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Effects of Rosiglitazone on Isoproterenol-induced Myocardial Infarction in Rats

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Abstract: The safety of rosiglitazone as it pertains to the cardiovascular system has been continuously debated. Because there is no strong evidence of an increased risk of myocardial infarction or cardiovascular mortality with rosiglitazone. This study aimed to investigate different doses of rosiglitazone effects to treat myocardial infarction in animal model specifically rats induced with isoperetenol used a high and low doses (6 and 12 mg kg^{-1}) determined the greatest influence and compare between doses. Spectrophotometric and chemical methods measured parameters specific to myocardial infarction presented a significant accumulation of lipid peroxidase, reduction of glutathione and increased of reduced lactate dehydrogenase levels were observed while the cardiac marker enzymes creatinine kinase, aspartate aminotransferase and alanine aminotransferase. None of these parameters changed significantly with rosiglitazone treatment (6 and 12 mg kg^{-1}) when compared to the group that was only treated with isoproterenol. Present results suggest that rosiglitazone does not potentiate the cardiac toxicity of isoproterenol-induced myocardial infarction in rats. These findings strengthen the notion that rosiglitazone has a lower risk of myocardial infarction than previously reported.

Key words: Rosiglitazone, myocardial infarction, isoproterenol, cardiac toxicity, antioxidant, PPAR-γ, cardiac marker

INTRODUCTION

Rosiglitazone, a specific agonist of the peroxisome Proliferator Activated Receptor-γ (PPARγ), improves insulin sensitivity in diabetic animals (Guerre-Millo *et al.*, 2000; Kramer *et al.*, 2001; Wang *et al.*, 2001) and in people with type 2 diabetes (DM) and insulin resistance syndrome (Mudaliar and Henry, 2001; Saltiel and Olefsky, 1996). PPARs are members of the nuclear hormone receptor super family and are activated by natural or synthetic fatty acids (Evans, 1988). Rosiglitazone has been found to mediate several other changes in cells that may contribute to its effectiveness in treating type 2 DM. Among these are an increase in adipocyte differentiation and a decrease in the release of free fatty acids from adipocytes through the blocking of tumour necrosis factor-alpha (Avandia, 1999; Spiegelman, 1998; Rosenbaum and Greenberg, 1998; Souza *et al.*, 1998).

A recent meta-analysis of 42 randomised clinical trials showed an increased risk of myocardial infarction in patients treated with rosiglitazone (Nissen and Wolski, 2007). A similar trend was observed for cardiac mortality, although this finding did not reach statistical significance. These results evoked doubts in the safety of rosiglitazone (Singh *et al.*, 2007). Subsequent analyses of patient-level data performed by the FDA Advisory Committee confirmed an increased risk of ischemic heart disease associated with the use of rosiglitazone (Rosen, 2007).

Conversely, there is evidence that several chemically distinct ligands of PPAR-γ reduce tissue necrosis associated with acute myocardial infarction. Wayman *et al.* (2002) showed that ligands of PPAR-γ. A recent study demonstrated that rosiglitazone therapy improves diastolic myocardial function in association with decreases in oxidative stress in asymptomatic patients with well-controlled, uncomplicated type 2 diabetes and no history of coronary artery disease or heart failure. Two other studies on rat models reported the effects of rosiglitazone cardioprotective on myocardial ischemia (Ito *et al.*, 2003; Yue *et al.*, 2001). Recent meta-analysis performed by Mannucci and colleagues, it was determined that figures for the rosiglitazone-associated risk for myocardial infarction could be lower than those previously reported in studies sponsored by GlaxoSmithKline that were based on of a small number of clinical trials (Mannucci *et al.*, 2008).

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Isoprenenol (ISO), a synthetic β-adrenoceptor agonist, has been found to induce myocardial infarction in rat by disturbing the physiological balance between the production of free radicals and the antioxidative defence system (Rathore *et al.*, 1998; Srivastava *et al.*, 2007). Balance disturbance leads to acute myocardium necrosis accompanied by increased cardiac marker enzymes, accumulated lipid peroxides and damaged cardiac function (Rajadurai and Prince, 2006, 2007a, b; Gupta *et al.*, 2004). ISO causes severe stress in myocardial tissue through a mechanism of action on the sarcolemmal membrane, stimulation of adenylate cyclase, activation of Na^+ and Ca^{++} channels, exaggerated Ca^{++} inflow and energy consumption which leads to cellular death (Milei *et al.*, 1978). It has been reported that the free radicals produced by ISO can initiate the peroxidation of membrane-bound Polyunsaturated Fatty Acids (PUFAs), leading to both functional and structural myocardial injury (Thompson and Hess, 1986).

