Detection of Left Ventricular Hypertrophy in Rats Administered a Peroxisome Proliferator–Activated Receptor α/γ Dual Agonist Using Natriuretic Peptides and Imaging

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Chronic treatment with suprapharmacologic doses of peroxisome proliferator–activated receptor (PPAR) agonists has a known potential for causing left ventricular hypertrophy (LVH). The mechanism by which LVH develops is not well understood nor are biomarkers of it well characterized. Natriuretic peptides are important regulators of cardiac growth, blood volume, and arterial pressure and may be useful biomarkers of LVH and hemodynamic changes that precede it. We measured amino-terminal pro-atrial natriuretic peptide (NTproANP), amino-terminal pro-brain natriuretic peptide (NTproBNP), and cardiac troponin I (cTnI) concentrations in serum and plasma, as well as transcripts in left ventricular heart tissue for atrial natriuretic peptide precursor (Nppa), brain natriuretic peptide precursor (Nppb), and myosin heavy chain-β (Myh7) as potential biomarkers of LVH induced by a PPARα/γ dual agonist in Sprague-Dawley rats. We used magnetic resonance imaging, echocardiography, and hemodynamics to identify structural and functional cardiovascular changes related to the biomarkers. Heart-to-brain weight ratios (HW:BrW) were correlated with NTproANP, NTproBNP, and cTnI concentrations in serum as well as fold change in expression of Nppa and Nppb. LVH was characterized by increased left ventricular wall thickness and inner diameter, increased cardiac output, decreased arterial blood pressure, and increased heart rate. In these studies, each end point contributed to the early detection of LVH, the ability to monitor its progression, and demonstrated the ability of NTproANP concentration in serum to predict LVH and hemodynamic changes.

Key Words: PPAR; natriuretic peptides; left ventricular hypertrophy; biomarkers.

Cardiac hypertrophy, an increase in cardiac mass, is an important risk factor for cardiac morbidity and mortality (Levy et al., 1990). Enlarged cardiac chambers with walls of normal proportion (during compensated phase) to somewhat decreased thickness (during decompensated heart failure) are characteristic of eccentric hypertrophy, whereas small chambers with thick walls are characteristic of concentric hypertrophy (Van Vleet and Ferrans, 1995). Cardiac hypertrophy is induced by a variety of factors, including persistent arterial hypertension and increased ventricular wall stress (Krauser and Devereaux, 2006). Clinical conditions that may result in cardiac hypertrophy include valvular regurgitation, stenosis, and components of the metabolic syndrome, including obesity, diabetes mellitus, and neurohormonal imbalances (Artham et al., 2009). Long-standing cardiac hypertrophy ultimately results in heart failure, the inability of the heart to supply sufficient blood flow to meet the body’s needs (Hunter and Chien, 1999).

Peroxisome proliferator–activated receptor (PPAR) α and γ agonists are medications that can prevent experimental cardiac hypertrophy both in vivo and in vitro (Asakawa et al., 2002), preserve left ventricular function in obese dyslipidemic mice (Verreth et al., 2006), and reduce cardiovascular risk factors in human patients with type 2 diabetes (Pfitzner et al., 2005). PPARα/γ dual agonists were developed in an attempt to combine the proven lipid-modulating effects of PPARα agonism with the insulin-sensitizing effects of PPARγ agonism (Miller and Etgen, 2003). LY510929 (Xu et al., 2004), a nonthiazolidinedione, binds human PPARα (Ki = 4nM) and PPARγ (Ki = 3nM) and displays potent agonist activity for both in cell-based cotransfection assays (half-maximal effective concentration [EC50] = 9 and 4nM, respectively; Etgen et al., 2003). In male Zucker Diabetic Fatty rats treated with LY510929, glucose normalization occurred at 0.03 mg/kg...
daily (half-maximal effective dose \[ED_{50} = 0.004 \text{ mg/kg}\]). Due to species differences in LY510929’s ability to activate PPAR\(\alpha\) (murine PPAR\(\alpha\) EC\(_{50}\) = 2\(\mu\)M) and differential regulation of apolipoprotein A-1 (apoA-1) in rodents (Berthou et al., 1996), lipid-modulating effects were tested in the human apoA-1 transgenic mouse, in which LY510929 increased high-density lipoprotein cholesterol in serum by 70% at 30 mg/kg and lowered triglycerides by 80% at 30 mg/kg.

