In Vivo Activation of Peroxisome Proliferator-Activated Receptor-δ Protects the Heart from Ischemia/Reperfusion Injury in Zucker Fatty Rats

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ABSTRACT

Peroxisome proliferator-activated receptor (PPAR)-δ is a transcription factor that belongs to the PPAR family. PPAR-δ is abundantly expressed in the heart, and its role in the heart is largely unknown. We tested whether pharmacological activation of PPAR-δ protects the heart from ischemia/reperfusion (I/R) injury in male Zucker fatty rats, a rodent model of obesity and dyslipidemia. A highly selective PPAR-δ agonist, [4-[[[2-[[3-fluoro-4-(trifluoromethyl)phenyl]-4-methyl-5-thiazolyl][methyl]thio]-2-methylphenoxy]acetic acid (GW0742), was administered for 7 days at 10 mg/kg/day (p.o., once a day). Ischemic injury was produced by occlusion of the left anterior descending artery for 30 min followed by reperfusion for up to 24 h. Treatment with GW0742 reduced serum levels of cardiac troponin-I and infarct size by 63% (p < 0.01) and 32% (p < 0.01), respectively, and improved left ventricular function. Treatment with GW0742 up-regulated gene expression involved in cardiac fatty acid oxidation, increased fat use in the heart, and reduced serum levels of free fatty acids. The enhanced cardiac expression of interleukin (IL)-6, IL-8, intercellular adhesion molecule-1, and monocyte chemoattractant protein-1 induced by I/R were significantly attenuated by GW0742. Treatment with GW0742 also reduced apoptotic cardiac myocytes by 34% and cardiac caspase-3 activity by 61% (both p < 0.01 versus vehicle). GW0742 differentially regulated Bcl family members, favoring cell survival, and attenuated I/R-induced cardiac mitochondrial damage. In addition, GW0742 treatment augmented the cardiac Akt signaling pathway, as reflected by enhanced phospho-3-phosphoinositide-dependent kinase-1 and p-Akt. The results indicate that activation of PPAR-δ protects the heart from I/R injury in Zucker fatty rats, and multiple mechanisms including amelioration of lipotoxicity, anti-inflammation, and up-regulation of prosurvival signaling contribute together to the cardioprotection.

Peroxisome proliferator-activated receptors (PPARs) belong to nuclear receptor superfamilies of ligand-activated transcription factors. Three PPAR isotypes, PPAR-α, PPAR-γ, and PPAR-β/δ (hereafter referred to as PPAR-δ) have been identified. Compared with PPAR-α and PPAR-γ, PPAR-δ is the least understood member of the PPAR family (Glass, 2006). PPAR-δ is ubiquitously expressed in most adult tissues and very early during embryogenesis (Escher et al., 2001). The multiplicity of phenotypes induced by PPARδ gene disruption in the mouse reflects the importance of this nuclear receptor in development (Peters et al., 2000), and early functional studies indicate PPAR-δ involvement in epidermal differentiation, maturation, and skin wound healing (Matsuura et al., 1999). Evidence has also suggested that activation of PPAR-δ promotes fatty acid (FA) catabolism in several tissues, such as skeletal muscle and adipose (Tanaka et al., 2003). More recent studies indicated a potential role of PPAR-δ in regulation of glucose metabolism and insulin sensitivity (Lee et al., 2006). These actions could explain the apparently beneficial effects of synthetic PPAR-δ agonists on circulating lipids, insulin resistance, and obesity that have been reported in some animal models (Oliver et al., 2001; Wang et al., 2003; Lee et al., 2006).

