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Cardiac peroxisome-proliferator-activated receptor expression in hypertension co-existing with diabetes

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ABSTRACT

Hypertension and DM (diabetes mellitus) are common chronic disorders that often co-exist. DM and PPAR (peroxisome-proliferator-activated receptor)-γ agonists may directly impair heart function. However, the effects of DM and PPAR-γ agonists on hypertensive myocardium are not known. Hence the aim of the present study was to investigate whether DM and a PPAR-γ agonist [RGZ (rosiglitazone)] modulated the effects of hypertension on myocardial expression of PPAR isoforms. Cardiac PPAR isoforms, TNF (tumour necrosis factor)-α and IL (interleukin)-6 were evaluated by real-time PCR and Western blotting in SHRs (spontaneously hypertensive rats), diabetic SHRs, diabetic SHRs treated with RGZ (5 mg/kg of body weight) and control WKY (Wistar–Kyoto) rats. Cardiac NADPH oxidase activity was quantified using a SOD (superoxide dismutase)-sensitive cytochrome *c* reduction assay. When compared with hearts from control WKY rats, hearts from SHRs had decreased PPAR-α and PPAR-δ mRNA and protein levels (39 and 44% respectively for PPAR-α, and 37 and 42% respectively for PPAR-δ), but had increased PPAR-γ mRNA and protein levels (1.9- and 1.4-fold respectively). The hypertension-induced changes in mRNA and protein of cardiac PPAR isoforms were enhanced in diabetic SHRs, which were attenuated in diabetic SHRs treated with RGZ. Cardiac TNF-α and IL-6 protein levels and NADPH oxidase activities were increased in SHRs and were increased further in diabetic SHRs. RGZ treatment decreased TNF-α and IL-6 protein levels and NADPH oxidase activities in hearts from diabetic SHRs. In conclusion, these findings suggest that DM and the PPAR-γ agonist modulated the hypertensive effects on cardiac PPAR isoform expression.

INTRODUCTION

PPAR (peroxisome-proliferator-activated receptor)-α, PPAR-γ and PPAR-δ are nuclear transcription factors expressed in the heart and modulate myocardial lipid metabolism, glucose and energy homoeostasis [1,2]. Several studies have shown that high non-esterified 'free' fatty acid oxidation and impaired glucose utilization in diabetic hearts may have detrimental effects on cardiac function [3,4]. In addition, cardiac-restricted

Key words: cardiomyocyte, diabetes mellitus, hypertension, NADPH oxidase, peroxisome-proliferator-activated receptor (PPAR), pro-inflammatory cytokine.

Abbreviations: ACE, angiotensin-converting enzyme; BP, blood pressure; DBP, diastolic BP; DM, diabetes mellitus; EF, ejection fraction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL, interleukin; PPAR, peroxisome-proliferator-activated receptor; qPCR, quantitative PCR; RGZ, rosiglitazone; ROS, reactive oxygen species; SBP, systolic BP; SHR, spontaneously hypertensive rat; SOD, superoxide dismutase; TNF, tumour necrosis factor; WKY, Wistar–Kyoto.

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overexpression of PPAR-α and PPAR-γ caused deranged myocardial energy metabolism, which contributed to cardiomyopathy [5,6]. Our previous study has also found that DM (diabetes mellitus) can modulate PPARs in cardiomyocytes through increased inflammatory cytokines and oxidative stress [7]. Therefore PPARs play a critical role in the pathogenesis of diabetic cardiomyopathy.

Hypertension is an important cause of cardiovascular morbidity and mortality [8]. Decreased expression of PPAR-α protein was found in hypertensive patients complicated with left ventricular hypertrophy and heart failure [9]. In contrast, increased expression of PPAR-α, PPAR-γ and PPAR-δ were found in hearts from SHRs (spontaneously hypertensive rats) [10]. Hypertension and DM frequently co-exist and lead to cardiac structural or functional dysfunction, which will accelerate the progression to morbidity and mortality [11]. Although previous studies have shown that PPARs have pivotal roles in the cardiac pathophysiology of DM or hypertension [5,6,9,10,12], it is not clear whether DM can modulate the cardiac effect of hypertension on PPARs.

