ROLE OF OXIDATIVE STRESS IN CARDIAC HYPERTROPHY INDUCED BY ROSIGLITAZONE IN DIABETIC RATS

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Abstract: Rosiglitazone is a member of thiazolidinediones (TZDs) which are insulin sensitizers used for treatment of diabetes. However, the use of rosiglitazone in diabetes has been associated with increased risk of cardiovascular (CV) disorders. The mechanisms for this increased CV risk of rosiglitazone are unclear. This study evaluates the involvement of oxidative stress, as an important mechanism of CV diabetic disorders, in rosiglitazone-induced cardiac hypertrophy in diabetic rats. Diabetes was induced in rats by streptozotocin (65 mg/kg i.v.) and rosiglitazone was administered orally (10 mg/kg/day) for 2 weeks before determination of serum glucose, CK-MB and LDH. Electrocardiogram (ECG) profile and cardiac markers of oxidative stress [malondialdehyde (MDA), reduced glutathione (GSH) and superoxide dismutase (SOD)] were also determined. Rosiglitazone caused improvement of hyperglycemia while it increased cardiac toxicity as shown by the elevation in cardiac biomarkers [CK-MB and LDH] of diabetic rats. Additionally, it caused widening of QRS intervals, prolongation in QT intervals and increased R wave amplitude suggesting abnormal ventricular conduction and left ventricular hypertrophy. Conversely, rosiglitazone did not affect the diabetic oxidative stress in the heart. In conclusion, rosiglitazone can induce cardiomyopathy in diabetes as shown by worsening of ECG profile and increasing cardiac biomarkers. These effects are not dependent on oxidative stress but rather may be related to abnormalities of cardiac ion channels and other mechanisms.

Keywords: Rosiglitazone, diabetes, cardiac hypertrophy, oxidative stress, Rat
INTRODUCTION

Diabetes is a rapidly expanding health problem. In 2025, the number of people with diabetes is expected to reach 300 million [1].

Diabetic patients have at least a 2- to 4-fold increased risk for cardiovascular (CV) disorders which are the leading cause of morbidity and mortality in diabetes [2].

Thiazolidinediones (TZDs) such as rosiglitazone and pioglitazone are insulin sensitizers that have been on the market for more than a decade. Their effect on insulin sensitivity is due to activation of the peroxisome proliferation-activated receptor-γ (PPAR-γ). Binding of TZDs to PPAR-γ activates transcription factors that modulate gene expression, leading to increased insulin sensitivity in peripheral tissues [3].

The first TZD to be marketed, troglitazone, was discontinued because of hepatic and cardiac toxic effects. In 2007, the FDA released a safety alert about possible increased risk of CV events with rosiglitazone. Rosiglitazone have been found to significantly increase the risk of myocardial infarction and possibly increases CV mortality [4]. In July 2010, an advisory committee of the FDA evaluated the safety of rosiglitazone and indicated that the drug raised the risk of CV events compared with other diabetes drugs but recommended leaving the drug on the market with various degrees of warnings or restrictions [5].

The mechanisms for this increased CV risk of TZDs such as rosiglitazone are unclear, and a better understanding of the effects of rosiglitazone on the diabetic heart is needed. This study evaluates the involvement of oxidative stress, as an important mechanism of CV diabetic disorders, in rosiglitazone-induced cardiac hypertrophy in diabetic rats.

METHODS AND MATERIALS

Drugs and chemicals

Streptozotocin, urethane, phosphate-buffered saline (PBS), heparin, trichloroacetic acid (TCA), pyrogallol and Ellman’s reagent were purchased from Sigma Aldrich chemical Co. (St. Louis, MO, USA). Rosiglitazone was obtained as Avandia® tablets (GlaxoSmithKline, UK).

Experimental animals.

Male Sprague Dawley rats, weighing 200 ± 20 g, were purchased from “Egyptian Organization for Biological Products and Vaccines”, Giza, Egypt. The animal care and experiments described in this study comply with the ethical principles and guidelines for the care and use of laboratory animals adopted by the “Research Ethics Committee” of Faculty of Pharmacy, Mansoura
University, Egypt which are in accordance with “Principles of Laboratory Animal Care” (NIH publication No. 85-23, revised 1985).

**Induction of diabetes and experimental protocol.**

Diabetes was induced in rats with a single i.v. injection in the tail vein of streptozotocin (65 mg/kg dissolved in 0.1 M citrate buffer). The control animals were injected with equal volumes of the vehicle. Forty-eight hours after streptozotocin injection, animals showing fasting serum glucose > 350 mg/dl were considered to be diabetic.

Rats were divided into four groups (6 animals each) as following:

1- Control group: normal rats receiving drug vehicle and single injection of citrate buffer.

