Stimulation of peroxisome-proliferator-activated receptor α (PPARα) attenuates cardiac fibrosis and endothelin-1 production in pressure-overloaded rat hearts

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ABSTRACT

Endothelin-1 (ET-1) production is increased in hypertrophied hearts accompanied with fibrosis. ET-1 is a potent mitogen of fibroblasts and ET receptor antagonists are reported to inhibit the proliferation of fibroblasts and cardiac fibrosis. Peroxisome-proliferator-activated receptor α (PPARα), one of the nuclear hormone receptors, suppresses activator protein-1 (AP-1), one of the nuclear transcription factors. Activation of PPARα is reported to inhibit thrombin-induced ET-1 production by repressing the AP-1 signalling pathway in vascular endothelial cells. We investigated effects of the PPARα activator fenofibrate (80 mg/kg per day, per os) on mRNA levels of ET-1, collagen type I and type III and histological features of myocardial fibrosis in hypertrophied rat hearts due to pressure-overload by abdominal aortic banding (AB). The treatment with fenofibrate or vehicle was started 7 days before the AB operation. Four days after the AB operation, fenofibrate treatment significantly reduced ET-1 mRNA expression compared with vehicle treatment in AB rat hearts. Collagen type I and type III mRNA expression, and interstitial and perivascular fibrosis were attenuated in the fenofibrate-treated AB rat group. Since the ET-1 gene has AP-1 response elements in the 5' flanking region, it is suggested that myocardial fibrosis is effectively inhibited by fenofibrate through suppression of AP-1-mediated ET-1 gene augmentation in the pressure-overloaded heart caused by aortic banding in rats.

INTRODUCTION

Cardiac fibrosis is an important adaptive physiological response to several haemodynamic overloads to the heart. Cardiac fibroblasts are the most abundant cell type present in the myocardium and are mainly responsible for the deposition of extracellular matrix (ECM) [1]. Collagen type I is the major collagens product of these cells representing 80% of the total of newly synthesized collagen that is secreted into the culture medium as procollagen [2]. About 20% of the total collagen synthesized is collagen type III and a small proportion is collagen type V (less than 5%). Interstitial fibrosis contributes to ventricular wall stiffness and consequently impairs cardiac compliance, contributing to impaired diastolic function [3,4]. And, since neither the

Key words: cardiac fibrosis, collagen, endothelin-1, PPARα, fenofibrate.

Abbreviations: ET, endothelin; PPARα, peroxisome-proliferator-activated receptor α; AB, abdominal aortic banding; ECM, extracellular matrix; AP-1, activator protein-1; LV, left ventricular; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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ECM nor the fibroblasts contribute to systolic contribution, increased ECM and fibroblast volume means that systolic work is being performed by a smaller proportion of the cardiac mass, contributing to systolic dysfunction.

Peroxisome-proliferator-activated receptor α (PPARα) is enriched in tissues with high oxidative energy demands that depend on mitochondrial fatty acid oxidation as a primary energy source, such as heart and liver. Fibric acid derivatives including fenofibrate are also thought to act as specific activators for PPARα [5–8]. PPARα together with PPARγ and PPARβ form a subgroup within the nuclear receptor superfamily [9]. PPARs regulate gene expression by binding with retinoid X receptors as a heterodimeric partner to specific DNA sequence elements termed peroxisome-proliferator response elements [10]. Interestingly, it has been reported that severe myocardial fibrosis was observed in PPARα-null mice compared with the wild-type mice in an age-dependent manner [11]. It is suggested that constitutive regulation of fatty acid metabolism through PPARα associates with age-dependent cardiac fibrosis.

Endothelin-1 (ET-1), a potent vasoconstrictor peptide first identified from the conditioned medium of vascular endothelial cell, is also synthesized and secreted by cardiac myocytes and fibroblasts [12]. Furthermore, ET-1 is a potent mitogen of fibroblasts, and ET receptor antagonists are reported to inhibit the proliferation of fibroblasts and cardiac fibrosis in vitro and in vivo. The expression of ET-1 mRNA is up-regulated by angiotensin II, ET-1 itself, and the activator of protein kinase C, phorbol ester [13]. These factors up-regulate activator protein (AP)-1 and the ET-1 gene has AP-1 response elements in the promoter region, suggesting that the ET-1 gene is partly induced by AP-1 binding.

It has been shown that protein–protein interactions of transcription factors are important in the regulation of expression. For instance, transcription of the ET-1 gene has been shown to be regulated through a cooperative interaction of GATA-2 and AP-1 transcription factors [14]. Delerive et al. also reported that PPAR activators negatively regulate the vascular inflammatory gene response by negative cross talk with transcription factors, nuclear factor κB and AP-1 [15].

