Effect of Rosiglitazone on Myocardial Ischemia-Reperfusion Injury in Rat Heart

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This study was undertaken to evaluate whether peroxisome proliferator-activated-receptor-gamma (PPAR-γ) agonist-rosiglitazone (ROSI) induces postischemic functional recovery in Langendorf heart model. Hearts isolated from normal rats were subjected to 20 min of normoxia or 25 min zero-flow ischemia followed by 50 min reperfusion. In this acute protocol, ROSI (20 μg/ml) administered 10 min before ischemia had no effect on hemodynamic cardiac function, but had protective effect on lipid peroxidation in in vitro experiments. In chronic protocol in which ROSI was given by daily gavage (4 mg/kg) for three consecutive days, ROSI could not prevent the hemodynamic alteration on cardiac performance, but has protective effect on the activity of superoxide dismutase (SOD). There was no significant difference in the contents of reduced glutathione (GSH) and catalase activity between ischemia-reperfusion (IR) and ROSI treated IR hearts. Although ROSI had no effect on hemodynamic factor, it had effect on antioxidant activity. Our results indicate that ROSI provides partial beneficial effects by inhibiting lipid peroxidation and/or recovering normal level of SOD activity in the ischemic reperfused heart.

Key Words: Peroxisome proliferator-activated-receptor-gamma, Rosiglitazone, Ischemia-reperfusion, Lipid peroxidation, Superoxide dismutase, Catalase

INTRODUCTION

Oxidative stress is a well established etiopathogenic factor of ischemic heart disease (IHD) and its consequences. Ischemia-reperfusion (IR) injury is known to be an important factor in several clinical disorders (Kloner et al, 1989). In the myocardium, IR injury occurs after a critical period of coronary occlusion followed by blood restoration, such events as myocardial infarction (MI) and procedures such as coronary bypass surgery and transplantation. The pathogenesis of IR injury is due to the production of reactive oxygen species (ROS). The generation of ROS immediately upon reperfusion has been documented in experimental conditions as well as in patients with acute myocardial infarction undergoing thrombolysis, coronary angioplasty or open heart surgery (Bolli, 1998). Upon reperfusion, molecular oxygen undergoes sequential reduction to form ROS, including superoxide anion and hydroxyl radical, in addition to hydrogen peroxide. In cardiac tissues, there is a protective system of endogenous anti-oxidants which includes several antioxidative enzymes [e.g., superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)] and non-enzymatic anti-oxidants. Among these antioxidants, the most important thing is glutathione system, which effectively hampers ROS-initiated damage under normal physiological conditions. However, when faced with severe oxidative stress after IR, the generation of ROS can exceed the capacity of this endogenous defense system, thus leading to pathophysiologic events. Moreover, tissue injury mediated by oxygen-derived free radicals is thought to be due to the activation of lipid peroxidation in cellular and subcellular membranes (Meerson et al, 1982). Several studies have demonstrated alterations in phospholipids metabolism following both ischemia and reperfusion (Chien et al, 1985; Burton et al, 1986). Lipid peroxidation is a self-propagating chain reaction, which forms lipid peroxides, which are then reduced to alcohols by glutathione peroxidase, resulting in further oxidation of GSH to GSSG.

The peroxisome proliferator-activated receptor-gamma (PPAR-γ) is a member of the nuclear receptor superfamily of ligand-activated transcription factors and is highly expressed in adipose tissue and, to a lesser degree, in several other mammalian tissues (Braissant et al, 1996; Mukherjee et al, 1997). Rosiglitazone (ROSI) is a PPAR-γ agonist and the most potent member of the thiazolidinedione antidiabetic agents. It is reported that pretreatment with the ROSI reduced infarct size in normal rat (Yue Tl et al, 2001)

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ABBREVIATIONS: PPAR-α, peroxisome proliferator-activated- receptor-gamma; ROSI, rosiglitazone; TBARS, thiobarbituric acid reactive substances; IR, ischemia-reperfusion; LVDP, left ventricular developed pressure; LVEDP, left ventricular end diastolic pressure; SOD, superoxide dismutase; GSH, reduced glutathione; CAT, catalase.

and reduced ischemic injury in isolated, perfused normal hearts (Sidell et al, 2002). Moreover, ROSI pre-treatment improved IR-induced myocardial contractile dysfunction (Yue Tl et al, 2001), and may (Uchida et al, 2000) or may not (Khandoudi et al, 2002) be a positive inotrope in isolated perfused rat hearts.

