave length of 530 nm. The standard curve was linear up to 21.2 nMol/ml. The concentration of sample was determined from standard curve [14].

2.3 Statistical Analysis

The results were analyzed using SPSS computer software package, version 10.0(Chicago-IL, USA). Data were presented as mean ± S.D. Differences among the three groups were compared by one-way ANOVA. To study the relationship between the variables, Pearson's correlation coefficient was calculated. The results were considered statistically significant at $P<0.05$.

3. RESULTS

Table 1 shows that untreated diabetic rats (group 2) had significantly higher level of fasting blood glucose (mmol/L), fasting plasma insulin (uIU/ml) and HOMA-IR compared to non diabetic control rats group 1. However, these levels decreased significantly in protected groups: pioglitazone protected diabetic rats group 3, vit E protected diabetic rats group 4 and metformin protected diabetic rats group 5 compared to untreated diabetic group 2 with no significant difference between the three treated groups. However, none of the measured parameters in the protected groups returned to control values.

Table 1. Levels of fasting blood glucose, fasting insulin and HOMA test in the studied groups

Calculated parameters	Group 1	Group 2	Group 3	Group 4	Group 5	P
Glucose (mmol/L)	4.81 ± 0.51 a	12.14 ± 1.44 b	9.45 ± 1.27 c	8.68 ±1.79 cd	7.08 ± 0±.47 d	0.00
Insulin (uIU/ml)	10.58 ± 0.57 a	18.2 ± 2.76 b	14.71 ± 1.78 c	15.13 ±1.54 c	13.81 ± 0.46 c	0.00
Homa	2.21 ± 0.21 a	9.79 ± 2.01 b	6.10 ± 1.00 c	5.80 ±1.28 cd	4.35 ± 0.28 d	0.00

Values are mean ± Standard deviation (SD)
Groups bearing the same initials are not statistically significant from each other at P<0.05.

As shown in Table 2:

The unprotected diabetic rats group 2 showed significant increase in the level of AT_2R and ACE_2 gene expression in cardiac tissue compared to control group 1. Pioglitazone treatment of diabetic rats almost doubled the level of AT_2R and ACE_2 gene expression in cardiac tissue compared to the untreated diabetic group and the other two protected groups (Vit E and metformin).

As reagards the level of MDA in cardiac tissue (nmol/mg ptn): The unprotected diabetic rats group 2 showed significant increase in the level of MDA in cardiac tissue (nmol/mg ptn)

compared to control group 1**.** Moreover, the protected groups: PPARγ protected diabetic rats group 3, vit E protected diabetic rats group 4 and metformin protected diabetic rats group 5 showed significant decrease in level of MDA in cardiac tissue compared to untreated diabetic group 2 although the level of the MDA remained significantly elevated compared to control group 1.

Table 2. AT2R and ACE2 expression and level of MDA in cardiac tissue in the studied groups

Calculated parameters	Group 1	Group 2	Group 3	Group 4	Group 5	P
AT_2R	0.18 ± 0.04a	0.508±0.06b	0.84 ±0.106d	0.408±0.07 b	0.503±0.061 b	0.00
ACE_2	0.108±0.031a	0.36± 0.085b	0.72±0.12d	0.35±0.026 b	0.39±0.018 b	0.00
MDA nmol/mg ptn	10.93±1.36 a	23.01±2.68b	16.28±1.97c	14.91±1.88 c	17.45±0.52 c	0.00

Values are mean± SD.
Groups bearing the same initials are not statistically significant from each other at P<0.05.

As shown in Table 3:

The unprotected diabetic rats group 2 showed significant decrease in the level of LVDP (mmHg) and dp/dt (mmHg/sec) compared to control group 1 but there was no significant difference in the level of HR (b/min) between the 2 groups.

Both pioglitazone protected diabetic rats (group 3) and vit E protected diabetic rats (group 4) showed significant improvement in LVDP and dp/dt but still not reaching control values of the nondiabetic rats. However, there was no significant difference between the metformin protected diabetic rats (group 5) and the unprotected diabetic rats (group 2).