The issue of the cardiac safety of rosiglitazone has been continuously debated. There is no strong evidence of an increased risk of myocardial infarction or cardiovascular mortality in patients treated with rosiglitazone.

The aim of this study was to investigate the effects of oral treatment with different doses of rosiglitazone on isoproterenol-induced myocardial infarction in rats.

MATERIALS AND METHODS

Animals: The present study conducted in 2011 within 4 weeks a study duration. Wistar rats (150-200 g) were maintained at constant room temperature (22-25°C) with access to food and water *ad libitum*, under a 12 h light/dark cycle. All experiments were carried out in accordance with the animal ethics recommendations of the King Saud University committee. The animals were housed in rectangular polypropylene cages (55 cm long x26 cm wide x24 cm high).

Drugs and chemicals: Standard chemical reagents of analytical grade and ISO were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Rosiglitazone was obtained from SmithKline Beecham Pharmaceuticals (Philadelphia, Pennsylvania, USA).

Induction of myocardial injury: ISO was dissolved in normal saline and injected subcutaneously into rats (85 mg kg^{-1}) for 2 consecutive days to induce experimental myocardial infarction (Rajadurai and Prince, 2007a).

Experimental protocol: Animals were randomly divided into four groups: (a) Untreated rats (control group, n = 12), (b) Rats injected subcutaneously with an ISO-induced myocardial infarction (ISO group, n = 12), (c) Rats treated orally with the low dose of rosiglitazone (6 mg kg^{-1} b.wt., n = 12) and finally, (d) Rats treated orally with the high dose of rosiglitazone (12 mg kg^{-1} b.wt., n = 12). Group a rats were administered normal saline orally for 2 weeks and rats in groups b, c and d were fasted and then injected subcutaneously with ISO (85 mg kg^{-1}) dissolved in normal saline for 2 consecutively days to induce experimental myocardial infarction. After the second day, group's c and d were fasted overnight and then treated with different oral doses of rosiglitazone (6 mg kg^{-1} for group c and 12 mg kg^{-1} for group d) for two weeks. At the end of the experiment (4 weeks), all animals were euthanized after fasting for 12 h and blood samples were collected in centrifuge tubes, allowed to stand at room temperature for 35 min and then centrifuged for 30 min at 4000 rpm. Hearts were removed rapidly, excised and washed with normal saline before being homogenised with tissue homogeniser in normal saline and then centrifuged at 3000×g for 10 min at 4°C. Serum, plasma and heart tissues samples were stored at -80°C until later use. Creatinine Kinase (CK), Lactate Dehydrogenase (LDH), Lipid Peroxidase (LPO), Glutathione (GSH), Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) were measured in these samples with a spectrophotometer.

Statistical analysis: All data were expressed as the Mean±SD and the statistical analysis of an observed value was performed using a one-way ANOVA ($p < 0.05$ was accepted as statistically significant).

RESULTS

Effects of rosiglitazone on cardiac marker enzymes: ISO-induced myocardial infarction was accompanied by a significant increase in the cardiac marker enzymes CK, AST and ALT. A significant effect appear clearly in ISO group for this enzymes compared with control and treated groups (Fig. 1). Neither 6 mg kg^{-1} nor 12 mg kg^{-1} rosiglitazone treatment induced changes in cardiac marker enzymes when compared to ISO treatment alone (Fig. 1).