The aims of this study, therefore, were: (1) to assess whether treatment with ROSI produces cardioprotective effects in the IR hearts and (2) to assess biochemically the effects of ROSI on oxidative stress and antioxidant defenses in the myocardium during IR, by measuring lipid peroxidation (TBARS), reduced glutathione (GSH) and superoxide dismutase (SOD).

METHODS

Chemicals

Rosiglitazone maleate (Avandia) was obtained from SB Pharmco (Puerto Rico Inc. USA), and other drugs were purchased from Sigma-Aldrich (St Louis, MO, USA).

Isolation of heart and perfusion

Sprague-Dawley male rats, weighing 250 ± 20 g, were used for perfusion experiments. The hearts were excised, the ascending aorta was cannulated, and immediately retrogradedly perfused with non-recirculating modified Krebs- Henseleit (K-H) solution (in mM: NaCl 118, KCl 4.7, $CaCl_2$ 1, HEPES 10, $MgSO_4$ 1.2, pyruvic acid 3, and glucose 11) at a constant pressure of 80 cm H_2O. The perfusion solution was bubbled with 100% O_2 and kept at 37°C. To assess contractile function, a latex balloon connected to a pressure transducer (Powerlab, ADI, USA) was inserted into the left ventricular cavity via the left atrium. Left ventricular end- diastolic pressure (LVEDP) was set at $8 \sim 12$ mmHg by inflating the balloon with physiological saline, and left ventricular pressure was continuously recorded. Coronary flow was measured by collecting the effluent. Pacing wires were fixed to the right atrium and left ventricular, and all hearts were paced at 300 beats/min (5.0 Hz, 2.0 V, and 2.0 ms).

Experimental protocol

There were 4 experimental groups of 6 rats each: group 1 (CTR), the hearts were perfused for 95 min with no ischemia-reperfusion; group 2 (CTR-IR), the hearts were allowed to stabilize for 20 min, followed by 25 min ischemia and then reperfusion for 50 min; group 3 (ROSI-P), ROSI (20 μg/ml) was added 10 min before ischemia in the perfused K-H buffer and during whole reperfusion period; and group 4 (ROSI-F), ROSI was fed three days before experiment and then the process was the same as in group 2.

Lipid peroxidation

The assessment of the extent of lipid peroxidation was based on individual determinations of the content of thiobarbituric acid reactive substance (TBARS) in heart homogenates prepared from the four groups of experimental rats (Ohkawa et al, 1979). TBARS reagent (1 ml) was added to a 0.5 ml aliquot of tissue homogenate and heated for 20 min at 100°C. The antioxidant, butylated hydroxy-

toluene, was added before heating of samples. After cooling on ice, the samples were centrifuged at $840 \times g$ for 15 min, and absorbance of the supernatant was read at 532 nm. Sample blanks for each sample were prepared and assessed in the above described way to correct for A_{532} contribution made by the sample. Duplicate determinations were made and the average of the two measurements was used in the subsequent statistical analysis. Using tetramethoxypropane as a standard, tissue lipid peroxide levels were calculated as nM/mg of protein.

Superoxide dismutase

Superoxide dismutase (SOD) activities of heart were measured with NBT reagent using a spectrophotometer. The preparations were homogenized for 60 sec in tissue lysis buffer containing 50 mM potassium phosphate, 1 mM EGTA, and 1% triton X-100. The homogenate was centrifuged at 4°C and 12,000 $\times$ g for 5 min, and the concentration of protein in the supernatant was determined by the Bradford method. For measurement of SOD activity, we diluted the supernatants (1 mg protein/ml) with 50 mM potassium phosphate buffer containing 50 mM nitroblue tetrazolium (NBT), 0.1 mM xanthine, and 0.002 U/ml xanthine oxidase. And then, SOD activity was determined from kinetics of absorbance at 560 nm.

Determination of catalase activity

The cardiac muscle was removed and homogenized in phosphate buffer (50 mM; pH 7.0) containing 0.1% Triton X-100. The homogenates were centrifuged at 10,000 $\times$ g for 10 min in an Eppendorf microcentrifuge. Aliquots of supernatant were used to determine catalase levels. Catalase activity in the supernatants was assayed spectrophotometrically by measuring the decrease of absorbance at 240 nm (Beers & Sizer, 1952). The specific activity was defined as units per milligram of protein. Protein contents in supernatants were determined by the Bradford method (1976).

Myocardial reduced glutathione

Myocardial reduced glutathione (GSH) was determined by the colorimetric method (Kum-Tatt & Tan, 1974). The hearts were homogenized with 10% TCA and centrifuged at 3,000 $\times$ g for 10 min. The reaction mixture contained 0.1 ml of supernatant, 2.0 ml of 0.3 M phosphate buffer (pH 8.4), 0.4 ml of double distilled water and 0.5 ml of 5,5-dithiobis 2-nirobenzoic acid (DTNB). The reaction mixture was incubated for 10 min, and the content was evaluated from absorbance together with the standard curve of DTNB's reaction obtained with commercially available GSH (Sigma chemical company, USA).