PPAR-δ is abundantly expressed in both adult heart and neonatal rat cardiomyocytes (Escher et al., 2001; Gilde et al., 2003; Cheng et al., 2004). In the presence of a PPAR-δ agonist, the expression of key enzymes in FA metabolism was up-regu-
lated, and FA oxidation (FAO) was enhanced in H9c2 cell line and in cultured rat cardiomyocytes (Gilde et al., 2003; Cheng et al., 2004). It was also reported that a PPAR-δ agonist inhibited phenylephrine-induced hypertrophy in neonatal rat cardiomyocytes (Planavila et al., 2005) and protected H9c2 cells from oxidative stress-induced apoptosis (Pesant et al., 2006). A recent study indicated that cardiac PPAR-δ deletion in mice resulted in cardiac dysfunction, hypertrophy, and congestive heart failure (Cheng et al., 2004).

Despite the progress in our understanding of PPAR-δ, its role in heart disease is largely unknown. This is an important consideration because PPAR-δ activators are in development for the treatment of dyslipidemia, obesity, and/or insulin resistance in patients with this metabolic syndrome, a population known to have an elevated risk of ischemic heart disease (Barish et al., 2006).

The aim of the present study was to investigate the effect of pharmacological activation of PPAR-δ on cardiac ischemia/reperfusion (I/R) injury in a model of metabolic syndrome. In particular, the cardioprotective effects and underlying mechanism of a potent and highly selective PPAR-δ agonist, GW0742 (van der Veen et al., 2005), were examined in obese/dyslipidemic Zucker fatty rats.

**Materials and Methods**

**Experimental Protocol.** The study was conducted in accordance with the Institute of Laboratory Animal Resources (1996). Male Zucker fatty rats (genetic models), 10 to 12 weeks old, were treated with vehicle or the PPAR-δ agonist, GW0742 (GlaxoSmithKline), at 10 mg/kg/day, once a day, via oral gavage for 7 days. GW0742 is a highly specific ligand for PPAR-δ. Its EC50 value for murine PPAR-δ, -α, and -γ is 28, 8900, and >10,000 nM, respectively. The EC50 for human PPAR-δ, -α, and -γ is 1, 1200, and 4100 nM, respectively (Sznайдman et al., 2003; van der Veen et al., 2005). The last dose was administered 1 h before ischemia. Left anterior descending artery (LAD) occlusion (30 min) and reperfusion (4 h unless otherwise indicated) were induced by inflating and then deflating a nontraumatic balloon occluder that was fixed on the LAD. The ischemic area was distinguished from nonischemic area by Evans blue dye staining, and the infarcted portion of the myocardium was determined by the triphenyl tetrazolium chloride method as described previously (Yue et al., 2001). Two groups of sham-operated rats treated with vehicle or GW0742 were also included in some studies.

**Assessment of Myocardial Function.** Cardiac function was determined in rats pretreated for 7 days with GW0742 (10 mg/kg/day) or vehicle and subjected to 30-min ischemia followed by reperfusion for 24 h or to sham surgery (control). To obtain stable and reproducible results, hemodynamic parameters were monitored at the 24-h postreperfusion time point. Left ventricle (LV) pressure and arterial blood pressure were measured via two 1.4 F Millar Mikrotip catheter transducers that were inserted into the LV via the right carotid artery and the right femoral artery, respectively. Mean arterial blood pressure, LV end diastolic pressure, positive, and negative dP/dt were measured (Yue et al., 2001).

**Biochemical Assays.** Blood or tissue samples were collected at time points indicated in respective figures (or see Supplemental Fig. 1). The serum levels of cardiac troponin-I (cTnI), IL-6, and IL-8 were determined by enzyme-linked immunosorbent assay from Life Diagnostics (West Chester, PA), R&D Systems (Minneapolis, MN), and Assay Designs (Ann Arbor, MI), respectively. Caspase-3 activity and prostaglandin synthesis were measured from Life Diagnostics (West Chester, PA), R&D Systems (Minneapolis, MN), and Assay Designs (Ann Arbor, MI), respectively. Blood lipid determinations were performed on the Olympus AU640 chemical analyzer (Olympus, Tokyo, Japan).