Increasing evidence suggests that circulating levels of pro-inflammatory cytokines are generally increased in patients with DM and hypertension [13]. Our previous study has shown that TNF (tumour necrosis factor)-α regulates PPAR-α and PPAR-γ through oxidative stress in cardiomyocytes, suggesting inflammation has a potential effect on PPARs [14]. Studies on PPAR agonists have shown that they decrease inflammatory markers such as TNF-α and IL (interleukin)-1β in atherosclerotic lesions [15]. In contrast, detrimental effects of PPAR-γ ligands, such as worsening of congestive heart failure and increased myocardial dysfunction, have also been reported [16,17]. However, the regulating effects of PPAR ligands on cardiac PPAR isoform expression in DM co-existing with hypertension have not been studied to date. Therefore the aim of the present study was to evaluate whether DM can modulate the effect of hypertension on myocardial expression of PPAR isoforms, and to investigate the effect of a PPAR-γ agonist on PPAR isoforms in cardiomyocytes.

MATERIALS AND METHODS

Animal and tissue preparations

The investigation conformed to the institutional Guide for the Care and Use of Laboratory Animals and the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication no. 85-23, revised 1996), and was approved by the Institutional Animal Care and Use Committee of Taipei Medical University (IACC no. LAC-97-0076). Rats were grouped into SHRs, diabetic SHRs, diabetic SHRs treated with RGZ (rosiglitazone) and WKY (Wistar–Kyoto) rats (control group). A total of 40 male SHRs (10 weeks of age) received an intraperitoneal injection of streptozotocin (65 mg/kg of body weight; Sigma–Aldrich) to induce DM with a fasting plasma glucose of $\geqslant$15 mM, as measured using a glucometer (Ascensia Elite; Bayer Health Care). Rats were housed under standard environmental conditions and maintained on commercial rat chow and tap water *ad libitum*. SBP [systolic BP (blood pressure)], DBP (diastolic BP) and heart rate were measured using a non-invasive BP tail-cuff method (MK-2000; Muromachi Kikai). SBP was monitored every 2 weeks from 10 to 14 weeks of age. At 12 weeks of age, 14 of the diabetic SHRs were treated with RGZ (5 mg/kg of body weight; GlaxoSmithKline) [18], and SHRs, diabetic SHRs and WKY rats were treated daily with a placebo by oral gavage for 2 weeks. Rats were killed at 14 weeks old. Before the animals were killed, echocardiography was performed using an HP Sonos 5500 system with a 15-6L probe (6–15 MHz; Agilent Technologies). M-mode tracing of the left ventricle was used to measure cardiac structures. Rats were anaesthetized intraperitoneally with sodium pentobarbital (40 mg/kg of body weight), and body weights were measured prior to death. Each heart was rapidly excised, weighed and dissected. Cardiac tissues were rinsed in a ice-cold physiological saline solution. Transverse tissue pieces from the left ventricle were snap-frozen in liquid nitrogen for RNA and protein isolation.

RNA extraction and reverse transcription

Total RNA was isolated from tissue using the TRIzol® reagent (Invitrogen), and mRNA concentration was determined spectrophotometry at 260 nm. Reverse transcription was performed using a superscript III cDNA synthesis kit (Invitrogen), according to the manufacturer's protocol. The resulting cDNAs were amplified by PCR using primers for rat PPAR isoforms.

Real-time qPCR (quantitative PCR)

Expression of PPAR isoforms was determined by using a SYBER Green-based qPCR with an ABI PRISM7300 system (Applied Biosystems). Primer sequences were as follows: PPAR-α forward, 5′-CAGGGACCTCTGAGGTCTGC-3′ and reverse, 5′-TGTGCAAATCCCTGCTCTCC-3′; PPAR-γ forward, 5′-CAGGCTTGCTGAACGTGAAG-3′ and reverse, 5′-GCAGCAGGTTGTCTTGGATGT-3′; PPAR-δ forward, 5′-ACGACCAGGTGACCCTCCTC-3′ and reverse, 5′-TGGCAAAGATGGCCTCATG-3′; and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) forward, 5′-ACCCAGCCCAGCAAGGATA-3′ and reverse, 5′-TCAGCAACTGAGGGCCTCTC-3′. Threshold cycle (C_t) values for PPAR isoforms were normalized with their respective C_t values for GAPDH.