Table 3. Left ventricular performance (developed pressure), heart rate, and contractility index (dp/dt) during the initial 30 minutes of perfusion in the studied groups

Calculated parameters	Group 1	Group 2	Group 3	Group 4	Group 5	P
LVDP mmHg	114.56±9.47 a	58.44±5.43 b	79.44±6.32 d	79.67±3.39 d	59.44±5.43 b	0.00
HR b/min	248.78±109.11a	224.00±46.56a	161.33±18.98 b	162.44±9.15 b	225.00±46.56 a	0.00
dp/dt mmHg/sec	116.44±7.126 a	65.78±4.969 b	97.89±7.688 cd	95.22±8.228 d	66.78±4.969 b	0.00

Values are mean± SD.
Groups bearing the same initials are not statistically significant from each other at P<0.05.

As shown in Table 4:

The percentage recovery of the left ventricle performance following ischemia-reperfusion (LVDP (mmHg), contractility index dp/dt (mmHg/sec) and heart rate(b/min) was significantly decreased in the unprotected diabetic rats (group 2) compared to control rats (group 1).

Regarding the protected groups of diabetic rats; PPARγ protected diabetic rats (group 3) showed significantly higher values of percentage recovery of LVDP and dp/dt compared to vit

E protected diabetic rats (group 4), metformin protected diabetic rats (group 5) and the untreated diabetic rats (group 2). Interestingly, pioglitazone prevented the effect of diabetes on % recovery of left ventricular performance after I/R that even returned to non diabetic control levels.

Table 4. The percentage recovery of the left ventricle performance following ischemia-reperfusion (developed pressure), heart rate and contractility index (dp/dt) in the five studied groups

Calculated parameters	Group 1	Group 2	Group 3	Group 4	Group 5	P
LVDP mmHg	78.144±1.93 ab	63.456±4.39 c	76.38±2.54 b	64.28±3.72 c	69.36±0.71 d	0.00
HR b/min	77.100±1.90 a	65.98±7.14 b	78.21±3.02 a	67.63±2.16 b	69.83±0.83 b	0.00
dp/dt mmHg/sec	77.256±2.83 a	71.55±4.14 b	79.56±1.43 a	66.87±2.90 d	69.68±0.91bd	0.00

Values are mean± SD.

Groups bearing the same initials are not statistically significant from each other at P<0.05.

When Pearson correlation was performed in studied groups, significant positive correlation was found between the level of AT2R expression in cardiac tissue and the percentage recovery of the left ventricle performance following ischemia-reperfusion "LVDP (r=..412**), heart rate(r=.546**) and contractility index dp/dt (r=.610**) & also, there was significant positive correlation between the level of ACE2 expression in cardiac tissue and the percentage recovery of the left ventricle performance following ischemia-reperfusion "LVDP (r= .465**) and contractility index dp/dt (r=.603**) and the percentage recovery the heart rate (r = .624**). In addition, there was significant negative correlation between the level of MDA in cardiac tissue and the percentage recovery of the left ventricle performance following ischemia-reperfusion "LVDP (r = -.439**), heart rate(r= -.363**) and contractility index dp/dt (r=-.353**)" (Table 5).

Table 5. Correlations between studied parameters

		AT2	ACE2	MDA
MDA	Pearson Correlation	.088	.115	
	Sig. (2-tailed)	.491	.371	
	N	63	63	
LVDP % recovery	Pearson Correlation	.412**	.465**	-.439**
	Sig. (2-tailed)	.001	.000	.000
	N	63	63	63
HR % recovery	Pearson Correlation	.546**	.624**	-.363**
	Sig. (2-tailed)	.000	.000	.003
	N	63	63	63
Dp/dt % recovery	Pearson Correlation	.610**	.603**	-.353**
	Sig. (2-tailed)	.000	.000	.003
	N	63	63	63

Correlation is significant at the 0.05 level (2-tailed).

4. DISCUSSION

The protective role of PPAR-γ agonist and vitamin E in diabetes was confirmed in the present study. However, the glucose level was significantly reduced in the metformin group compared to other protected groups.

A recent study found that tocopherols dramatically enhanced adiponectin expression and that this effect was mediated through a PPAR-γ-dependent process. These findings illustrate a possible mechanistic link between vitamin E and insulin sensitivity [15] It is likely that the diabetic milieu and associated changes in the myocardium sensitize the diabetic heart to dysfunction after ischemic injury [16].

Vitamin E-protected rats showed significantly higher values of left ventricular performance and contractility index.