**Assessment of Relative Cardiac Carbohydrate and Free FA/Ketone Oxidation.** Rats subjected to I/R were placed in restraining tubes after implantation of a jugular vein catheter. A continuous [1-13C]glucose (10 mg/kg/min/somatostatin (1.5 μg/min) clamp was initiated 1 h postreperfusion and run for 120 min. This period was sufficient for glycolytic and tricarboxylic cycle intermediates to achieve steady-state enrichments and is also when significant metabolic changes occur during early postreperfusion (Yue et al., 2003). At the end of the clamp experiment, the ischemic myocardium was rapidly removed and prepared for [4-13C]glutamate, [3-13C]lactate, and [3-13C]alanine enrichment measurement as described in detail previously (Jucker et al., 1997; Yue et al., 2003, 2005). Relative cardiac carbohydrate (including glucose, glycerogen, pyruvate, and lactate) and free FA (FFA/ketone oxidation in terms of relative substrate contribution to acetyl-CoA oxidation was assessed from the metabolite pool enrichments. The labeling schematic and additional study details may be found in Supplemental Fig. 2.

**Quantitative Real-Time Reverse Transcriptase Polymerase Chain Reaction Analysis.** Total RNA was extracted from myocardial tissues collected from vehicle- and GW0742-treated rats using the QIAGEN RNeasy Mini kit (QIAGEN, Valencia, CA). The animals were sacrificed at four time points (sham baseline, 30 min of ischemia, and reperfusion 2 and 4 h). All samples were subjected to DNase treatment using DNase I (Ambion, Austin, TX). The efficiency of the DNase procedure was validated in a standard TaqMan assay using the glyceraldehyde-3-phosphate dehydrogenase primer set and RNAs that were not subjected to a reverse transcription step. The DNA-free total RNA samples were quantified using RiboGreen.
GW0742 Reduces I/R-Induced Myocardial Injury.

Based on a pilot study showing that a significant increase in serum level of cTn-I appears at 30 min and peaks at 2 h after reperfusion (data not shown), these two time points were chosen to test the effect of the PPARδ agonist. As shown in Fig. 1A, I/R-induced serum level of cTn-I was reduced by 47 and 63% at 0.5 and 2 h after reperfusion, respectively, in the GW0742-treated group versus the vehicle (p < 0.01).

Results

GW0742 and Cardiac Metabolism.

The relative copy number of a given mRNA detected in each sample was calculated using a standard curve followed by statistical analysis.

**Measurement of Cardiac Fatty Acid β-Oxidation Activity.**

Cardiac tissues were collected from sham-operated rats treated with vehicle or GW0742 or the rats subjected to I/R (4 h) and treated with vehicle or GW0742. FAO activity was measured by the method reported previously (Yue et al., 2003), with [1-14C]palmitic acid and [1-14C]lauric acid (American Radiolabeled Chemicals, St. Louis, MO) as the substrates. FAO activity was expressed as picomoles per minute per milligram of protein.

**DNA Fragmentation (Ladder).** DNA ladder formation in myocardial tissue was examined at 4 h after I/R or in a sham surgery group as described previously (Yue et al., 2005).

**Terminal Deoxynucleotidyl Transferase dUTP Nick-End Labeling Assay.** Ischemic cardiac tissues were collected 4 h after reperfusion, and the tissue sections were processed and prepared for terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay using a Cell Death Detection Kit (Roche Applied Science, Indianapolis, IN). The apoptotic cardiomyocytes were counted in 10 randomly chosen fields per section and four sections per block (Yue et al., 2005).

**Western Blot Analysis.** Cardiac tissues were collected at 4 h postreperfusion, and Western blot analysis was performed using primary antibodies against Akt-pS473, phospho-3-phosphoinositide-dependent kinase-1, Bcl-xl, Bid (Cell Signaling Technology Inc., Danvers, MA), Bax (Santa Cruz Biotechnology Inc., Santa Cruz, CA), and cytochrome c (BD Biosciences Pharmingen, San Diego, CA). Blots were incubated with horseradish peroxidase-conjugated second antibody, and the signal was detected with LumiGlo chemiluminescence reagent (Cell Signaling Technology Inc.). The membranes were stripped and immunoblotted with the anti-glyceraldehyde-3-phosphate dehydrogenase antibody (Millipore Bioscience Research Reagents) for normalization.