Western blot analysis

Equal amounts of proteins were resolved by SDS/PAGE, followed by electrophoretic transfer of proteins on to nitrocellulose membranes. Blots were probed with antibodies against PPAR-α (Santa Cruz Biotechnology), PPAR-γ (Santa Cruz Biotechnology), PPAR-δ (Affinity Bio Reagent), TNF-α (AbD Serotec; MorphoSys UK), IL-6 (Bender MedSystems) and secondary antibodies conjugated with HRP (horseradish peroxidase; Leinco Technology). Bound antibodies were detected with an enhanced chemiluminescence detection system (Millipore) and analysed with AlphaEaseFC software (Alpha Innotech). Targeted bands were normalized to cardiac α-sarcomeric actin (Sigma–Aldrich) to confirm equal protein loading.

Quantification of NADPH oxidase activity

Left ventricles from the different groups were homogenized separately with a homogenizer (Qiagen Instruments) in lysis buffer containing 20 mM Hepes, 2 mM EGTA and protease inhibitor cocktail (Sigma–Aldrich) at 4 °C, and then centrifuged at 2000 *g* for 10 min at 4 °C. The supernatant was centrifuged further at 100 000 *g* for 1 h at 4 °C. The supernatant represented the cytosolic fraction and the pellet contained the membrane fraction. The pellet fraction was resuspended in lysis buffer with 0.2 % Triton X-100 (Sigma–Aldrich) and stored at − 80 °C for further studies.

The enzymatic activity of NADPH oxidase in the membrane fraction was measured using a SOD (superoxide dismutase)-inhibitable cytochrome *c* reduction assay (Sigma–Aldrich). Cytochrome *c* (80 μM; Sigma-Aldrich) and NADPH (100 μM; Merck) were added to tissue homogenates in the presence or absence of SOD (10 units) and incubated at room temperature (25 °C) for 30 min. Cytochrome *c* reduction was measured photometrically at 550 nm and was calculated from the difference between the absorbance without and with SOD.

Statistical analysis

All quantitative data are expressed as means ± S.E.M. Statistical significance among the different groups was determined by ANOVA with Fisher's least-significant difference for post-hoc test analysis of multiple comparisons. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Physical characteristics and echocardiograms of SHRs, diabetic SHRs and WKY rats

The mean fasting blood glucose levels of SHRs and WKY rats were similar at 10 weeks of age, whereas blood glucose levels were elevated in diabetic SHRs and diabetic SHRs treated with RGZ compared with WKY rats and SHRs at 14 weeks of age (Table 1). A decrease in body weight was observed in diabetic SHRs and RGZ-treated diabetic SHRs compared with WKY rats and SHRs. The absolute heart weights in SHRs, diabetic SHRs and RGZ-treated diabetic SHRs differed statistically compared with WKY rats. In addition, the heart/body weight ratios were greater in SHRs, diabetic SHRs and RGZ-treated diabetic SHRs compared with WKY rats. Furthermore, RGZ-treated diabetic SHRs had a statistically significant lower mean SBP and DBP than the age-matched SHRs and diabetic SHRs (Table 1). As shown in Table 2, diabetic SHRs and RGZ-treated diabetic SHRs had a dilated left atrium and left ventricle with a lower EF (ejection fraction) than SHRs and WKY rats.

Differential cardiac expression of PPAR isoforms in SHRs, diabetic SHRs, RGZ-treated diabetic SHRs and WKY rats

Figures 1 and 2 show mRNA and protein expression respectively of different cardiac PPAR isoforms in each group. Compared with WKY rats, cardiac PPAR-α mRNA and protein expression decreased by 39 and 44 % respectively in SHRs. Changes in cardiac PPAR-α mRNA and protein expression were enhanced in diabetic SHRs. However, the changes in cardiac PPAR-α mRNA and protein levels in diabetic SHRs were attenuated in RGZ-treated diabetic SHRs (Figures 1A and 2A).

