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Effect of Fenofibrate in Pressure Overload-induced Experimental Cardiac Hypertrophy

[1,2]Pitchai Balakumar, [2]Madhankumar Rose and [1,2]Manjeet Singh
[1]I.S.F. Institute of Pharmaceutical Sciences and Drug Research, Moga, Punjab, India
[2]Department of Pharmaceutical Sciences and Drug Research,
Punjabi University, Patiala, India

Abstract: The study has been designed to investigate the effect of fenofibrate, a peroxisome proliferator activated receptor PPARα agonist in partial abdominal aortic constriction (PAAC)-induced cardiac hypertrophy in rats. Rats were subjected to PAAC for 4 weeks to produce cardiac hypertrophy. The fenofibrate (3 mg kg^{-1} day^{-1}, p.o.) was administered 3 days before PAAC and continued for 4 weeks after PAAC. The development of cardiac hypertrophy was assessed in terms of measuring ratio of left ventricular (LV) weight to body weight (LVW/BW), LV wall thickness (LVWT), LV protein content and LV collagen content. Further, the collagen accumulation in left ventricle was analyzed using picrosirius red staining. Moreover, the cardiomyocyte diameter was assessed using hematoxylin-eosin staining and measured using NIH Scion image analyzer. The PAAC has produced cardiac hypertrophy by increasing LVW/BW, LVWT, LV protein content, LV collagen content and mean cardiomyocyte diameter. However, treatment with fenofibrate significantly attenuated PAAC-induced increase in LVW/BW, LVWT, LV protein content, LV collagen content and mean cardiomyocyte diameter. Thus, it may be concluded that fenofibrate has prevented PAAC-induced cardiac hypertrophy, which may be due to activation of PPARα.

Key words: Fenofibrate, PPARα, aortic banding, cardiac hypertrophy

INTRODUCTION

Chronic heart failure has been associated with high morbidity and mortality in industrialized nations (Lips *et al*., 2003). Cardiac hypertrophy is an adaptive response of the heart, during which terminally differentiated cardiomyocytes increase in size without undergoing cell division. Initially, the hypertrophic response may be adaptive; however, prolonged hypertrophy may accelerate cardiac dysfunction and heart failure (Balakumar and Singh, 2006a). Peroxisome proliferator activated receptors are ligand activated transcription factors belonging to nuclear receptor superfamily, which comprise of three subtypes such as PPARα, PPARγ and PPARδ (Balakumar *et al*., 2007a). PPARα is abundant in tissues with high fatty acid oxidative capacity like heart and liver and is involved in regulation of fatty acid uptake and oxidation thereby maintaining energy homeostasis (Ferre, 2004). Activation of PPARα has been noted to inhibit various inflammatory mediators such as tumor necrosis factor α (TNF-α) and interleukin-6 (Staels and Fruchart, 2005), which are involved in progression of cardiac hypertrophy. Further, PPARα activation has been reported to inhibit redox regulated transcription factors like nuclear factor kappa B (NF-κB) and cell adhesion molecules (Irukayama-Tomobe *et al*., 2004; Ichihara *et al*., 2006; Balakumar *et al*., 2007b), which may play a pivotal role in the pathogenesis of cardiac hypertrophy. Fenofibrate has been shown to be an activator of PPARα

Corresponding Author: Dr. Pitchai Balakumar, Department of Pharmaceutical Sciences and Drug Research, Cardiovascular Pharmacology Division, Punjabi University, Patiala-147002, India Tel: +91-9815557265 Fax: +91-175-2286622

(Diep *et al*., 2004; Irukayama-Tomobe *et al*., 2004; Ogata *et al*., 2004; Ichihara *et al*., 2006). Therefore, the present study was undertaken to investigate the effect of fenofibrate, an agonist of PPARα in pressure overload-induced cardiac hypertrophy.

MATERIALS AND METHODS

The experimental protocol used in the present study was approved by the Institutional Animal Ethical Committee. Male Wistar albino rats weighing about 175-225 g were employed in the present study. They were maintained on rat feed (Kisan Feeds Ltd., Chandigarh, India) and water *ad libitum* and were exposed to 12 h light and 12 h dark cycle.