**Preparation of Cardiac Mitochondrial and Cytosolic Extracts.** Cardiac mitochondrial and cytosolic fractions were isolated from cardiac tissues collected at 4 h postreperfusion or the sham control using differential centrifugation (Huang et al., 2005; Kujoth et al., 2005). In brief, the myocardium was minced and gently homogenized in 1:10 (w/v) ice-cold isolation buffer with a Potter Elvejem Teflon homogenizer. The homogenate was centrifuged at 750 g for 5 min, and the supernatant was retrieved and centrifuged at 10,000 g for 10 min to sediment the mitochondrial fraction. The supernatant was filtered through a 0.22-μm filter and used for measurement of cytochrome c by Western blot (Narula et al., 1999). Supplemental Fig. 1 indicating the different protocols and time points for tissue collection in the present study is included for reader’s convenience.

**Statistical Analysis.** Data are expressed as mean ± S.E.M. and analyzed by one-way analysis of variance with subsequent post hoc-paired comparisons or by an unpaired Student’s t test. Differences with a value of p < 0.05 were considered statistically significant.
The body weight was increased from 366 to 403 g in the vehicle group as 1. Rats were treated with vehicle or GW0742 (10 mg/kg/day) for 7 days. Cardiac tissues were collected before (basal) or after I/R at the two groups of rats after LAD occlusion. However, treatment with GW0742 reduced basal circulating levels of TG from 83 to 384 mg/dl (p < 0.05) and enhanced levels of high-density lipoprotein cholesterol from 49 ± 5.4 to 54 ± 6.4 mg/dl (p < 0.01) before I/R. In addition, the basal serum levels of FFA were also reduced by 35% in GW0742-treated animals compared with the vehicle group (p < 0.01) (Fig. 3A, basal). I/R elevated serum levels of FFA in the vehicle group by 2.6- and 2.9-fold, respectively, at 2 and 4 h postreperfusion compared with the basal level (Fig. 3A). However, I/R-elevated serum levels of FFA were attenuated in the GW0742-treated group (p < 0.01 and p < 0.05 versus vehicle at 2 and 4 h postreperfusion, respectively).

GW0742 enhanced cardiac basal levels of expression of genes involved in FFA uptake (CD36), transport (M-CPT-1), and β-oxidation (HADHA and ACACα) and attenuated their down-regulation by I/R (Table 1). The ex vivo measurement of cardiac FA β-oxidation showed that overall FAO activity was significantly higher in GW0742-treated groups (sham or with I/R) compared with respective vehicle groups (Table 2).

Figure 3B shows the effect of GW0742 on cardiac relative carbohydrate versus FFA/ketone oxidation in the ischemic myocardium. Although FFA/ketone oxidation remained the major metabolic substrate source for the heart, GW0742 treatment increased the relative FFA/ketone oxidation by 20% (p < 0.05). In addition, GW0742 treatment reduced hepatic TG content from 2.2 ± 0.13 (vehicle) to 1.7 ± 0.08 (GW0742) mg/mg tissue (p < 0.05, n = 14).

GW0742 Suppresses I/R-Induced Inflammatory Response in the Heart. I/R significantly elevated the expression of mRNAs of proinflammatory cytokines IL-1β and IL-6. In addition, two genes involved in recruitment and activation of inflammatory cells, monocyte chemoattractant protein (MCP-1) and intercellular adhesion molecule (ICAM)-1, were also enhanced in ischemic myocardium (Table 3). In contrast, the expression of these genes was significantly attenuated in the GW0742-treated group. Measurement of serum levels of IL-6 and IL-8 further demonstrated that GW0742 suppressed I/R-enhanced production of both cytokines (Fig. 4, A and B).