Statistical analysis

All values were expressed as mean $\pm$ SE. Paired or unpaired Student's t-test was applied to test for significance of results of the different groups. *Significance is set at $p < 0.05$.

RESULTS

Effects of rosiglitazone (ROSI) on postischemic myocardial functions

During the pre-ischemic period, all parameters measured, such as LVDP, LVEDP, heart rate, and coronary flow, were comparable among the groups. In the normal rat hearts, the time course of changes in LVDP, LVEDP and coronary flow in the CTR and ROSI-P or ROSI-F groups are shown in Fig. 1. As evident in the figure, there were no significant protective effects on coronary flow when ROSI treated hearts were reperfused. One group included rats which were fed with ROSI for three days (ROSI-F), whereas the other group consisted of rats whose hearts were perfused with ROSI (ROSI-P). The post-ischemic recoveries of LVDP and LVEDP showed no difference in the extent of functional recovery between ROSI groups and control hearts (Fig. 1A and B). There was also no significant difference in heart rate (data not shown).

Effects of rosiglitazone (ROSI) on GSH

Significant decrease of reduced glutathione (GSH) level in myocardium was observed in CTR-IR group, compared with CTR group. In the ROSI-P and ROSI-F groups, there was no significant change of myocardial GSH levels, compared with CTR-IR (Fig. 2).
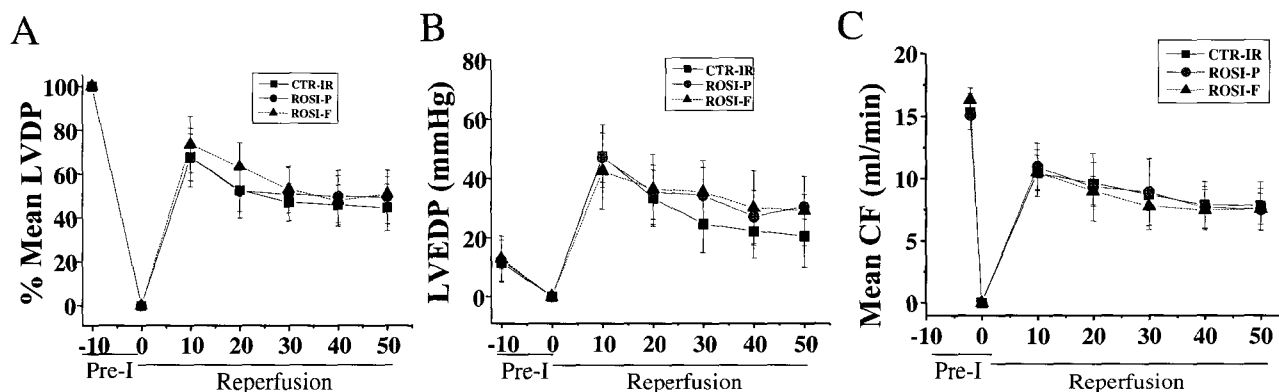

Fig. 1. The effect of rosiglitazone (ROSI) on cardiac functions. Time course of changes in LVDP (A), LVEDP (B), and CF (C) during 20 min equilibration period and 25 min of zero-flow ischemia followed by 50 min of reperfusion in the CTR-IR (■) and ROSI-P (●) and ROSI-F (▲) rat hearts. ROSI (20 µg/ml) was added to the perfusion solution 10 min before ischemia and was maintained throughout reperfusion. LVDP was measured before and after ischemia.
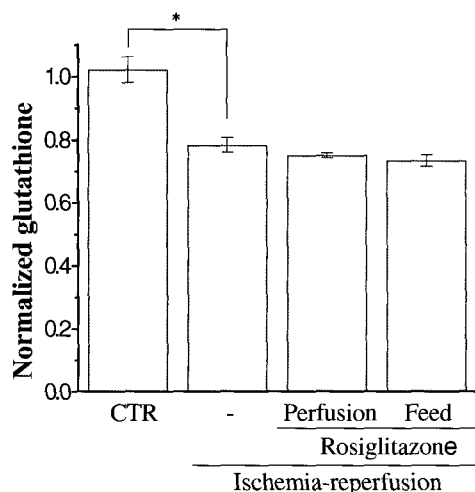