Effects of rosiglitazone on antioxidant levels: ISO treatment caused the accumulation of lipid peroxides, the reduction of glutathione and increased of reduced LDH levels significantly compared with control group (Fig. 2).
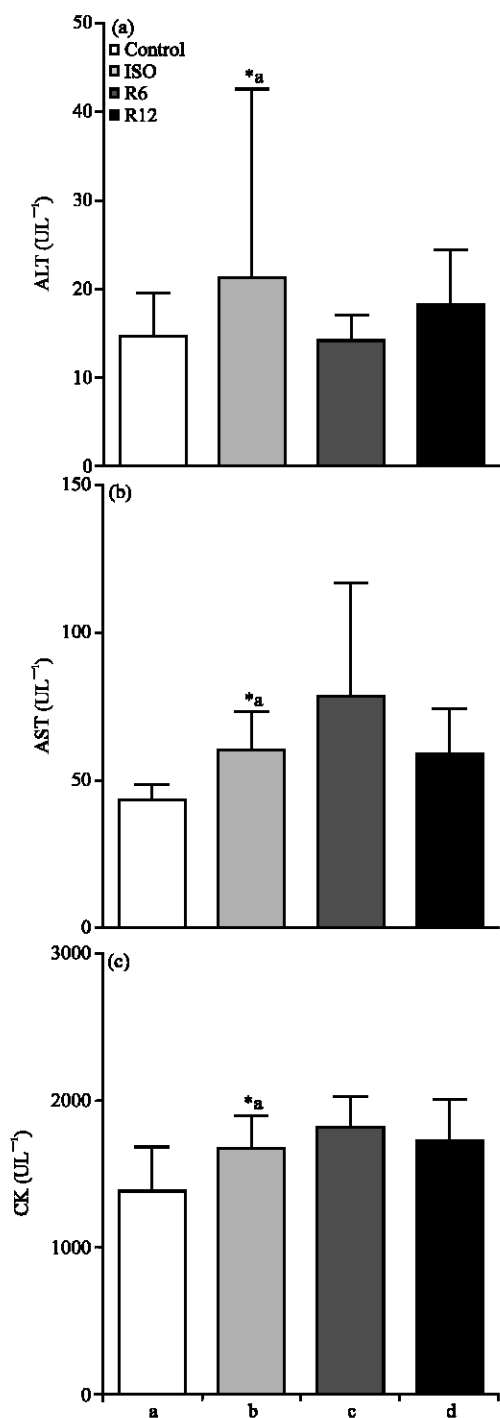
Fig. 1(a-c): Effect of rosiglitazone (12 and 6 mg kg^{-1}, respectively) on (a) Creatinine kinase, (b) Aspartate aminotransferase and (c) Alanine aminotransferase levels in control and isoperetenol-treated rats, a: Control, b: Isoperetenol, c: Rosiglitazone 6 mg kg^{-1} and d: Rosiglitazone 12 mg kg^{-1}, (*a = p<0.05, compared to the control group)
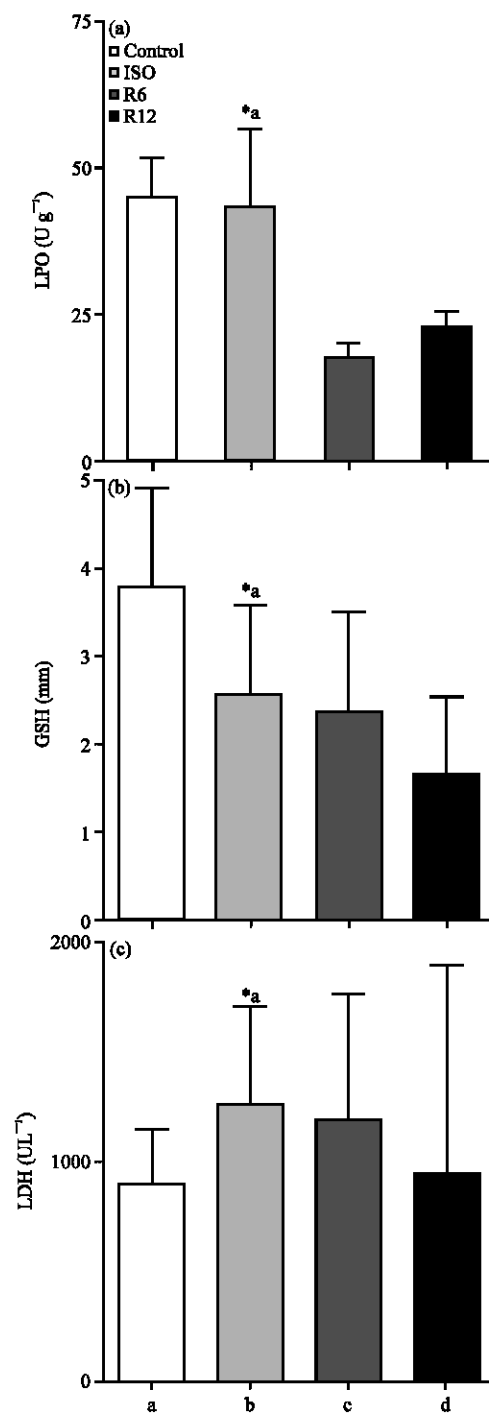
Fig. 2(a-c): Effect of rosiglitazone (6 and 12 mg kg^{-1}) on (a) Lipid peroxidase, (b) Glutathione and (c) Lactate dehydrogenase levels in control and isoperetenol-treated in rats, a: Control, b: Isoperetenol, c: Rosiglitazone 6 mg kg^{-1} and d: Rosiglitazone 12 mg kg^{-1} (*a = p<0.05, compared to the control group)

Neither 6 mg kg^{-1} nor 12 mg kg^{-1} rosiglitazone treatment induced changes in antioxidant levels when compared to ISO treatment alone (significants appeared in high level of this markers for the ISO group) (Fig. 2).