When compared with hearts from WKY rats, cardiac PPAR-δ mRNA and protein expression were also down-regulated by 37 and 42 % respectively in SHRs. The reduced cardiac PPAR-δ mRNA and protein expression in SHRs were decreased further in diabetic SHRs (Figures 1C and 2C). Cardiac PPAR-δ mRNA and protein expression in diabetic SHRs were attenuated in RGZ-treated diabetic SHRs (Figures 1C and 2C). In contrast, cardiac PPAR-γ mRNA and protein expression were increased by 1.9- and 1.4-fold respectively in SHRs compared with hearts from WKY rats. Up-regulation of cardiac PPAR-γ mRNA and protein expression in SHRs was enhanced in diabetic SHRs. Cardiac PPAR-γ mRNA and protein expression in diabetic SHRs were attenuated in RGZ-treated diabetic SHRs (Figures 1B and 2B).

Effects of hypertension and DM on pro-inflammatory cytokines and oxidative stress

As shown in Figure 3, cardiac TNF-α and IL-6 protein levels were increased by 4.3-fold and 84 % respectively in SHRs compared with hearts from WKY rats. Cardiac TNF-α and IL-6 protein expression were enhanced more in diabetic SHRs. RGZ attenuated the up-regulation of cardiac TNF-α and IL-6 protein levels in diabetic SHRs.

Figure 4 shows NADPH oxidase activities in hearts from WKY rats, SHRs, diabetic SHRs and RGZ-treated

Table 1 Physical characteristics of WKY rats, SHRs, diabetic SHRs and RGZ-treated diabetic SHRs at 14 weeks of age

Values are expressed as means ± S.E.M, $n = 5$. *$P < 0.05$ compared with WKY rats; †$P < 0.05$ compared with SHRs; ‡$P < 0.05$ compared with diabetic SHRs. FBG, fasting blood glucose; BW, body weight; HR, heart rate; HW, heart weight.

	Group			
Parameter	WKY rats	SHRs	Diabetic SHRs	RGZ-treated diabetic SHRs
FBG (mM)	5 ± 0.2	4.5 ± 0.3	25 ± 2*†	24 ± 1*†
BW (g)	285 ± 2	301 ± 10	186 ± 11*†	183 ± 6*†
SBP (mmHg)	131 ± 4	186 ± 9*	181 ± 7*	159 ± 4*†‡
DBP (mmHg)	73 ± 8	131 ± 11*	135 ± 19*	89 ± 6†‡
HR (beats/min)	430 ± 20	418 ± 14	398 ± 32	320 ± 16*†‡
HW (g)	1.4 ± 0.02	1.6 ± 0.04*	1 ± 0.03*†	1.1 ± 0.06*†‡
HW/BW ratio (g/kg)	4.6 ± 0.1	5.3 ± 0.1*	5.9 ± 0.9*†	5.7 ± 0.1*†

Table 2 Echocardiogram results from WKY, SHR, diabetic SHR and RGZ-treated diabetic SHR at 14 weeks of age

Values are expressed as means ± S.E.M, $n = 5$. *$P < 0.05$ compared with WKY rats; †$P < 0.05$ compared with SHRs. LA, left atrium; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension.

	Group			
Parameter	WKY rats	SHRs	Diabetic SHRs	RGZ-treated diabetic SHRs
LVEDD (mm)	7.5 ± 0.2	7.8 ± 0.3	8.8 ± 0.3*†	8.4 ± 0.2*
LVESD (mm)	4 ± 0.2	3.5 ± 0.2	5.1 ± 0.2*†	4.9 ± 0.2*†
LA (mm)	4 ± 0.07	4 ± 0.2	4.9 ± 0.1*†	4.6 ± 0.2*†
EF (%)	72 ± 0.03	80 ± 0.02*	67 ± 0.01†	68 ± 0.01†

diabetic SHRs. NADPH oxidase activity in cardiomyocytes was significantly increased by 42 % in SHRs and was augmented in diabetic SHRs. NADPH oxidase activities in diabetic hearts were attenuated in hearts from RGZ-treated diabetic SHRs.