GW0742 Attenuates I/R-Induced Myocardial Apoptosis. The effect of GW0742 on I/R-induced myocardial apoptosis was determined basing on three parameters: DNA ladder, TUNEL assay, and caspase-3 activity. As shown in Fig. 5A, a clear DNA ladder, the hallmark of apoptosis, was observed in myocardial tissues from the vehicle group (lanes 5–10). In contrast, the DNA ladder was markedly attenuated in the myocardium from GW0742-treated rats (Fig. 5A, lanes 11–15).

### TABLE 1

Effect of GW0742 on the expression of mRNAs of FAO enzyme genes in myocardium subjected to I/R

<table>
<thead>
<tr>
<th>Gene</th>
<th>Group</th>
<th>Basal</th>
<th>I/R, 2 h</th>
<th>I/R, 4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD36</td>
<td>Vehicle</td>
<td>1.0 ± 0.08</td>
<td>0.91 ± 0.04</td>
<td>0.88 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>GW0742</td>
<td>1.2 ± 0.03**</td>
<td>1.26 ± 0.05**</td>
<td>1.30 ± 0.04**</td>
</tr>
<tr>
<td>M-CPT 1</td>
<td>Vehicle</td>
<td>1.0 ± 0.06</td>
<td>0.83 ± 0.09</td>
<td>0.72 ± 0.10††</td>
</tr>
<tr>
<td></td>
<td>GW0742</td>
<td>1.27 ± 0.06**</td>
<td>1.21 ± 0.06**</td>
<td>1.35 ± 0.05**</td>
</tr>
<tr>
<td>HADHA</td>
<td>Vehicle</td>
<td>1.0 ± 0.07</td>
<td>0.78 ± 0.1*</td>
<td>0.76 ± 0.03**</td>
</tr>
<tr>
<td></td>
<td>GW0742</td>
<td>1.18 ± 0.02**</td>
<td>1.49 ± 0.12**</td>
<td>1.93 ± 0.15**</td>
</tr>
<tr>
<td>ACACα</td>
<td>Vehicle</td>
<td>1.0 ± 0.14</td>
<td>0.83 ± 0.12</td>
<td>0.72 ± 0.08*</td>
</tr>
<tr>
<td></td>
<td>GW0742</td>
<td>1.2 ± 0.06</td>
<td>1.1 ± 0.04*</td>
<td>1.2 ± 0.04**</td>
</tr>
</tbody>
</table>

* p < 0.05 vs. the vehicle.
** p < 0.01 vs. the vehicle.
†† p < 0.001 vs. the basal level in the same treatment.
## p < 0.001 vs. the basal value in the same treatment.
### TABLE 2

Effect of GW0742 on cardiac fatty acid β-oxidation in rats subjected to I/R or sham control

Cardiac tissues were collected from rats treated with vehicle or GW0742 and subjected to ischemia (30 min)/reperfusion (4 h) or sham-operated rats as indicated. 1-14C Lauric acid and 1-14C palmitic acid were used as substrates as described under Materials and Methods.

<table>
<thead>
<tr>
<th>Group</th>
<th>FA β Oxidation Activity</th>
<th>Lauric acid pmol/min/mg protein</th>
<th>Palmitic acid pmol/min/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham + vehicle</td>
<td></td>
<td>45.1 ± 1.8**</td>
<td>49.3 ± 0.7**</td>
</tr>
<tr>
<td>Sham + GW0742</td>
<td></td>
<td>61.7 ± 1.5</td>
<td>67.2 ± 2.9</td>
</tr>
<tr>
<td>I/R + vehicle</td>
<td></td>
<td>36.9 ± 1.0††</td>
<td>45.5 ± 1.4††</td>
</tr>
<tr>
<td>I/R + GW0742</td>
<td></td>
<td>46.5 ± 1.3</td>
<td>62.8 ± 1.9</td>
</tr>
</tbody>
</table>