PPAR agonists are also capable of causing cardiac toxicity when used at suprapharmacologic doses in healthy animals (Duan et al., 2007). The toxicity induced by PPAR\(\alpha\) and PPAR\(\gamma\) agonists resembles that caused by overexpression of PPAR\(\alpha\) and PPAR\(\gamma\) genes in transgenic mouse models. Cardiac-specific overexpression of PPAR\(\alpha\) causes upregulation of fatty acid oxidation pathways in mitochondria, while downregulating glucose transport pathways and expression of glycolysis enzymes in transgenic mice, which develop cardiac hypertrophy and chamber dilation (Finck et al., 2003). Cardiac volume overload, due to sodium retention by the kidneys, and increased glucose utilization in cardiomyocytes, caused by reduced plasma-free fatty acids due to uptake into adipose tissue, may contribute to the development of PPAR\(\gamma\) agonist–induced cardiac hypertrophy (Arakawa et al., 2004; Edgley et al., 2006).

Natriuretic peptides are likely biomarkers of cardiac hypertrophy. They are important regulators of cardiac growth, blood volume, and arterial pressure. Expression of atrial natriuretic peptide precursor (Nppa) and brain natriuretic peptide precursor (Nppb) was upregulated in human cardiac hypertrophy (Schirmer and Omland, 1999). Increased plasma concentrations of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) have been found in human patients with heart disease (Doust et al., 2004; Sagnella, 1998).

While ANP and BNP have proven useful biomarkers in the clinic, they have very short half-lives, on the order of minutes (Kimura et al., 2007). Due to their important physiological roles, they are rapidly cleared from circulation through receptor-mediated clearance and proteolytic degradation, resulting in relatively low concentrations that are challenging to measure. Amino-terminal pro-atrial natriuretic peptide (NTproANP) and amino-terminal pro-brain natriuretic peptide (NTproBNP) are N-terminal cleavage fragments of their respective pro-hormones and are cosecreted in equimolar amounts with ANP and BNP (Schirmer and Omland, 1999). In addition, expression of myosin heavy chain-\(\beta\) (Myh7), part of the fetal genotype, has been reported in hypertrophied hearts (Young et al., 2001).

In these studies, suprapharmacologic doses (16–1666 times the dose required for glucose normalization; Etgen et al., 2003) of LY510929 were used to reproducibly induce LVH in rats. The model was used to assess the relative sensitivity of several approaches for premortem diagnosis of LVH in rats and to gain insight into the physiological changes underlying its pathogenesis. We compared relative heart weights with transcripts in heart tissue and biomarker concentrations in serum and plasma from cardiac imaging and hemodynamic studies in instrumented rats.

### MATERIALS AND METHODS

#### Animals.
Animal protocols were approved by Eli Lilly and Company’s Institutional Animal Care and Use Committee under accreditation from the Association for Assessment and Accreditation of Laboratory Animal Care (Memphis, TN). Male and female Sprague-Dawley rats were obtained at 6–7 weeks of age from Charles River Laboratories (Wilmington, MA) or Harlan Sprague Dawley, Inc. (Indianapolis, IN), for studies 1 and 2, respectively, and male rats at 10–11 weeks of age from Harlan Sprague Dawley, Inc., for studies 3 and 4. Rats were housed singly in ventilated stainless steel racks with access to food (study 1: PMI Certified Rodent Diet #5002; Purina Mills International, Inc., Brentwood, MO or studies 2–4: Teklad Global Rodent Diet #2014; Harlan Sprague Dawley, Inc.) and water ad libitum and allowed to acclimate for at least 1 week to caging, feeding, and watering conditions before further handling. Serial blood samples were collected from the tail vein. Terminal samples were collected under isoflurane anesthesia via abdominal aorta. Rats were euthanized either by exsanguination under anesthesia, followed by organ removal, or by carbon dioxide inhalation, followed by cervical dislocation.