2- RGN group: normal rats receiving single injection of citrate buffer and 10 mg/kg/day rosiglitazone (RGN) by oral gavage for 2 weeks.

3- Diabetic group: diabetic rats receiving drug vehicle and single injection of citrate buffer.

4- Diabetic + RGN group: diabetic rats receiving 10 mg/kg/day rosiglitazone for 2 weeks.

Blood glucose was measured via the glucose oxidase method, using tail blood and a portable blood glucose monitor (Accu-chek® Active, Roche Diagnostic, Germany).

After 2 weeks, rats were anesthetized with i.p. injection of 1.8 g/kg urethane. Electrocardiogram (ECG) was recorded and blood samples were collected from the retro-orbital venous plexus to obtain serum for biochemical measurements.

**Electrocardiogram (ECG).**

Electrocardiograms were recorded from standard lead II limb leads using a single channel ECG (Fukuda ME Kogyo Co. Ltd., Model: 501-B III, Tokyo, Japan). The ECG was standardized before each tracing to get 2 mV sensitivity pulse that produces 20 mm height, with speed 50 mm/sec.

**Determination of serum creatine kinase-MB isozyme (CK-MB) activity.**

CK-MB activity was determined according to the method of Wurzburg et al. [6] using a commercial kit (Centronic GmbH, Germany). The method is based on measuring CK activity in the presence of an antibody to the CK-M monomer that does not affect the activity of CK-B subunits. CK-MB activity was measured at 340 nm wavelength and expressed as a unit per liter (U/L),
Determination of serum lactate dehydrogenase (LDH) activity.

LDH activity was assessed according to the method of Henry et al. [7]. The experimental procedure works by monitoring the disappearance of NADH⁺ which absorbs at 340 nm. By time, NADH⁺ is consumed and its consumption is directly proportional to serum LDH concentration. LDH activity was expressed as U/L.

Preparation of heart homogenate:

Hearts were isolated at the end of the experiment, weighed, and homogenized in PBS buffer containing heparin (0.16 mg /ml) as 10% (w/v) using Omni-125 hand held homogenizer (Omni international, USA). The homogenates were centrifuged at 2000×g/4ºC for 15 min, and supernatant was used for fresh assay of oxidative biomarkers (MDA, GSH and SOD).

Determination of cardiac malondialdehyde (MDA) concentration, reduced glutathione (GSH), Superoxide dismutase (SOD) activity.

Thiobarbituric acid reactive substance was measured as MDA, the end product of lipid peroxidation, according to the method of Ohkawa et al. [8]. The absorbance was determined at 532 nm spectrophotometrically and expressed as nmol/mg protein.

To determine the GSH, TCA-deproteinized serum was used to measure non-protein sulfhydryl compound by the method of Ellman [9] which is based on the reaction of GSH with Ellman’s reagent to give a compound that absorbs at 412 nm and expressed µmol/mg protein.

Superoxide dismutase activity was measured by the method of Marlund and Marklund [10]. The degree of inhibition of the auto oxidation of pyrogallol at an alkaline pH by SOD was used as a measure of the enzyme activity and expressed as U/mg protein.

Statistical Analysis

Data are expressed as mean ± standard error of the mean (SEM), where n= no. of rats. Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test. The level of significance was set at (p< 0.05). Statistical tests and graphs were performed with GraphPad Prism V 5.02 (GraphPad Software Inc., San Diego, CA, USA).
RESULTS

Effect of rosiglitazone on serum glucose in diabetic rats:

Diabetes caused a significant (p<0.05, n=6) increase of serum glucose level by 442% compared to control group (Figure 1). Rosiglitazone had no significant effect on serum glucose level in control rats. However, in diabetic rats, rosiglitazone treatment caused a significant (p<0.05, n=6) decrease of serum glucose level to 327% compared with diabetic group.

![Graph showing effect of rosiglitazone on serum glucose in diabetic rats](image)

Figure (1): Effect of rosiglitazone on serum glucose in diabetic rats:

Diabetes was induced in rats by streptozotocin (65 mg/kg i.v.) and rosiglitazone (RGN) was administered orally (10 mg/kg/day) for 2 weeks before determination of serum glucose.

Data are expressed as mean ± SEM; n=6. * p<0.05 and $ p<0.05, significantly different from control and diabetic groups respectively using one-way ANOVA, followed by Bonferroni post hoc test.
Effect of rosiglitazone on serum CK-MB in diabetic rats:

Diabetes caused a significant (p<0.05, n=6) increase of serum CK-MB by 210% compared to control group (Figure 2). Rosiglitazone treatment in normal rats increased serum CK-MB by 134% compared to control group. Moreover, rosiglitazone treatment significantly caused a further increase of serum CK-MB level to 311% compared with diabetic group.