Increased ROS generation may activate maladaptive signaling pathways, which may lead to cell death, which could contribute to the pathogenesis of diabetic cardiomyopathy [17] Increased ROS-mediated cell death could promote abnormal cardiac remodeling, which ultimately may contribute to the characteristic morphological and functional abnormalities that are associated with diabetic cardiomyopathy[18] Increased ROS also might contribute to mitochondrial uncoupling, which could impair myocardial energetics in diabetes. Strategies that either reduce ROS or augment myocardial antioxidant defense mechanisms might have therapeutic efficacy in improving myocardial function in diabetes mellitus [19].

PPAR-γ agonists may exert their anti-inflammatory effects by negatively regulating the expression of pro-inflammatory genes induced during macrophage differentiation and activation. Several lines of evidence suggest that TZDs protect the heart and other organs against the tissue injury caused by ischemia/reperfusion (I/R) injury and shock [20].

The present study showed that PPAR- γ ligands improved cardiac performance after I/R. This agrees with Shimabukuro and colleagues [21]. The authors showed that this cardioprotective effect is associated with inhibition of the JNK/AP-1 pathway [22].

Zhu and coworkers [23], showed that chronic troglitazone administration significantly improved recovery of left ventricular systolic and diastolic function as well as increasing net myocardial lactate uptake, suggesting enhanced myocardial carbohydrate oxidation [24].

A significant increase in the level of MDA in cardiac tissue was detected in the unprotected diabetic rats. However, the MDA level was equally decreased in all protected diabetic groups.

Under physiological states, most of the ROS generated within cells arises from mitochondria. Relatively few studies to date have directly measured mitochondrial ROS production in mitochondria obtained from diabetic hearts [25] ROS are produced from nonmitochondrial sources such as NADPH oxidase or reduced neuronal nitric oxide synthase (NOS1) activity coupled with increased activation of xanthine oxidoreductase [26] Antioxidant inhibits NF-kB activation by blocking LPS-induced IkB degradation and nuclear translocation of the cytosolic NF-kB p65 subunit [27].

Potential mechanisms for cardioprotection afforded by ACE2 are unclear. They include increased Ang II degradation and increased formation of Ang-(1-7) which activates the G protein–coupled Mas receptor. Mas receptor activation by Ang-(1-7) opposes many of the AT_1 receptor-mediated actions (vasoconstriction, hypertrophy, fibrosis), thereby improving cardiac function and remodeling and attenuating heart failure. Mas receptors are also localized to cardiac myocytes and activate NO production [28].

In the present work, % recovery of cardiac performance after I/R injury was positively correlated with levels of AT2R and ACE2 gene expression and negatively correlated with levels of MDA.

It has been shown that TZDs could inhibit the oxidative stress indirectly through the inhibition of the promoter activity of the chemokine genes and the inhibition of NF-κB activation on these promoter with the inhibition of the expression of proinflammatory adhesion molecules (ICAM-1, VCAM-1, E-selectin)[32]. Moreover PPAR- γ agonists prevent the phosphorylation of NF-κB p65 and inhibit the activities of NF-κB and AP-1 and thus reducing the generation of oxygen-derived free radicals in diabetic hearts [29].

In our study, lowering glucose level by metformin induced significant decrease in MDA. Metformin prevent ROS formation by preventing the build-up of mNADH through insulin-stimulated nutrient uptake and preventing the entrance of energetic substrates (pyruvate, fatty acids) into the mitochondria [30].

Furthermore, metformin has been shown to reduce ROS levels in human leukocytes by either directly scavenging the free radicals or modulating their intracellular production [31].

Comparing the levels of HOMA-IR and cardiac tissue MDA in our studied groups confirms the role of oxidative stress in pathophysiology of diabetic hearts and also confirms the antioxidant effect of PPAR-γ agonists which surprisingly enough was not different from either vitamin E or metformin.

The unprotected diabetic rats showed significant increase in the level of AT_2R and ACE_2 gene expression. This might indicate a protective role for both AT2R and ACE2 [32].

The levels of AT_2R and ACE2 gene expression in cardiac tissue were significantly elevated in pioglitazone protected groups compared to other groups.

There is good evidence that PPAR-γ agonists mediate inhibition of the AT1R expression as well as Ang II-mediated signaling pathways which may explain the increase in the expression of AT2R and ACE2 [33,34].