Fig. 2. The effects of Rosiglitazone (ROSI) on GSH in the IR hearts. Significant decrease in myocardial GSH level was observed in the CTR-IR group, when compared with CTR group. In the ROSI-P and ROSI-F groups, there was no significant change of myocardial GSH levels, when compared with CTR-IR. Results were presented as means±SE of 6 hearts in each group (*p<0.05 versus CTR).
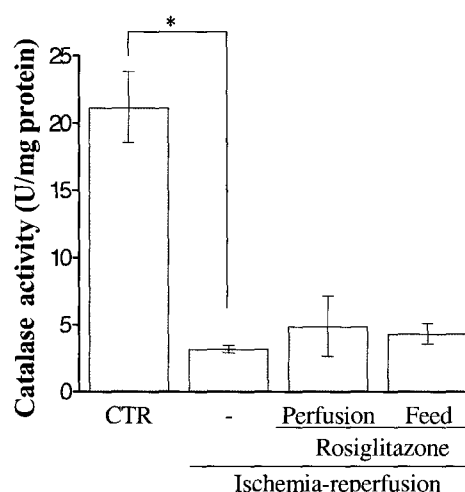
Fig. 3. The effects of rosiglitazone (ROSI) on catalase activity in the IR hearts. In the CTR-IR group, there was significant (p<0.05) decrease in myocardial catalase activity, compared with CTR group. In the ROSI-P and ROSI-F groups, there were no significant changes of myocardial catalase activity, compared with CTR-I/R group. Results were presented as means±SE of 6 hearts in each group (*p<0.05 versus CTR).
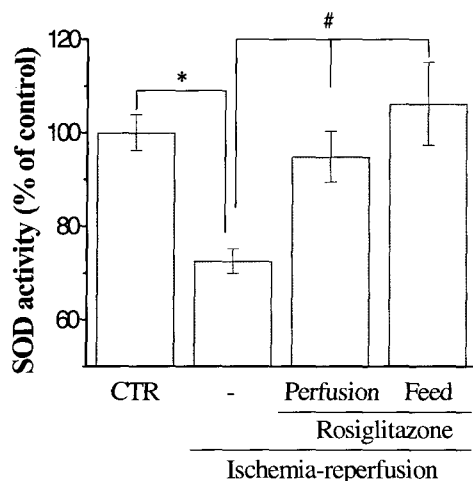
Fig. 4. The effects of rosiglitazone (ROSI) on myocardial SOD in the IR hearts. In the CTR-I/R group, there was a significant reduction in myocardial SOD activity, when compared with CTR group. Significant increases in myocardial SOD activities were observed in the ROSI-F and ROSI-P groups, compared with CTR-I/R group. Results were presented as means (SE of 6 hearts in each group (*p<0.05 versus CTR, #p<0.05 versus CTR-IR).

Fig. 5. The effects of rosiglitazone (ROSI) on lipid peroxidation in the IR hearts. There was significant increase of myocardial TBARS in the CTR-IR group, when compared with the CTR group. Significant decrease in the level of myocardial TBARS was observed in the ROSI-P group. And there was no significant difference in the ROSI-F group. Results were presented as means±SE of 6 hearts in each group (*p<0.05 versus CTR, #p<0.05 versus CTR-IR).

Effects of rosiglitazone (ROSI) on catalase activity

In the CTR-IR group, there was significant decrease in myocardial catalase activity, compared with the CTR group. Also, there was no significant change of myocardial catalase activity in the ROSI-P group, compared with the CTR-IR group. The catalase activity did not change also in the ROSI-F group (Fig. 3).

Effects of rosiglitazone (ROSI) on myocardial SOD

In the CTR-IR group, there was a significant reduction of myocardial SOD activity, compared with the CTR group. SOD activity was increased in the ROSI-P group, compared with the CTR-IR group. Significant increase of myocardial SOD activities was also observed in the ROSI-F group, compared with the CTR-IR group (Fig. 4).

Effects of rosiglitazone (ROSI) on lipid peroxidation

Fig. 5 illustrates the levels of lipid peroxidation products in cardiac tissues of experimental rats. There was significant increase of myocardial TBARS in the CTR-IR group, when compared with the CTR group. Significant decrease in the level of myocardial TBARS was observed also in the ROSI-P group. However, the ROSI-F group showed no significant difference in TBARS, compared with the CTR-IR group (Fig. 5).