** p < 0.01 vs. sham + GW0742.
†† p < 0.001 vs. I/R + GW0742 (n = 6–7).

### TABLE 3

Effect of GW0742 on expression of mRNAs of proinflammatory cytokines in myocardium subjected to I/R

<table>
<thead>
<tr>
<th>Gene</th>
<th>Group</th>
<th>Basal</th>
<th>I/R, 2 h</th>
<th>I/R, 4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>Vehicle</td>
<td>1.0 ± 0.2</td>
<td>1098 ± 59</td>
<td>1020 ± 240</td>
</tr>
<tr>
<td></td>
<td>GW0742</td>
<td>0.8 ± 0.1</td>
<td>621 ± 89**</td>
<td>457 ± 51*</td>
</tr>
<tr>
<td>IL-18</td>
<td>Vehicle</td>
<td>1.0 ± 0.2</td>
<td>24 ± 4</td>
<td>30 ± 7</td>
</tr>
<tr>
<td></td>
<td>GW0742</td>
<td>0.9 ± 0.1</td>
<td>12 ± 2.6*</td>
<td>13 ± 2.9*</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Vehicle</td>
<td>1.0 ± 0.1</td>
<td>27 ± 3.4</td>
<td>30 ± 5</td>
</tr>
<tr>
<td></td>
<td>GW0742</td>
<td>1.0 ± 0.1</td>
<td>14 ± 2.4*</td>
<td>13 ± 3.4**</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Vehicle</td>
<td>1.0 ± 0.1</td>
<td>6 ± 0.6</td>
<td>4.4 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>GW0742</td>
<td>0.9 ± 0.1</td>
<td>3.1 ± 0.3**</td>
<td>2.4 ± 0.4*</td>
</tr>
</tbody>
</table>

* p < 0.05 vs. the vehicle (n = 6–8).
** p < 0.01 vs. the vehicle (n = 6–8).
The sham-operated rats exhibited no DNA ladder (Fig. 5A, lanes 2–4). The antiapoptotic effect of GW0742 was quantified using TUNEL assay (Fig. 5B). Evidence of cardiomyocyte apoptosis was very low in the sham groups. I/R resulted in a significant increase in apoptotic cardiomyocytes (20% of total counted myocytes) in the vehicle group. The apoptotic myocytes were localized mainly in salvaged areas surrounding the infarct zone (data not shown). Treatment with GW0742 reduced the number of apoptotic myocytes by 34% \( (p < 0.01) \) versus vehicle (Fig. 5C).

Effect of GW0742 on Expression of Bcl Family Genes in Ischemic Myocardium. I/R up-regulated cardiac expression of the proapoptotic genes, Bax and Bid, by 39 and 76%, respectively, and down-regulated the antiapoptotic gene, Bcl-xL, by 33% at 4 h after reperfusion (Fig. 6A). However, this expression pattern of Bcl family genes was reversed in the GW0742-treated group (Fig. 6A). Measurement of cardiac protein levels of Bax and Bcl-xL was in agreement with the mRNA expression results showing that I/R-induced elevation of Bax and suppression of Bcl-xL were reversed by GW0742 (Fig. 6B).

**Effect of GW0742 on I/R-Induced Cardiac Mitochondrial Damage.** Because Bcl family proteins play a critical role in regulation of cytochrome c release from mitochondria into cytosol, we further studied the effect of GW0742 on I/R-induced mitochondrial injury. As shown in Fig. 7, I/R resulted in a significant elevation in levels of myocardial cytosolic cytochrome-c versus the sham, and treatment with GW0742 reduced the cytosolic cytochrome c level by 60% \( (p < 0.05) \).