Molavi et al., [35] reported a reduction of infarct size, attenuation of myocardial AT1R and increase in AT2R mRNA expression in the TZDs group.

5. CONCLUSION

Taken together, these results clearly suggest a cross talk between ROS, PPAR -γ and RAS systems which interplay in the pathogenesis of diabetes and its cardiac complications.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Guzik TJ, Mussa S, Gastaldi D, Sadowski J, Ratnatunga C, Pillai R, et al. Mechanisms of increased vascular superoxide production in human diabetes mellitus. Circulaion. 2002;105:1656–1662.
2. Marchioli R, Schweiger C, Levantesi G, Gavazzi L, Valagussa F. Antioxidant vitamins and prevention of cardiovascular disease: epidemiological and clinical trial data. Lipids. 2001;36:S53–S63.
3. Wang K, Zhou Z, Zhang M, Fan L, Forudi F, Zhou X, et al. Peroxisome proliferators-activated receptor gamma down-regulates receptor for advanced glycation end products and inhibits smooth muscle cell proliferation in a diabetic and nondiabetic rat carotid artery injury model. J Pharmacol Exp Ther. 2006;317(1):37-43.
4. El Midaoui A, Wu L, Wang R, de Champlain J. Modulation of cardiac and aortic peroxisome proliferators-activated receptor-gamma expression by oxidative stress in chronically glucose-fed rats. Am J Hypertens. 2006;19(4):407-12.
5. Frustaci A, Kajstura J, Chimenti C, Jakoniuk I, Leri A, Maseri A, et al. Myocardial cell death in human diabetes. Circ Res. 2000;87:1123–1132.
6. Nita AA, Nita V, Spulber St, Moldovan M, Popa DP, Zagrean AM, et al. Oxidative damage following cerebral ischemia depends on reperfusion – a biochemical study in rat. J. Cell Mol. 2001;5(2):163-170.
7. Geng DF, Wu W, Jin DM, Wang JF, Wu YM. Effect of peroxisome proliferators-activated receptor gamma ligand. Rosiglitazone on left ventricular remodeling in rats with myocardial infarction. Int J Cardiol. 2006;113(1):86-91.
8. Kintscher U, Lyon CJ, Law RE. Angiotensin II, PPAR-gamma and atherosclerosis. Front Biosci. 2004;9:359-69.
9. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. Pharmacol Res. 2005;52(4):313-20.
10. Kraegen EW, James DE, Storlien LH, Burleigh KM, and Chisholm DJ. In vivo insulin resistance in individual peripheral tissues of the high fat fed rat: assessment by euglycaemic clamp plus deoxyglucose administration. Diabetologia. 1986;29:192–198.
11. Skrzypiec-Spring M, Grotthus B, Szelag A, Schulz R. Isolated heart perfusion according to Langendorff---still viable in the new millennium.J PharmacolToxicol Methods. 2007;55(2):113-26. Epub 2006 May 26.
12. Trinder P. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. J Clin Pathol. 1969;Mar;22(2):158-61.
13. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985 ;28(7):412-9.
14. Buege JA, Aust JD. Microsomal lipid peroxidation. Methods Enzymol. 1978;52:302–310.
15. Gray B, Swick J, Ronnenberg AG. Vitamin E and adiponectin: proposed mechanism for vitamin E-induced improvement in insulin sensitivity. Nutr Rev. 2011;69(3):155-61.