DISCUSSION

The present study was performed to determine the effects of ROSI, a PPAR-γ agonist, on IR injury in langendorff apparatus heart. The results indicated that ROSI had protective effects on IR heart: ROSI reduced the lipid per-
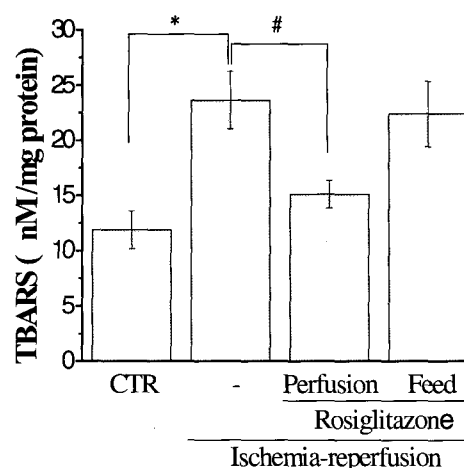
oxidation when ROSI was added in the perfusion buffer and SOD activity in the heart was increased, indicating that the beneficial effects of ROSI may be associated with its antioxidant property. However, the pronounced cardioprotection of ROSI is not likely mediated through its hemodynamic effects, because there was no change in mean LVDP, LVEDP and coronary flow.

Expression of PPAR-γ in the heart and cardiomyocytes has been reported, but the function of PPAR-γ in the heart is little known (Bishop-Bailey, 2000; Takano et al, 2000). An earlier in vitro study (Shimabukuro et al, 1996), using perfused hearts isolated from streptozotocin-induced diabetic rats, found that pretreatment of the rats with troglitazone, a PPAR-γ agonist, for 6 weeks improved the postischemic heart rate and cardiac work. We also observed in a study of nondiabetic rats that ROSI prevented myocardial infarction after ischemia (no-flow) and reperfusion injury. Short- or long-term treatment with ROSI resulted in a significant reduction of infarct size subjected to IR. However, in the present study, the pronounced cardioprotection of ROSI was not likely mediated through its hemodynamic effects, since no changes in LVDP, LVEDP and coronary flow were observed during experimental periods, when compared with the CTR-IR group (Fig. 1).

The cardioprotective effect of ROSI does not seem to be related to a direct hemodynamic effect, since our results showed no improvement in LVDP, LVEDP and coronary flow. In the absence of a hemodynamic explanation for the cardioprotective effects of ROSI, another mechanistic explanation for the observed effects could be the ability of drugs to improve lipid metabolism and antioxidant enzyme systems. Several mechanisms have been proposed to explain the myocardial injury observed after IR. Dormandy (1978) demonstrated that production of free radicals contributed to myocardial cell injury. Free radicals have been shown to initiate lipid peroxidation, resulting in an alteration of membrane integrity, fluidity and permeability

(Sevanian & Hochstein, 1985). Increased levels of lipid peroxidation product (TBARS) in the hearts of IR rats were observed. The increased peroxidation results from the increased oxidative stress in cardiac tissues induced by IR. In the present study, IR injury was found to be associated with increased oxidative stress, as evidenced by increase of myocardial TBARS and depletion of myocardial endogenous antioxidants such as SOD, catalase and GSH. Similar observations were made earlier by others, using similar models (Borchgrevink et al, 1989; Ambrosio et al, 1991; Singal et al, 1993; Maulik et al, 1999). Increased oxidative stress might be responsible for such myocyte injuries, and chronic ROSI administration prevented the oxidative stress. The mechanism of such protection of chronic ROSI administration could be due to myocardial adaptation.

Myocardial adaptation against oxidative stress is mediated through augmentation of a number of cellular antioxidants, such as SOD, catalase and GSH (Das et al, 1995; Engelman et al, 1995; Schaefer et al, 1998). Since IR injury is a common sequel of ischemic heart disease and oxidative stress plays a central role in its etiopathogenesis, protection against oxidative stress through myocardial adaptation holds a promise as an effective therapeutic approach. Myocardial adaptation occurs in response to various obnoxious stimuli, including ischemia (Lawson et al, 1993), certain endotoxins (Maulik et al, 1995), and free radicals (Sun et al, 1996), and protects heart from subsequent exposure to injuries of similar or more severe nature (Asimakis et al, 1992). Although protective in nature, the basic mechanisms of adaptation are harmful in them and, therefore, cannot be considered as acceptable therapeutic methods. Therefore, pharmacological as well as transgenic approaches of myocardial adaptation have recently become the focus of scientific interest.

In the present study, protection against IR-induced oxidative stress in ROSI treated rat hearts was evidenced by preservation of endogenous antioxidant and prevention of TBARS from rise. These findings suggest that ROSI could provide a novel therapeutic approach to reduce or prevent IR injury in the myocardium. The present results together with the recent findings showed that ROSI prevents the cardiac damage through lipid peroxidation and SOD activity, suggesting a potential beneficial action of the drug for the patients with substantially high risk of cardiovascular events.

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