Partial Abdominal Aortic Constriction (PAAC)-Induced Cardiac Hypertrophy

The pressure overloaded cardiac hypertrophy was produced by partial abdominal aortic constriction as previously described (Balakumar and Singh, 2006b, c). Rats were anaesthetized with thiopentone sodium (35 mg kg^{-1} i.p.) and a midline incision of 3-4 cm was made in the abdomen to expose the aorta between the diaphragm and celiac artery. The 4-0 silk suture was passed beneath the abdominal aorta and was tied along with a needle of 0.7 mm in diameter. The needle was withdrawn to leave the abdominal aorta partially constricted. The incision was sutured in layers and neosporin antibiotic powder (GlaxoSmithKline, Mumbai, India) was applied locally. Rats were allowed to recover and were kept under observation for 4 weeks. Sham operated animals have undergone the same surgical procedures except PAAC. Body weight was monitored weekly for 4 weeks.

Assessment of Cardiac Hypertrophy

Morphological Assessments

After 4 weeks, the rat was sacrificed, thorax was opened and the heart was excised. The left ventricular weight including interventricular septum and the right ventricular weight was noted separately and expressed as milligram per gram of body weight. The left ventricle was then divided into three slices and the wall thickness of each slice was noted at eight different points using an ocular micrometer. The mean values of all three slices were calculated and expressed in millimeter.

Biochemical Assessments

The left ventricular protein content was measured by method of Lowry *et al*. (1951). The tissue was homogenized in saline and protein content was determined spectrophotometrically at 750 nm and expressed as mg g^{-1} of wet weight of left ventricle.

The left ventricular collagen content was estimated by measuring left ventricular hydroxyproline content as previously described (Simko *et al*., 2002; Balakumar and Singh, 2006c, d). The dry left ventricular mass was hydrolysed in 6N hydrochloric acid at 100°C for 12 h and then dissolved in phosphate buffer of pH 7. Ehrlich reagent (p-dimethyl amino benzaldehyde) was added to form a pink coloured complex with hydroxyproline and the intensity of colour was measured spectrophotometrically at 558 nm. The hydroxyproline concentration of left ventricle was taken as an index of left ventricular collagen and expressed as mg g^{-1} of dry weight of left ventricle.

Histopathological Studies

The left ventricular cardiomyocyte diameter was measured as previously described (Mori *et al*., 2004). The heart was excised and immediately immersed in 4% paraformaldehyde. The sample was fixed in 10% formaldehyde, dehydrated in graded concentrations of ethanol, immersed in xylene and then embedded in paraffin. From the paraffin blocks, 4 μm thin sections were cut, stained with hematoxylin-eosin to analyze cardiomyocyte diameter and examined by light microscopy. Using

an image analysis program (NIH Scion image analyzer, USA), the diameter of at least 100 cardiomyocytes were determined in randomly selected visual fields at 400-fold magnification and the cardiomyocyte diameter was expressed in micrometer. Moreover, the collagen deposition was assessed in the left ventricular sections using picrosirius red staining.

Experimental Protocol

Five groups were employed in the present study and each comprising of 12 animals. Group 1 (Sham control), surgery was performed to expose the abdominal aorta but it was not constricted. Group 2 (PAAC control), abdominal aorta was exposed and partially constricted. Group 3 (CMC *per se*), sham group rats were administered 1 mL of carboxy methyl cellulose (0.5% w/v p.o., day^{-1}) for 4 weeks. Group 4 (Fenofibrate *per se*), sham group rats were administered fenofibrate (3 mg kg^{-1} p.o., day^{-1}) suspended in 0.5% CMC for 4 weeks. Group 5 (Fenofibrate treated), rats subjected to partial abdominal aortic constriction were treated with fenofibrate (3 mg kg^{-1} p.o., day^{-1}), which was started 3 days before surgery and was continued for 4 weeks after surgery.

Statistical Analysis

Results were expressed as mean±SEM. The data obtained from various groups were statistically analyzed using one-way ANOVA followed by Tukey's Multiple Range test. The p-value <0.05 was considered to be statistically significant.

Drugs and Chemicals

Fenofibrate was obtained from Franko Indian Pharmaceutical Ltd., Mumbai, India. Ehrlich reagent and Folin ciocalteu reagent were purchased from SRL, Mumbai, India. Eosin and hematoxylin were procured from Sd fine-chem limited, Mumbai, India. Picrosirius red was obtained from Sigma-Aldrich, USA. All other reagents used in this study were of analytical grade.