#### Histology.
Hearts were excised, fixed in 4% paraformaldehyde for 24 h, and then transferred into methanol-free ethanol prior to trimming. Hearts were trimmed in cross section at a level immediately below the junction of atria and ventricles. Both the atria and the attached great vessel outflow tract were oriented longitudinally, and the ventricles were oriented in cross section. Heart tissues were paraffin embedded, sectioned at a thickness of 5 \(\mu\)m, and stained with hematoxylin and eosin (H&E). Sections were examined by light microscopy for lesions and changes in myofiber thickness or arrangement.

#### Echocardiography.
Cardiac 2-dimensional (2D) ultrasound images were acquired using a Vevo 770 high-resolution in vivo microimaging system using a 710 probe at 20 MHz (Visualsonics, Inc., Toronto, Ontario, Canada). Rats were shaved and depilated on the ventral thorax prior to imaging. During image acquisition, rats were maintained under isoflurane anesthesia and fixed in the supine position. Measurements of left ventricular inner diameter, anterior, and posterior walls at systole and diastole were made in M-mode from long-axis acquisition, rats were maintained under isoflurane anesthesia and fixed in the supine position. Measurements of left ventricular inner diameter, anterior, and posterior walls at systole and diastole were made in M-mode from long-axis acquisitions as previously described (Hanton et al., 2008).

#### Magnetic resonance imaging.
Magnetic resonance imaging (MRI) was performed on isoflurane-anesthetized rats using a Varian DirectDrive MRI spectrometer operating at 7 T with the Vnmr 2.1B software (Varian, Inc., Palo Alto, CA). The spectrometer was equipped with a Magnex 205/120/HD actively shielded gradient coil set and a Varian 63-mm inner diameter quadrature radiofrequency (RF) transmitter coil (Varian, Inc.). Positioning of the animal and the RF coil was adjusted and maintained with a custom-built support system (High Field NMR Systems, Inc., Birmingham, AL). Monitoring of respiration, heart rate (HR), and magnet bore temperature were performed with a dedicated system (Small Animal Instruments, Inc., Stony Brook, NY). This system was also used to generate gating pulses to strobe MRI data acquisition to the heart cycle. Two sets of localizer scans were acquired to allow visualization of the long axis of the left ventricle. Next, a cine mode sequence was used to acquire multislice, multiphase short-axis images of the heart. Seven to nine slices were obtained using the following: echo time 1.45 ms, number of images averaged 4, matrix 128 \(\times\) 128, field of view 40 \(\times\) 40 mm, slice thickness 1 mm, slice gap 1 mm, and flip angle 20°. Acquisition
was initiated at every other R-wave, so the effective repetition time for each phase-encode step was two heart cycles (~330 ms). Twelve phases of the heart cycle were obtained per slice. The total acquisition time for the procedure (induction of anesthesia to removal from the magnet) was about 30–40 min.