In the relationship between ET-1 gene regulation and PPARα activation, AP-1 is a common molecule, because several stimuli up-regulate the ET-1 gene via AP-1 activation and PPARα is reported to interfere negatively with AP-1 in endothelial cells. We have hypothesized that PPARα activation may reduce ET-1 production and ameliorate cardiac fibrosis. Therefore, we investigated the effect of the PPARα activator fenofibrate on the development of cardiac fibrosis in a rat pressure-overload (by abdominal aortic banding; AB) model, which is detected by collagen deposition in the left ventricle.

**METHODS**

**Experimental pressure-overloaded heart and study protocol**

The operation of aortic banding was carried out according to the method described by Jouannot and Hatt, and our laboratory with minor modification [16,17]. Nine-week-old male Sprague–Dawley rats (body weights 320–350 g) were anaesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal) and the abdominal aorta was exposed through a midline abdominal incision. To be certain the narrowness of the aorta was constant, stainless steel wire (diameter 0.2 mm) was shaped into a coil (inside diameter 0.75 mm, one pitch 1.15 mm, length of the coil 3.45 mm) by means of an instrument (COIL-95, Yamashita-Giken, Tokushima, Japan), and aseptically placed around the abdominal aorta above the renal arteries by the method originally described by Kudo et al. [18]. Sham-operated control rats underwent an identical procedure using a coil, the diameter of which was greater than that of the aorta.

Rats were sacrificed 4 days after the surgery. On the day of the experiments, each rat was anaesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal). The right carotid artery was cannulated, and the arterial blood pressure was measured directly with a pressure transducer and recorded on a polygraph system (AP-601G amplifier and WT-687G thermal pen recorder, Nihon Kohden, Tokyo, Japan), as described previously [19–21]. After measurement of the arterial pressure, the heart was excised and the atria were removed. The ventricles were divided into the left ventricle including the intraventricular septum and the right ventricle, and they were rinsed with a cold saline, weighed, and quickly frozen in liquid nitrogen, as described previously [19–21]. These specimens were stored at −80 °C until they were required for isolation of total RNA. Left ventricular (LV) hypertrophy was evaluated by the LV wet weight to body weight ratio (LV mass index).

The effect of the PPARα activator, fenofibrate, on the development of cardiac hypertrophy due to pressure-overload in rats with aortic banding was investigated. Oral administration with fenofibrate (80 mg/kg per day; Kaken Seiyaku Ltd., Tokyo, Japan) or vehicle [3 % (w/v) arabic gum; Wako Ltd., Osaka, Japan] was treated for 1 week before the surgery. The rats were divided into the following three groups:

1. Sham rats + vehicle, sham-operated rats receiving vehicle (3 % arabic gum);
2. AB rats + vehicle, rats with aortic banding receiving vehicle;
3. AB rats + fenofibrate, rats with aortic banding receiving fenofibrate (80 mg/kg per day per rat).

Rats were sacrificed 4 days after the surgery. Haemodynamic parameters and tissue weights were evaluated and the tissues were stored. This study was approved by...
University of Tsukuba and conformed to the “Position of the American Heart Association on Research Animal Use” adapted by the Association in November 1984.

**Histological analysis**

For histological analysis, hearts were subsequently fixed in 4% (v/v) paraformaldehyde and embedded in paraffin. Coded slices stained with Masson trichrome were microscopically evaluated. Cardiac fibrotic area was photographed by using a digital microscope camera (PDMC 1e, Nihon Poladigital K. K., Tokyo, Japan).

**Quantification of mRNA levels by reverse transcription (RT–PCR)**

To evaluate whether PPARα activator ameliorates cardiac hypertrophy not only at the tissue weight level and cardiac fibrotic area, but also at the molecular level, we investigated mRNA expression of collagen type I and type III, and ET-1 in the LV of the three groups. The mRNA expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was also determined as an internal control. Total RNA from the LV was isolated by the method of acid guanidinium thiocyanate/phenol/chloroform extraction with ISOGEN (Nippon Gene Ltd., Tokyo, Japan) according to methods described in our previous papers. RT–PCR was also performed according to our previous papers [22]. The sequences of the specific primers were as follows:

- Collagen type I (sense), 5′CATAAAGGTCATCG-TGGCTTC3′ and Collagen type I (antisense), 5′GTGATAGGTATGTTCTGGGAG3′;
- Collagen type III (sense), 5′TGACAGAGGTGAAAAGAGGATC3′ and Collagen type III (antisense), 5′CTGTCTCTCTGCTCCCTCA-TTACAGT3′; ET-1 (sense), 5′CTGTCTCTCGTCTGTTG3′ and ET-1 (antisense), 5′TATTTTCTTCCCTCCACC3′; GAPDH (sense), 5′GCCATCCACCCCTTATTG3′ and GAPDH (antisense), 5′TGCCAGTGAAGCTCCCTCGGTT3′.