RESULTS

Effect of Fenofibrate on Morphological and Biochemical Assessments

The fenofibrate and CMC (used as vehicle for fenofibrate) did not produce any significant per se effect on all parameters employed in the present study. There was no significant change in body weight of rats subjected to sham surgery and 4 weeks of PAAC with or without fenofibrate treatment (Table 1). The PAAC has increased the ratio of left ventricular weight to body weight (LVW/BW) (mg/g) and left ventricular wall thickness (LVWT) (mm) when compare with sham group. However, treatment with fenofibrate (3 mg kg^{-1} p.o., day^{-1}) significantly attenuated the PAAC-induced increase in LVW/BW and LVWT (Table 1). There was no significant change in ratio of right ventricular weight to body weight (RVW/BW) (mg g^{-1}) of rats subjected to sham surgery and PAAC with or without fenofibrate treatment (Table 1). Further, the left ventricular protein content and collagen content have been observed to increase in rats subjected to PAAC, which were markedly attenuated by fenofibrate (3 mg kg^{-1} p.o., day^{-1}) treatment (Table 1 and Fig. 1).

Effect of Fenofibrate on Histo-pathological Studies

The PAAC was noted to increase the mean diameter of cardiomyocytes. However, treatment with fenofibrate significantly attenuated PAAC-induced increase in cardiomyocyte diameter (Table 1 and Fig. 2). Moreover, the cardiomyocytes of PAAC rat heart were observed to be bizarre, disorganized and was associated with myofibrillar lysis (Fig. 2).

Table 1: Effect of fenofibrate on morphological and biochemical assessments

Parameters	Sham control	Fenofibrate *per se*	PAAC control	Fenofibrate treated
BW (g)	202.40±4.64	199.30±4.19	198.70±3.96	205.50±3.02
LVW/BW (mg g^{-1})	2.27±0.04	2.24±0.03	3.22±0.06[a]	2.74±0.04[b]
LVWT (mm)	2.43±0.08	2.54±0.12	3.74±0.11[a]	3.22±0.10[b]
RVW/BW (mg g^{-1})	0.45±0.01	0.45±0.01	0.50±0.01	0.46±0.01
LV Protein (mg g^{-1})	107.10±3.40	109.40±4.20	195.60±6.20[a]	150.40±4.80[b]
LV Collagen (mg g^{-1})	1.67±0.11	1.65±0.10	4.14±0.12[a]	3.14±0.12[b]
Cardiomyocyte Diameter (μm)	15.52±0.58	15.01±0.42	19.57±0.51[a]	17.54±0.38[b]

PAAC indicates partial abdominal aortic constriction, BW indicates body weight, LVW indicates left ventricular weight, RVW indicates right ventricular weight and LVWT indicates left ventricular wall thickness. LV protein content was expressed as mg g^{-1} of wet weight of left ventricle, LV collagen content was expressed as mg g^{-1} of dry weight of left ventricle. Values are expressed as mean±SEM. a = $p<0.05$ vs Sham control; b = $p<0.05$ vs PAAC control

Fig. 1: Effect of fenofibrate on collagen accumulation assessed by picrosirius red staining of section of left ventricles. The left ventricular collagen was increased in PAAC heart (B) when compared with sham operated rat heart (A). Treatment with fenofibrate markedly attenuated the PAAC-induced increase in left ventricular collagen accumulation (C)