DISCUSSION

Similar to previous reports [9,10], the results of the present study have shown an increase in protein and mRNA expression of PPAR-γ in hypertensive hearts, but a decrease in protein and mRNA expression of PPAR-α and PPAR-δ. Reduced expression of PPAR-α and PPAR-δ were also found previously in both human hypertrophy and animal cardiac hypertrophy models, and these were associated with lipid accumulation and diminished fatty acid oxidation [9,19]. Increased PPAR-γ expression in cardiomyocytes could be due to accumulation of intracellular triacylglycerols (triglycerides) that resulted in the increase in lipid uptake [6], and may also be a compensatory response to cardiac hypertrophy and failure [20], thereby compromising cardiac function.

For the first time, we found greater reductions in cardiac PPAR-α and PPAR-δ mRNA and protein expression in diabetic SHRs than in SHRs. These results imply that increased deactivation of PPAR-α and PPAR-δ in diabetic hypertensive hearts leads to a more diminished fatty acid oxidation and reduced production of ATP, resulting in more compromised cardiac function. On the contrary, PPAR-γ mRNA and protein expression were found to be greatly increased in diabetic hypertensive hearts. These effects might have resulted from accelerated intramyocardial lipid accumulation caused by cardiac glucolipotoxicity in the cardiomyoctes [6]. These factors may play an important role in the clinical pathophysiology of hypertension complicated by diabetic cardiomyopathy. In addition, on the basis of the echocardiographic results, we found a significant increase in left ventricular size and a decrease in EF in diabetic SHRs. The results were in agreement with the results of PPAR expression. However, similar to results in a previous study [21], the structural changes in diabetic SHRs did not improve after treatment with RGZ. Although the present study did not evaluate PPAR function and activity, previous studies have shown a correlation between the changes in the PPAR function and activity [6,22,23].

Hypertension injures blood vessels causing end-organ damage, and inflammation plays an important role in the damage caused by hypertensive disease. Hypertension and DM increase inflammation and oxidative stress, accelerating atherosclerosis and inducing cytokines secretion [24,25], which may contribute to the genesis of cardiomyopathy. NADPH oxidase is a major source of ROS (reactive oxygen species) in cardiomyocytes [26,27]

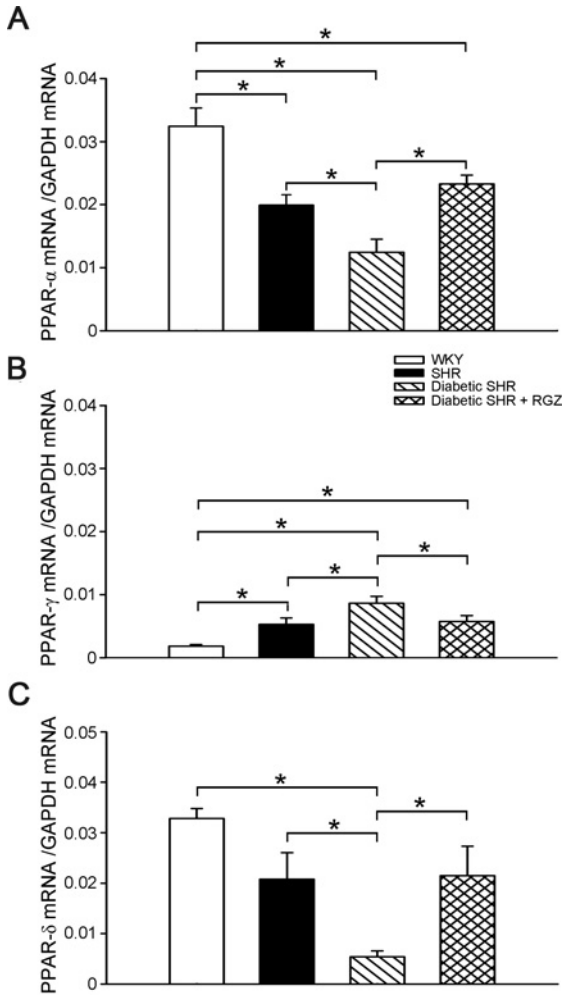


Figure 1 Cardiac PPAR-α (A), PPAR-γ (B) and PPAR-δ (C) mRNA expression from WKY rats, SHRs, diabetic SHRs and RGZ-treated diabetic SHRs as determined by real-time qPCR