GW0742 Up-Regulates Akt Signaling in Ischemic Myocardium. Cardiac phospho-Akt (Akt-pS473) levels were similar between the vehicle and the sham control (Fig. 8A).
However, the myocardial p-Akt signaling was increased by 57.5% in the GW0742-treated group (p < 0.05 versus vehicle). I/R resulted in a mild, albeit not significant, decrease in the cardiac p-PKD1, an upstream regulator of Akt, compared with the sham group (Fig. 8B). Treatment with GW0742 increased cardiac level of p-PDK by 2.3-fold versus the vehicle (p < 0.05 versus sham or vehicle). In the absence of I/R, GW0742 had no effect on the basal levels of p-Akt or phospho-3-phosphoinositide-dependent kinase-1 (data not shown).

**Discussion**

In this study, we used a highly selective PPAR-δ agonist and demonstrated for the first time that in vivo pharmacological activation of PPAR-δ protects the heart against I/R injury in a dyslipidemic animal model. This novel cardioprotective effect of the PPAR-δ agonist reduced I/R-induced cardiac infarct size and preserved cardiac contractile function. The study explored the potential role of PPAR-δ in three main biological events associated with cardiac I/R injury, namely lipotoxicity, inflammation, and apoptosis.

Studies using different transgenic mouse models have demonstrated that lipid accumulation due to mismatch between lipid import and use in the heart leads to cell death and systolic ventricular dysfunction (Schaffer, 2003; Park et al., 2007). An increased cardiac lipid accumulation and cardiomyopathy was observed in mice with conditional cardiac-specific deletion of PPAR-δ (Cheng et al., 2004). The present study was performed on relatively young Zucker fatty rats that were euglycemic and largely normotensive, with no impaired basal cardiac function (Paulson and Tahiliani 1992). However, the rats had already presented with enhanced circulating levels of FFA and TG and an elevated cardiac content of TG. An increased cardiac susceptibility to ischemic
injury, as reflected by a greater infarct size versus Zucker lean rats, was previously demonstrated in young Zucker fatty rats (Paulson and Tahirian, 1992; Jordan et al., 2003). It is believed that this enhanced cardiac susceptibility to ischemia is mainly due to an imbalance between cellular FFA import and use and subsequent lipid accumulation in the heart. When the heart is exposed to I/R, the activated cardiac lipases cause a marked increase in FFA release from the accumulated lipid, further aggravating the preexisting imbalance and resulting in a lipotoxic environment in the heart (Young et al., 2002; Vikramadithyan et al., 2005). A close relation between peak plasma FFA levels and infarct size has been observed in patients with acute myocardial infarction (Opie et al., 1977). The present study demonstrated that PPAR-δ activation up-regulated cardiac expression of nuclear genes involved in FAO, enhanced cardiac FAO enzyme activity, and reduced circulating and cardiac lipid (FFA and TG), especially I/R-elevated plasma FFA. Furthermore, treatment with GW0742 increased relative FFA/ketone versus carbohydrate substrate use in the heart after I/R. The enhanced peripheral PPAR-δ activation could also contribute to the decrease in serum FFA levels after reperfusion. Our data are consistent with previous studies showing activation of FAO enzymes and attenuation of lipid accumulation in skeletal muscle by the PPAR-δ agonist (Tanaka et al., 2003). It is conceivable that the enhanced FAO activity and increased lipid metabolism by GW0742 would ameliorate the imbalance between FFA availability and FFA oxidation in the heart after I/R and diminish the detrimental effect of increased availability of FFA to the heart and thereby contribute in part to the cardioprotection by the PPAR-δ agonist. However, the additional studies would be required to provide more evidence supporting the direct causal link between the amelioration of lipotoxicity and cardioprotection by the PPAR-δ agonist.