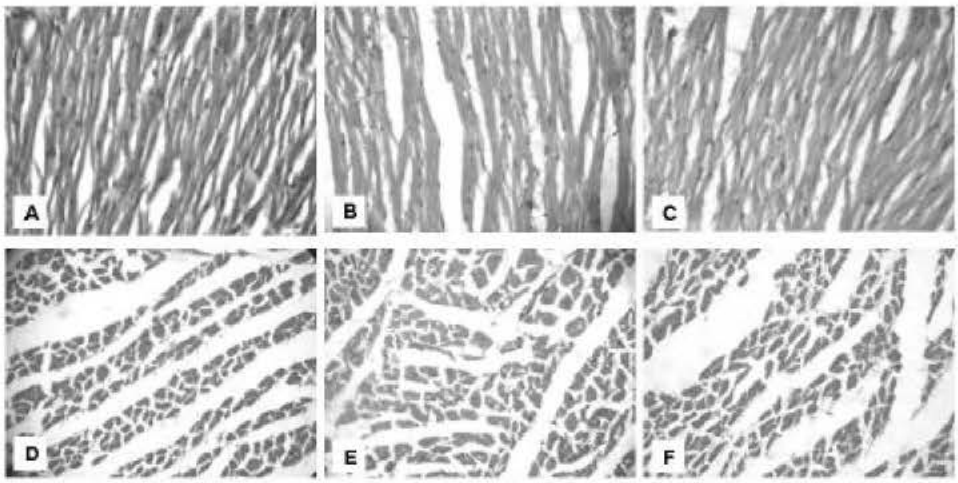

Fig. 2: Effect of fenofibrate on cardiomyocyte diameter assessed by hematoxylin-eosin staining of transverse (A, B and C) and longitudinal (D, E and F) section of left ventricles. The cardiomyocyte size was increased in PAAC hearts (B and E) when compared with sham control (A and D). Treatment with fenofibrate markedly attenuated the PAAC-induced increase in cardiomyocyte diameter (C and F)

DISCUSSION

Hypertrophy of cardiac myocytes occurs to meet the increased demand of mechanical energy utilization (Wakatsuki *et al.*, 2004). It is basically a response to sustained hemodynamic overload such

as pressure or volume overload (Balakumar *et al*., 2007d). Initially, the hypertrophic response may be beneficial as it tends to normalize wall stress (Lips *et al*., 2003). However, during the prolonged course of time it leads to contractile dysfunction and heart failure (Wang, 2001; Ritter and Neyses, 2003; Balakumar *et al*., 2007d). The cardiac hypertrophy is associated with increase in left ventricular mass and it involves various signaling pathways such as p38 mitogen activated protein kinase, c-Jun NH_2 terminal Kinase, phosphatidylinositol 3-kinase (PI3Kγ) (Wakatsuki *et al*., 2004), calcineurin (Frey *et al*., 2004), Raf kinase, protein kinase D (Luedde *et al*., 2006), Rho-kinase (Wang *et al*., 2005; Balakumar and Singh, 2006c), poly (ADP-ribose) polymerase (Balakumar and Singh, 2006b; Pillai *et al*., 2006), Caspase (Frey *et al*., 2004; Balakumar and Singh, 2006e) and inflammatory cytokines (Manabe *et al*., 2002; Balakumar and Singh, 2005, 2006d). The increase in ratio of left ventricular weight to body weight (van den Bosch *et al*., 2006), left ventricular wall thickness (Wakatsuki *et al*., 2004), left ventricular protein content (Lips *et al*., 2003; Balakumar and Singh, 2006b), left ventricular collagen content (Simko *et al*., 2002; Balakumar and Singh, 2006c, d) and mean cardiomyocyte diameter (Hayashi *et al*., 2003) have been documented to be an index of experimental cardiac hypertrophy. In the present study, PAAC has been noted to produce cardiac hypertrophy assessed in terms of increase in LVWT, LVW/BW, LV protein content, LV collagen content and mean cardiomyocyte diameter. Treatment with fenofibrate markedly attenuated PAAC-induced cardiac hypertrophy. Fenofibrate has been shown to be an activator of PPARα (Diep *et al*., 2004; Irukayama-Tomobe *et al*., 2004; Ogata *et al*., 2004; Ichihara *et al*., 2006). PPARα activation has been demonstrated to inhibit various transcription factors such as nuclear factor kappa B (NF-κB), activator protein-1 (AP-1) and signal transducer and activators of transcription 1/3 (STAT1/3) (Staels and Fruchart, 2005). Moreover, activation of PPARα has been suggested to inhibit inflammatory mediators such as inducible nitric oxide synthase (iNOS), C-reactive protein (CRP), monocyte chemotactic protein-1 (MCP-1), matrix metalloproteinase-9 (MMP-9), interleukin (IL)-1β, IL-6, IFN-inducible protein-10 (IP-10) and tumour necrosis factor-α (TNFα) (Staels *et al*., 1998; Cabrero *et al*., 2002; Kleemann *et al*., 2004; Staels and Fruchart, 2005; Blaschke *et al*., 2006; Balakumar *et al*., 2007c). Therefore the ameliorative effect of fenofibrate in PAAC-induced cardiac hypertrophy may be due to activation of PPARα and consequent inhibition of various transcription factors and inflammatory mediators.

CONCLUSION

On the basis of above discussion it may be concluded that fenofibrate prevented PAAC-induced cardiac hypertrophy, which may be due to its PPARα agonistic property.

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