(A) Cardiac PPAR-α mRNA expression was decreased most in diabetic SHRs ($n = 6$) compared with WKY rats ($n = 6$), SHRs ($n = 7$) and RGZ-treated diabetic SHRs ($n = 7$). (B) Cardiac PPAR-γ mRNA expression was enhanced most in diabetic SHRs ($n = 6$) compared with WKY rats ($n = 7$), SHRs ($n = 7$) and RGZ-treated diabetic SHRs ($n = 7$). (C) Cardiac PPAR-δ mRNA expression was significantly decreased in diabetic SHRs ($n = 6$) compared with WKY rats ($n = 6$), SHRs ($n = 6$) and RGZ-treated diabetic SHRs ($n = 6$). Results were normalized to GAPDH as an internal control. $^*P < 0.05$.

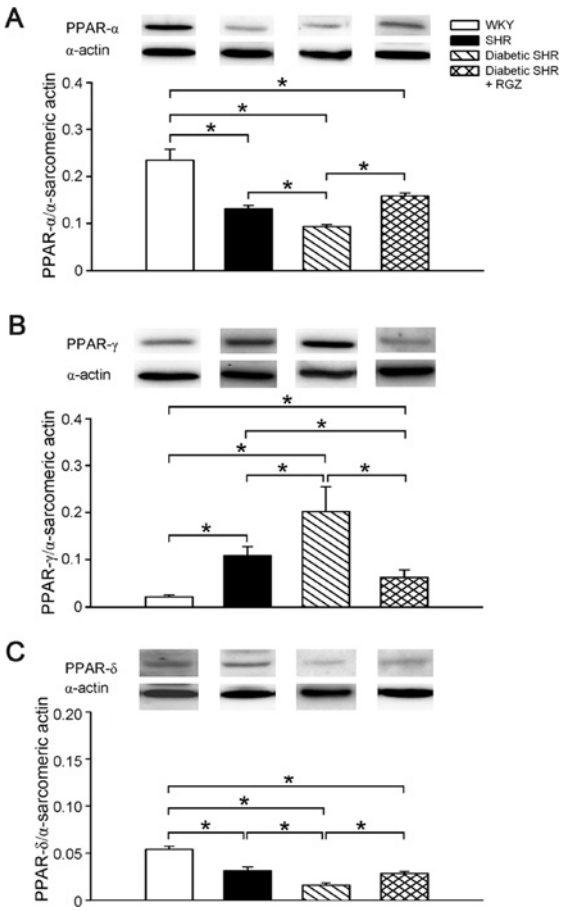
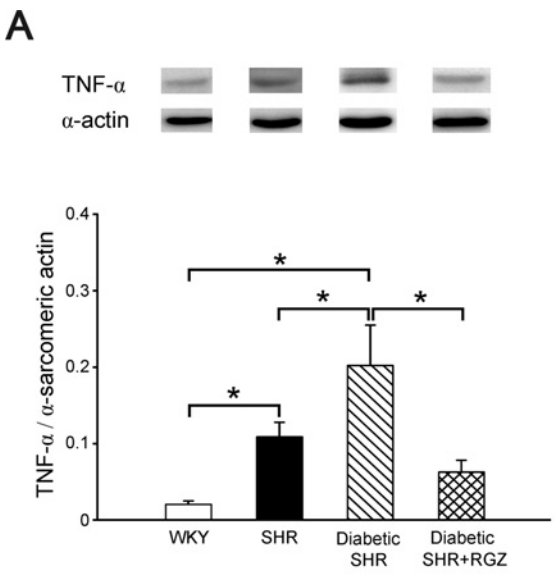


Figure 2 Cardiac PPAR-α (A), PPAR-γ (B) and PPAR-δ (C) protein expression from WKY rats, SHRs, diabetic SHRs and RGZ-treated diabetic SHRs as determined by Western blotting