![Graph showing effect of rosiglitazone on serum CK-MB in diabetic rats](image)

Figure (2): Effect of rosiglitazone on serum CK-MB in diabetic rats:

Diabetes was induced in rats by streptozotocin (65 mg/kg i.v.) and rosiglitazone (RGN) was administered orally (10 mg/kg/day) for 2 weeks before determination of serum CK-MB.

Data are expressed as mean ± SEM; n=6. * p<0.05 and $ p<0.05, significantly different from control and diabetic groups respectively using one-way ANOVA, followed by Bonferroni post hoc test.
Effect of rosiglitazone on serum LDH in diabetic rats:

Diabetes caused a significant (p<0.05, n=6) increase of serum LDH by 378% compared to control group (Figure 3). Rosiglitazone treatment in normal rats increased serum LDH by 155% compared to control group. Moreover, rosiglitazone treatment significantly caused a further increase of serum LDH level to 559% compared with diabetic group.

![Graph showing the effect of rosiglitazone on serum LDH in diabetic rats.](image)

**Figure (3): Effect of rosiglitazone on serum LDH in diabetic rats:**

Diabetes was induced in rats by streptozotocin (65 mg/kg i.v.) and rosiglitazone (RGN) was administered orally (10 mg/kg/day) for 2 weeks before determination of serum LDH.

Data are expressed as mean ± SEM; n=6. * p<0.05 and $ p<0.05, significantly different from control and diabetic groups respectively using one-way ANOVA, followed by Bonferroni post hoc test.
Effect of rosiglitazone on ECG parameters in diabetic rats:

Heart rate (HR) fell rapidly and dramatically after administration of rosiglitazone in diabetic group (Table 1).

Widening of QRS intervals, a sign of abnormal intra-ventricular conduction, was found to be significant in diabetic rats compared to control rats (Table 1). Rosiglitazone treatment showed a significant widening in QRS intervals when compared to control group.

QT interval, a measure of ventricular repolarization, was longer in diabetic rats treated with RGN when compared to either control or diabetic groups, respectively.

R wave amplitude, suggesting left ventricular hypertrophy was significantly increased in diabetic rats compared to control. Treatment of diabetic rats with rosiglitazone showed a further increase in R wave amplitude when compared to diabetic group.

For PR interval, administration of rosiglitazone in diabetic group caused a significant prolongation in PR interval compared to control group.

Table (1): Effect of rosiglitazone on ECG parameters in diabetic rats:

<table>
<thead>
<tr>
<th></th>
<th>HR (bpm)</th>
<th>PR interval (msec)</th>
<th>QRS interval (msec)</th>
<th>QT interval (msec)</th>
<th>R voltage (μ volt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>279 ± 10</td>
<td>47 ± 2</td>
<td>45 ± 2</td>
<td>182 ± 6</td>
<td>571 ± 22</td>
</tr>
<tr>
<td>RGN group</td>
<td>292 ± 6</td>
<td>45 ± 2</td>
<td>66 ± 5*</td>
<td>204 ± 7</td>
<td>628 ± 26</td>
</tr>
<tr>
<td>Diabetic group</td>
<td>311 ± 9</td>
<td>51 ± 2</td>
<td>64 ± 4*</td>
<td>204.1±6.68</td>
<td>808 ± 41*</td>
</tr>
<tr>
<td>Diabetic+RGN group</td>
<td>231 ± 7$</td>
<td>58 ± 3*</td>
<td>69 ± 6*</td>
<td>244.3±7.7$</td>
<td>987 ± 65$</td>
</tr>
</tbody>
</table>

Diabetes was induced in rats by streptozotocin (65 mg/kg i.v.) and rosiglitazone (RGN) was administered orally (10 mg/kg/day) for 2 weeks before measuring ECG.

Data are expressed as mean ± SEM; n=6. * p<0.05 and $ p<0.05, significantly different from control and diabetic groups respectively using one-way ANOVA, followed by Bonferroni post hoc test.
Effect of rosiglitazone on cardiac MDA and GSH content and SOD activity in diabetic rats:

Diabetes caused a significant (p<0.05, n=6) increase of lipid peroxidation as shown by the elevation in cardiac MDA content by 302% compared to control group (Figure 4A). Additionally, diabetes significantly (p<0.05, n=6) decreased cardiac GSH content and SOD activity by 58 and 52 % respectively compared to control group (Figure 4B and 4C). Rosiglitazone treatment had no significant effect on these cardiac oxidative parameters in normal rats. Furthermore, rosiglitazone treatment did not significantly affect the diabetes-induced changes in cardiac MDA, GSH or SOD.