The inflammatory response after I/R plays an important role in the development of tissue injury. I/R triggers a cytokine cascade, up-regulates adhesion molecules and chemokines, enhances neutrophil and monocyte infiltration into myocardium, and as a result causes cardiac damage (Huang et al., 2002). Compared with PPAR-α and PPAR-γ, the role of PPAR-δ in inflammation is unclear, and some paradoxical observations exist (Kostadinova et al., 2005). The present study showed that cardiac I/R-induced expression of inflammatory cytokine mRNAs was significantly reduced by treatment with GW0742. The suppression of cardiac cytokine gene expression was associated with a reduction in plasma levels of IL-6 and IL-8. Likewise, I/R-enhanced expression of ICAM-1, an adhesion molecule for leukocyte, and MCP-1, a chemokine for recruiting monocytes, was markedly decreased by GW0742. Our results are consistent with previous in vitro data (Rival et al., 2002; Lee et al., 2003) and would be expected to contribute to the cardioprotective effects of PPAR-δ activation.

Apoptosis is an evolutionarily conserved suicide process, and its role in I/R-induced cardiomyocyte death has been widely demonstrated (Crow et al., 2004). Some lipid metabolites and inflammatory cytokines are known to induce cell apoptosis. The present study provides several lines of evidence indicating that up-regulation of prosurvival signaling and suppression of apoptotic cell death are associated with the cardioprotective effect of the PPAR-δ agonist. First, treatment with GW0742 reversed the detrimental effect of I/R on the role of PPAR-δ in inflammation is unclear, and some paradoxical observations exist (Kostadinova et al., 2005). The present study showed that cardiac I/R-induced expression of inflammatory cytokine mRNAs was significantly reduced by treatment with GW0742. The suppression of cardiac cytokine gene expression was associated with a reduction in plasma levels of IL-6 and IL-8. Likewise, I/R-enhanced expression of ICAM-1, an adhesion molecule for leukocyte, and MCP-1, a chemokine for recruiting monocytes, was markedly decreased by GW0742. Our results are consistent with previous in vitro data (Rival et al., 2002; Lee et al., 2003) and would be expected to contribute to the cardioprotective effects of PPAR-δ activation.

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The expression of Bel family genes. I/R-enhanced expression of proapoptotic genes, Bax and Bid, was diminished, and I/R-down-regulated antiapoptotic gene, Bel-XL, was reversed by GW0742 treatment. The present results are consistent with previous studies demonstrating cardioprotection associated with loss- and gain-of-function of cardiac Bax and Bel-xl (Yamamura et al., 2001; Hochhauser et al., 2003; Huang et al., 2005). Second, the present study demonstrated that GW0742 treatment ameliorated I/R-induced cardiac mitochondrial damage, a critical step in cell apoptotic death controlled by Bel family proteins (Crow et al., 2004). Third, the improvement in mitochondrial dysfunction by GW0742 was further supported by reduced myocardial caspase-3 activity, a downstream target of cytochrome c (Narula et al., 1999).

Finally, GW0742 treatment augmented cardiac p-Akt and its upstream regulator, p-PDK1, a central kinase signaling pathway regulating cell survival and growth (Matsui et al., 2001; Mangi et al., 2003). Studies have suggested that Akt signaling pathway may relate to the mitochondrial apoptotic pathway and act at multiple levels to regulate downstream targets including the Bcl-2 family (Datta et al., 1997; Kiriti et al., 2002). Therefore, enhanced Akt signaling could relate to the beneficial effect of GW0742 on the Bcl-2 family. Taken together, this evidence supports the conclusion that PPAR-δ-dependent cardioprotection is associated with inhibition of apoptosis.

In summary, PPAR-δ activation in obese rats protects the heart against I/R injury. Multiple mechanisms including reducing lipotoxicity, inflammation, and up-regulating prosurvival signaling in the heart contribute together to the cardioprotection by the PPAR-δ agonist. Given the fact that the PPAR-δ agonists are promising new agents for treatment of metabolic syndrome and that patients with metabolic syndrome are associated with an elevated risk of ischemic heart disease, the cardioprotecive effect of the PPAR-δ agonist shown in this study would be expected to provide additional benefit in this patient population.

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