(A) Cardiac PPAR-α protein expression was decreased most in diabetic SHRs ($n = 6$) compared with WKY rats ($n = 7$), SHRs ($n = 7$) and RGZ-treated diabetic SHRs ($n = 7$). (B) Cardiac PPAR-γ protein expression was enhanced most in diabetic SHRs ($n = 6$) compared with WKY rats ($n = 7$), SHRs ($n = 7$) and RGZ-treated diabetic SHRs ($n = 7$). (C) Cardiac PPAR-δ protein expression was significantly decreased in diabetic SHRs ($n = 6$) compared with WKY rats ($n = 6$), SHRs ($n = 6$) and RGZ-treated diabetic SHRs ($n = 6$). Upper panels, representative immunoblots of PPAR isoforms in the different groups; lower panels, quantification of the Western blot analysis, with densitometry normalized to α-sarcomeric actin as the internal control. $^*P < 0.05$.

and may enhance inflammation. Previous studies have shown that inflammation can regulate PPARs [7,14,28–30]. In the present study, hypertension significantly increased pro-inflammatory cytokine expression and NADPH oxidase activity, and was even enhanced when complicated by DM. Differential levels of PPAR isoform expression were also found and were enhanced further when complicated by DM. These findings indicate further that DM can modulate PPAR expression through pro-inflammatory cytokines and oxidative stress.

Emerging findings have demonstrated the anti-inflammatory effect of PPAR agonists by decreasing inflammatory markers [15]. It has also been reported that activation of PPAR-γ plays a preventive cardiovascular role by offsetting the cardiac inflammatory response in SHRs [31]. However, PPAR ligands as therapeutic targets in cardiovascular disease have received widespread attention due to their potential adverse effects, such as the dual PPAR-α/γ agonist muraglitazar and the PPAR-γ agonist RGZ, which have been reported to



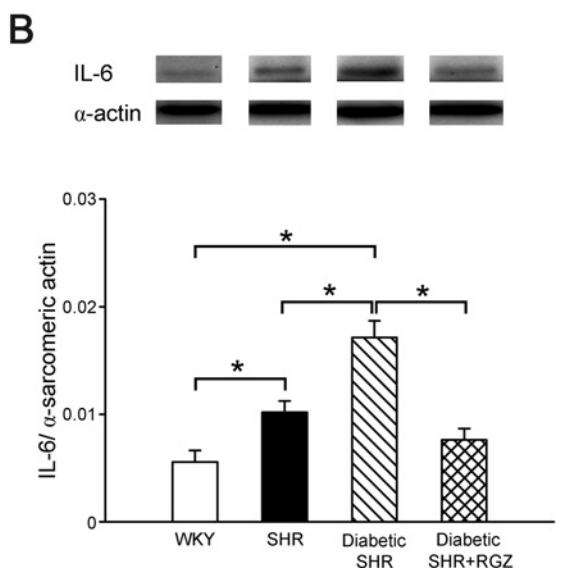


Figure 3 Cardiac TNF-α (A) and IL-6 (B) protein expression from WKY rats, SHRs, diabetic SHRs and RGZ-treated diabetic SHRs

(A) Representative immunoblot and quantification of TNF-α levels from WKY rats ($n = 8$), SHRs ($n = 8$), diabetic SHRs ($n = 8$) and RGZ-treated diabetic SHRs ($n = 8$). (B) Representative immunoblots and quantification of IL-6 levels from WKY rats ($n = 8$), SHRs ($n = 8$), diabetic SHRs ($n = 8$) and RGZ-treated diabetic SHRs ($n = 8$). Densitometry was normalized to α-sarcomeric actin as the internal control. $^*P < 0.05$.