Figure (4): Effect of rosiglitazone on cardiac MDA, GSH and SOD in diabetic rats:

Diabetes was induced in rats by streptozotocin (65 mg/kg i.v.) and rosiglitazone (RGN) was administered orally (10 mg/kg/day) for 2 weeks before determination of cardiac (A): malondialdehyde (MDA), (B): reduced glutathione (GSH) and (C): superoxide dismutase (SOD).

Data are expressed as mean ± SEM; n=6. * p<0.05, significantly different from control group using one-way ANOVA, followed by Bonferroni post hoc test.
DISCUSSION

The major findings of the present study were as follows: improvement of hyperglycemia by rosiglitazone, the increased abnormalities of ECG and increased cardiac biomarkers such as CK-MB and LDH of diabetic rats treated with rosiglitazone and the inability of rosiglitazone treatment to correct diabetic oxidative stress in the heart.

The most important goal in the treatment of patients with diabetes is to prevent the risk of CV disease as the first cause of mortality in these patients. TZDs improve insulin sensitivity which is known to be effective in both counteracting the onset and reducing the detrimental impact of cardiovascular risk factors. However, controversial reports about the role of TZDs use in CV disorders are present with some showing the increase in CV outcomes [4,11] while others showing no association between TZDs use and CV disorders [12-14].

To measure whether rosiglitazone causes cardiotoxic effects or not, cardiac biomarkers such as CK-MB and LDH have been used as indicators for cardiac disorders. It has been previously reported that serum LDH and CK-MB activities were found to be increased in cardiomyopathy in diabetic patients and may serve as a marker for cardiovascular risk and cardiac muscular damage [15]. In our study, serum LDH and CK-MB activities were found to be increased in diabetic rats, possibly due to myocardial dysfunction. Moreover, there was a significant increase in serum LDH and CK-MB levels observed with rosiglitazone treatment which is marked in diabetic than normal rats indicating the cardiotoxic effect of rosiglitazone.

In addition to cardiac biomarkers, we confirmed the cardiotoxic effects of rosiglitazone using ECG. Rosiglitazone treatment in both normal and diabetic rats caused widening of QRS intervals and prolongation in QT intervals representing abnormal ventricular conduction. Moreover, rosiglitazone increased R wave amplitude, suggesting left ventricular hypertrophy. Previous studies have shown similar cardiac cycle abnormalities which may be explained by lengthening of the action potential duration due to a decrease in potassium currents [16,17].

The underlying mechanisms for the development of diabetic cardiomyopathy involves hyperglycemia, hyperinsulinemia and hyperlipidemia [18,19]. These risk factors initiate significant cellular and molecular changes, such as oxidative stress, endothelial dysfunction, inflammation and apoptosis. These changes ultimately leading to cardiac fibrosis and hypertrophy, which are major structural changes associated with cardiac dysfunction inherent to diabetic cardiomyopathy [20,21].

In diabetes, oxidative stress has been recognized as an important player in the mechanism underlying diabetic cardiomyopathy. Diabetes leads to reduction of antioxidant defense
mechanisms and, consequently, to toxic effects induced by the oxidative stress aggravating cardiac function \(^{[22]}\). Results of this study revealed significant increase in cardiac oxidative stress biomarkers such as the increase in MDA and the decrease in GSH and SOD in diabetic rats.

One possible mechanism for rosiglitazone toxic cardiac effects could be due to its effects on the cardiac antioxidant defense mechanism. Palee et al. \(^{[23]}\), proved that in rat isolated cardiac mitochondria, rosiglitazone could not prevent oxidative damage caused by H\(_2\)O\(_2\). Additionally, rosiglitazone caused cardiotoxicity via PPAR\(\gamma\)-independent mitochondrial oxidative stress in mouse hearts \(^{[24]}\). However, rosiglitazone treatment in our study did not affect the oxidative stress induced by diabetes in rats. This indicates that oxidative stress may not represent a major pathway through which rosiglitazone causes CV disorders.

It has been proposed that rosiglitazone blocks K\(\text{ATP}\) channels, and thus alters the action potential and may promote arrhythmia \(^{[16,17]}\). Another possible mechanism of the profibrillatory effect of rosiglitazone could be due to its effects on the cardiac mitochondria. Increased ROS production and oscillation of the mitochondrial membrane potential have been shown to play an important role in the genesis of cardiac arrhythmias \(^{[25]}\).

In conclusion, rosiglitazone can induce cardiomyopathy in diabetes as shown by worsening of ECG profile and increasing cardiac biomarkers. These effects are not dependent on oxidative stress but rather may be related to abnormalities of cardiac ion channels and other mechanisms.

REFERENCES