Analysis of the short-axis magnetic resonance images was conducted using custom-written programs for the Image Processing and Analysis in Java (ImageJ) platform (Rasband, W. S., U.S. National Institutes of Health, Bethesda, MD, USA, http://rsb.info.nih.gov/ij/, 1997–2005). First, from a given set of multislice multiphase images, the two phases corresponding to systole and diastole were identified based on the size of the left ventricular lumen. Next, regions of interest were defined for each of the slices within each phase by manual segmentation of the epicardial and endocardial borders. The endocardial borders included the papillary muscles. Several parameters were calculated from these regions of interest. These included average wall thickness and maximum wall thickness (in millimeters) and wall area and lumen area (in square millimeters). The wall volume and lumen volume (in cubic millimeters) were computed by summing the wall area or lumen volume, respectively, and multiplying by the slice separation (equal to the sum of the slice thickness and gap). Ejection fraction was calculated by subtracting systolic from diastolic lumen volume and dividing by the diastolic lumen volume. Cardiac output (CO) was calculated by multiplying heart by stroke volume, defined as the difference in lumen volume from diastole to systole.

Transcript analysis by TaqMan real-time PCR. Total RNA was isolated from left ventricular tissue using RNA Stat-60 (Tel-Test, Friendswood, TX) according to the manufacturer’s protocol. Further purification of total RNA was done using RNeasy columns (Qiagen, Valencia, CA). Reverse transcription was performed using Applied Biosystems’ (Foster City, CA) High Capacity cDNA Archive Kit (Cat# 4322171). Quantitative PCR was performed using probe and primer sets for Nppa (cat# Rn00561661_m1), Nppb (cat# Rn00580641_m1), Myh7 (Rn00568328_m1), and glyceraldehyde-3-phosphate dehydrogenase (Gapdh). Data were normalized to Gapdh and used for relative quantification as previously described (Livak and Schmittgen, 2001).

Hemodynamic measurements. Data Sciences International (DSI, St Paul, MN) transmitters and catheters were surgically implanted using aseptic technique under isoflurane anesthesia in either the descending aorta or left ventricle. Buprenorphine hydrochloride (Reckitt Benckiser Pharmaceuticals Inc., Parsippany, NJ) was administered im at 0.01 mg/kg prior to surgery for analgesia. Abdominal implants were performed as previously described (Greene et al., 2007) and used to measure ejection time, HR, systolic pressure (SYS), and diastolic pressure (DIA). For thoracic implants, rats were intubated and anesthesia maintained using a ventilator and vaporizer during the surgical procedure. After a ventral incision, the left ventricle was exposed via the diaphragm. The catheter was placed through a small puncture into the left ventricle and secured with surgical glue. Animals were allowed to recover for at least 5 days prior to being placed on study. Left ventricular catheters were used to measure left ventricular systolic pressure (LV SYS), HR, and change in pressure over time (dP/dt max, maximal rate of pressure rise). Data were acquired and analyzed using DSI’s Ponemah Physiology Platform (P3) acquisition and analysis system.

Biomarker measurement in serum and plasma. N-terminal proANP was measured by ELISA (Cat# 04-BI-20892; Alpco Diagnostics, Salem, NH) according to the manufacturer’s protocol. N-terminal proBNP was measured by mass spectrometry as previously described (Berna et al., 2008). Cardiac troponin I (cTnI) was measured using the Access Immunoassay System (Beckman Coulter, Inc., Fullerton, CA).

Study 1. Rats were administered LY510929, suspended in 1% carboxymethylcellulose, 0.5% sodium lauryl sulfate, 0.085% Povidone, and 0.05% Dow Corning Antifoam 1510-US in purified water via oral gavage at 0, 0.5, 5, or 50 mg/kg daily. There were 26 animals in each group, consisting of equal numbers of males and females. The body weights of all animals were periodically monitored throughout the study and at the time of necropsy. Five rats per sex per group were killed after 14 daily administrations and eight after 28 daily administrations. Blood was collected, processed for serum, stored at −80°C, and the hearts and brains excised and weighed at both time points from five rats per sex per group. In rats receiving 28 daily administrations, ultrasonad 2D echocardiography was performed prior to the first dose and then after 6, 13, and 27 days, and left ventricles were dissected from hearts, frozen in liquid nitrogen, and stored at −80°C for transcript analysis. In the remaining three rats per sex per group, heart tissue was collected for histology and blood was collected via orbital plexus 1, 2, 4, and 8 and via abdominal aorta 24 h after the 28th dose and processed for plasma using EDTA for determination of LY510929 concentrations by mass spectrometry.