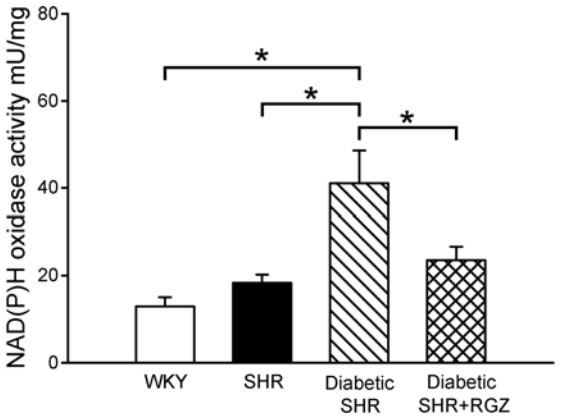


Figure 4 Cardiac NADPH oxidase activities from WKY rats, SHRs, diabetic SHRs and RGZ-treated diabetic SHRs

NADPH-dependent superoxide production were measured in WKY rats ($n = 5$), SHRs ($n = 6$), diabetic SHRs ($n = 6$) and RGZ-treated diabetic SHRs ($n = 6$). $^*P < 0.05$. mU, milli-units.

increase the risk of death, cardiovascular events and myocardial infarction [16,32]. In the present study, the effectiveness of RGZ in attenuating increased protein levels of TNF-α and IL-6 in diabetic SHRs indicated that the PPAR-γ agonist is a potential anti-inflammatory therapeutic agent for hypertension complicated by DM. The inhibition of NADPH oxidase activity after RGZ treatment suggests that oxidative stress may contribute to the effects of hypertension co-existing with DM on PPARs. In addition, antioxidant treatment with ascorbic acid can reverse the changes in PPAR expression in diabetic rats [7], and atorvastatin also ameliorates the reduced expression of PPAR-α and PPAR-β/δ in cardiac hypertrophy [19]. Moreover, coenzyme Q10 reversed PPAR changes in TNF-α-treated cardiomyocytes [14], and resveratrol can attenuate the changes in PPAR expression in oxygen glucose deprivation-exposed neurons [33]. In the present study, we also found that RGZ reversed the decrease in PPAR-α and PPAR-δ protein expression in hearts from diabetic SHRs and attenuated the increase in PPAR-γ mRNA and protein expression in hearts from diabetic SHRs. These results confirm further that oxidative stress and inflammation may play important roles in regulating cardiac PPAR isoforms and may explain why RGZ only decreased PPAR-γ expression in DM and hypertension complicated with DM, but not in HL-1 cells [14]. Furthermore, similar to previous studies, we found a lower BP in the RGZ-treated diabetic SHRs, which may also contribute to a decrease in inflammation and ROS in this group. The mechanism by which RGZ lowers BP has been proposed to be caused by direct vessel dilatation [34], reduction of oxidative stress [35] and inhibition of ACE (angiotensin-converting enzyme) [36]. However, the effects of RGZ on ACE were not evaluated in the present study. Moreover, it has been shown that the antihypertensive agent nifedipine did not affect PPAR-γ expression [37], and telmisartan or irbesartan (angiotensin type 1 receptor blocker) can lower BP and increase PPAR-γ activity [38], which was different from the effects of RGZ reported in the present study. Therefore

changes in PPAR expression after RGZ treatment might not be caused by its BP-lowering effect. To the best of our knowledge, the present study is the first to link the mechanisms of a hypertensive heart model complicated with DM to the regulation of cardiac PPARs, pro-inflammatory cytokines and NADPH oxidase activity.

In conclusion, our findings suggest that DM may modulate the hypertensive effects on PPAR isoform expression through pro-inflammatory cytokines and oxidative stress, which may be attenuated by RGZ treatment.

AUTHOR CONTRIBUTION

Ting-I Lee contributed to the experimental design, performed the *in vivo* experiments, analysed the results and wrote the paper. Yu-Hsun Kao provided the concept and experimental design of the study, handled the *in vivo* functional experiments and reviewed the paper prior to submission. Yao-Chang Chen collected the samples, performed the *in vivo* functional experiments and provided technical assistance. Nan-Hung Pan contributed to the *in vivo* experiments and technical assistance of the study. Yung-Kuo Lin gave various contributions to the statistical analysis and interpretation of the results and discussion. Yi-Jen Chen contributed to the experimental design, the analysis of the results and the final revision of the paper.

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