16. Boudina S, Sena S, Theobald H, Sheng X, Wright JJ, Hu XX, et al. Mitochondrial energetics in the heart in obesity-related diabetes: direct evidence for increased uncoupled respiration and activation of uncoupling proteins. Diabetes. 2007;;56(10):2457-66. Epub 2007 Jul 10.
17. Aliciguzel Y, Ozen I, Aslan M, Karayalcin U. Activities of xanthine oxidoreductase and antioxidant enzymes in different tissues of diabetic rats. J Lab Clin Med. 2003;142:172–177.
18. Boudina S, Abel ED. Mitochondrial uncoupling: a key contributor to reduced cardiac efficiency in diabetes. Physiology (Bethesda). 2006;21:250–258.
19. Matsushima S, Kinugawa S, Ide T, Matsusaka H, Inoue N, Ohta Y, et al. Overexpression of glutathione peroxidase attenuates myocardial remodeling and preserves diastolic function in diabetic heart. Am J Physiol Heart Circ Physiol. 2006;291:H2237–H2245.
20. Abdelrahman M, Sivarajah A, Thiemermann C. Beneficial effects of PPAR-γ ligands in ischemia–reperfusion injury, inflammation and shock. Cardiovasc Res 2005;65(4):772-781.
21. Shimabukuro M, Higa S, Shinzato T, Nagamine F, Komiya I, Takasu N. Cardioprotective effects of troglitazone in streptozotocin-induced diabetic rats. Metabolism. 1996;45:1168–1173.
22. Khandoudi N, Delerive P., Berrebi-Bertrand I, Buckingham RE, Staels B, Bril A. Rosiglitazone, a peroxisome proliferator-activated receptor-gamma, inhibits the Jun NH(2)-terminal kinase/activating protein 1 pathway and protects the heart from ischemia/reperfusion injury. Diabetes. 2002;51:1507–1514.
23. Schupp M, Janke J, Clasen R, Unger T, Kintscher U. Angiotensin Type 1 Receptor Blockers Induce Peroxisome Proliferator–Activated Receptor-Activity Circulation. 2004;109:2054-2057
24. Zhu P, Lu L, Xu Y, Schwartz GG. Troglitazone improves recovery of left ventricular function after regional ischemia in pigs. Circulation. 2000;101:1165–1171.
25. Boudina S, Abel ED. Diabetic cardiomyopathy revisited. Circulation. 2007;115(25):3213-23.
26. Li SY, Yang X, Ceylan-Isik AF, Du M, Sreejayan N, Ren J. Cardiac contractile dysfunction in Lep/Lep obesity is accompanied by NADPH oxidase activation, oxidative modification of sarco(endo)plasmic reticulum Ca2+-ATPase and myosin heavy chain isozyme switch. Diabetologia. 2006;49:1434–1446.
27. Bai1 SK, Lee SJ, Na HJ, Ha KS, Han JA, Lee H, et al. Carotene inhibits inflammatory gene expression in lipopolysaccharide-stimulated macrophages by suppressing redox-based NF-□B activation. Experimental and medicine. 2005;37(4):323-334.
28. Stewart JA Jr, Lazartigues E, Lucchesi PA. The angiotensin converting enzyme 2/Ang-(1-7) axis in the heart: a role for MAS communication? Circ Res. 2008;103(11):1197-9.
29. Sasaki M, Jordan P, Welbourne T, Minagar A, Joh T, Itoh M, et al. Troglitazone, a PPAR-gamma activator prevents endothelial cell adhesion molecule expression and lymphocyte adhesion mediated by TNF-alpha. BMC Physiol. 2005;5:3.

30. Yoshida T, Okuno A, Tanaka J, Takahashi K, Nakashima R, Kanda S, et al. Metformin primarily decreases plasma glucose not by gluconeogenesis suppression but by activating glucose utilization in a non-obese type 2 diabetes Goto-Kakizaki rats. Eur J Pharmacol. 2009;25;623(1-3):141-7.
31. Behzad M, Negah R, Neda R. A review of thiazolidinediones and metformin in the treatment of type 2 diabetes with focus on cardiovascular complications. Vasc Health Risk Manag. 2007;3(6):967-973.
32. Bindom and Lazartigues The sweeter side of ACE2: Physiological evidence for a role in diabetesMol Cell Endocrinol. 2009;302(2):193–202.
33. Sugawara A., Akira Uruno, Masataka Kudo, Ken Matsuda, Chul Woo Yang, Sadayoshi Ito. PPARγ Agonist Beyond Glucose Lowering Effect. /kjim.2011.26.1.19
34. Sugawara A., Akira Uruno, Masataka Kudo, Ken Matsuda, Chul Woo Yang, Sadayoshi Ito Effects of PPARγ on hypertension, atherosclerosis, and chronic kidney disease. Endocrine Journal. 2010;57(10):847-852
35. Molavi B, Chen J, Mehta JL. Cardioprotective effects of rosiglitazone are associated with selective over expression of type 2 angiotensin receptors and inhibition of p42/44 MAPK. Am J Physiol Heart Circ Physiol. 2006;291(2):H687-93.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=279&id=3&aid=2570