PCR was performed with the annealing temperature and required cycles for each template as follows: 64 °C and 23 cycles for collagen type I, 62 °C and 23 cycles for collagen type III, 64 °C and 30 cycles for ET-1, 62 °C and 23 cycles for GAPDH. PCR was performed in the range that demonstrates a linear correlation between the amount of cDNA and the yield of PCR products. The ratio of the RT–PCR product for each gene to GAPDH was quantified and compared [23].

The amplified products on agarose gel were stained with ethidium bromide, visualized by UV transilluminator and photographed. The photographs were scanned by a scanner (CanoScan 600, Canon Ltd., Tokyo, Japan), and the quantification was done with MacBAS (FUJI FILM Ltd., Tokyo, Japan).

**Statistical analysis**

Data were expressed as the mean±S.E.M. One-way ANOVA followed by a post hoc test was used for statistical comparison among the various treatment groups. Values of \( P < 0.05 \) were considered statistically significant.

**RESULTS**

**Tissue weights**

Four days after operation, LV mass index was significantly increased in AB rats with vehicle compared with sham rats with vehicle (\( P < 0.01 \)), and it did not differ between AB rats with vehicle and with fenofibrate 4 days after surgery (2.03±0.04, 2.97±0.16, 2.88±0.10 mg/g in sham rats with vehicle, AB rats with vehicle, AB rats with fenofibrate respectively).

**Effect of fenofibrate treatment on expression of ET-1 mRNA in LVs of rats with pressure-overload due to aortic banding**

The expression of ET-1 mRNA in LVs was markedly higher in AB rats with vehicle than in sham rats with vehicle (Figure 1). In contrast, it was significantly lower in fenofibrate-treated AB rats than in vehicle-treated AB rats (Figure 1).

**Perivascular and interstitial fibrosis in LVs of rats**

Cardiac fibrosis was evaluated by staining Masson trichrome. Fibrotic area, which contains perivascular and interstitial fibrosis, was remarkably increased in vehicle-treated AB rats compared with sham-operated rats. Administration of fenofibrate resulted in inhibition of
PPAR\(\alpha\) activator attenuates cardiac fibrosis and ET-1 production

**DISCUSSION**

ET-1 is a bioactive peptide first described as a potent vasoconstrictor [24]. It is now known to have growth-promoting properties in different cell types and recent reports have suggested a mitogenic effect of this peptide on cardiac fibroblasts. In cardiac tissue, ET-1 is synthesized and secreted from cardiomyocytes and fibroblasts [25]. The production of ET-1 has also been shown to be increased in hypertrophied hearts in various models of pressure-overload [16,25]. Furthermore, it has been reported that ET receptor antagonists have been shown to inhibit the cardiac hypertrophy and fibrosis in AB rats [26]. It was reported that ET-1 stimulated myocardial collagen deposition in DOCA-salt hypertensive rats via ET\(_\alpha\) receptor and that an ET\(_\alpha\) receptor antagonist inhibited cardiac fibrosis [27]. These data suggest that the activation of the ET-1 pathway is involved in cardiac fibrosis induced by pressure-overload.

The present study demonstrated that PPAR\(\alpha\) activator fenofibrate inhibited the increase of myocardial ET-1 mRNA expression and left ventricle fibrosis in rat hearts with pressure-overload which is induced by AB. These findings suggest that PPAR\(\alpha\) negatively regulates the ET-1 gene expression, and PPAR\(\alpha\) activator reduces cardiac fibrosis by inhibiting ET-1 production in the pressure-overloaded heart.

Activation of PPAR\(\alpha\) is reported to inhibit thrombin-induced ET-1 production by repressing the AP-1 signalling pathway in vascular endothelial cells [28]. It is considered that activated PPAR\(\alpha\) binds to AP-1 or its cofactor and AP-1 is forced not to bind to the cis-elements of the ET-1 gene, which results in impairment of ET-1 gene induction. These findings and the present study suggest PPAR\(\alpha\) activator effectively inhibits cardiac fibrosis through suppression of the ET-1 pathway in vivo.

In summary, our study has demonstrated that the PPAR\(\alpha\) activator, fenofibrate, inhibits ET-1 mRNA expression in LVs of AB rats, and decreases cardiac fibrosis during the subacute phase of pressure-overload.
These findings suggest that the PPARα activator may improve clinical outcomes of pressure-overloaded hearts.

ACKNOWLEDGMENTS

This study was supported by grants-in-aid for scientific research from the Ministry of Education, Science, Sports and Culture of Japan (00005167, 11357019, 11557047, 12470147 and 12680012), a grant from The Ueda Memorial Trust Fund for Research of Heart Disease, and a grant from the Miyauchi Project of Centre for Tsukuba Advanced Research Alliance in University of Tsukuba.

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