DISCUSSION

Rosiglitazone is an agonist of PPAR-γ which is found in insulin-dependent, glucose-requiring tissues, such as adipose, skeletal muscle and liver (Young *et al.*, 1998; Spiegelman, 1998; Lehmann *et al.*, 1995). Avandia and others have found that rosiglitazone mediates several changes in cells through the blocking of tumour necrosis factor, causing an increase in adipocyte differentiation and a decrease in the release of free fatty acids from adipocytes and through its high level of binding to plasma proteins, especially albumin, *in vivo* (Avandia, 1999; Spiegelman, 1998; Rosenbaum and Greenberg, 1998; Souza *et al.*, 1998). Wayman *et al.* (2002) discovered that various chemically distinct ligands of PPAR-γ (including the TZDs rosiglitazone, ciglitazone and pioglitazone, as well as the cyclopentanone prostaglandins 15D-PGJ2 and PGA1) substantially reduce the size of myocardial infarcts in the rat. The most pronounced reduction in infarct size was observed with the endogenous PPAR-γ ligand, 15-deoxyΔ^{12,14}-prostaglandin J$_2$ (15D-PGJ$_2$). The mechanisms of the cardioprotective effects of 15D-PGJ$_2$ may include: (1) The activation of PPAR-α, (2) The activation of PPAR-γ, (3) The expression of HO-1 and (4) The inhibition of the activation of NF-κB in the ischemia-reperfused heart. Wayman *et al.* (2002) speculated that ligands of PPAR-γ and PPAR-α may be useful in the therapy of conditions associated with ischemia-reperfusion of the heart and other organs. The insulin sensitizer rosiglitazone is the most potent and selective PPAR-γ agonist (Murphy and Holder, 2000; Lehmann *et al.*, 1997; Young *et al.*, 1998). In the Wayman *et al.* (2002) study, the administration of rosiglitazone caused a dose-related reduction in myocardial infarct size (a maximum reduction of infarct size was achieved at the highest dose) due to its ability to activate PPAR-γ. The findings of author support the view that various chemically distinct agonists of PPAR-γ reduce myocardial infarct size in the rat *in vivo*. Thus, Wayman *et al.* (2002) proposed that (1) The activation of either PPAR-α or PPAR-γ can protect the heart against ischemia-reperfusion injury and (2) The activation of both PPAR-α and PPAR-γ contributes to the substantial cardioprotective effects of 15D-PGJ$_2$.

A recent meta-analysis of 42 randomised clinical trials showed an increased risk of myocardial infarction in patients treated with rosiglitazone (Nissen and Wolski, 2007). A similar trend was observed for cardiac mortality, although this result did not reach statistical significance. These results evoked doubts on the safety of rosiglitazone (Singh *et al.*, 2007). Subsequent analyses of patient-level data performed by the FDA Advisory Committee confirmed an increased risk of ischemic heart disease associated with the use of rosiglitazone (Rosen, 2007). An analysis by Mannucci *et al.* (2007) however, suggested that the risk of myocardial infarction and ischemic heart disease attributable to rosiglitazone could be lower and the risk of heart failure is higher than what had been reported previously (Mannucci *et al.*, 2010; Nissen and Wolski, 2007; (http://ctr.gsk.co.uk/welcome.asp, 2007; http://ctr.gsk.co.uk/welcome.asp, 2007). Our results show that rosiglitazone treatment neither improves nor exacerbates the cardiac injury caused by ISO in rats. These findings conflict with previous studies that noted beneficial effects of rosiglitazone in animal models of myocardial infarctions (Bagi *et al.*, 2004; Hetzel *et al.*, 2005; Yue *et al.*, 2001). Present results also conflict with a previously published meta-analysis that determined the estimated risk for myocardial infarction associated with rosiglitazone to be between 1.2 and 1.4 (Nissen and Wolski, 2007). These findings strengthen the notion that rosiglitazone treatment has a lower associated risk of myocardial infarction than previously reported (Nissen and Wolski, 2007; Rosen, 2007).

CONCLUSION

In conclusion, the findings of our study suggest that rosiglitazone does not potentiate the cardiac toxicity of ISO in rats. Treatment with rosiglitazone lower the risk of myocardial infarction than previously as reported in previous studies.

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