Study 2. Sixteen male rats were divided into two equal-sized groups and dosed orally with LY510929 at 0 or 50 mg/kg daily for 14 days. MRI of the left ventricles of all rats was performed pre-dose and after 4 and 14 doses. Blood was collected at necropsy after 14 daily LY510929 administrations. Hearts and brains were excised and weighed at necropsy from all rats. After weighing the whole heart, left ventricles were excised and weighed.

### TABLE 1

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>14-day treatment</th>
<th>28-day treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HW (g)</td>
<td>H:BW (× 10⁻³)</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.99 ± 0.03</td>
<td>3.43 ± 0.09</td>
</tr>
<tr>
<td>0.5</td>
<td>1.17 ± 0.07</td>
<td>4.09 ± 0.18*</td>
</tr>
<tr>
<td>5</td>
<td>1.31 ± 0.05**</td>
<td>4.52 ± 0.10**</td>
</tr>
<tr>
<td>10</td>
<td>1.39 ± 0.07**</td>
<td>4.83 ± 0.14**</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.72 ± 0.02</td>
<td>4.05 ± 0.16</td>
</tr>
<tr>
<td>0.5</td>
<td>0.94 ± 0.05*</td>
<td>4.71 ± 0.19</td>
</tr>
<tr>
<td>5</td>
<td>1.12 ± 0.02***</td>
<td>5.17 ± 0.07***</td>
</tr>
<tr>
<td>10</td>
<td>1.05 ± 0.08***</td>
<td>5.10 ± 0.25***</td>
</tr>
</tbody>
</table>

Note. Data are mean ± SE. n = 5 except as noted, data analyzed by ANOVA; HW, heart weight (grams); H:BW, heart-to-body weight (grams) ratio; H:BrW, heart-to-brain weight (grams) ratio.

*a n = 4.

* p < 0.05 and ** p ≤ 0.01 compared to time-matched controls; *** p ≤ 0.05 compared to 0.5 mg/kg group; and **** p < 0.001 compared to 5 mg/kg group.
Study 3. Twenty male rats were divided into two equal size groups and administered LY510929 at 0 or 50 mg/kg via oral gavage daily 14 days. Pressure transducers implanted in the descending aorta were used to continuously measure hemodynamics in the first five rats from each group. Serum from the remaining five rats of each group was obtained and frozen at −80°C after 2, 4, and 8 daily doses from blood collected by venipuncture of the tail vein. Hearts and brains were excised and weighed at necropsy for the second group. Necropsies were performed 24 h after the final dose in studies 1, 2, and 3.

Study 4. Male Sprague-Dawley rats were administered LY510929 for 4 days at 0 (n = 3) or 50 (n = 4) mg/kg via oral gavage daily. Pressure transducers were implanted in the left ventricle as described above to continuously measure hemodynamics.

Statistics. Echocardiograms and MRI measurements were evaluated using a repeated measures analysis. A linear mixed-effects model was fitted to the data, and pre-dosing baseline measurements were included in the model as a covariate. Treatment, time, and their interaction were tested by overall F-tests at the 0.01 significance level. Monotonicity of dose response was examined using a sequential trend test based on ordinal spacing of dose levels when appropriate (Tukey et al., 1985). When the trend test was not significant but the overall F-test was, Dunnett’s t-test (Dunnett, 1964) was performed at the 0.05 significance level to evaluate nonmonotonic dose response. The mean of each treatment group was compared to that of the vehicle control group using a Bonferroni-adjusted t-test (Miller, 1981) at the 0.05 significance level. Biomarkers, organ weights, transcripts, and hemodynamic data were analyzed by a one-factor ANOVA model for each time point with treatment group as the main effect (Winer, 1971). Correlation coefficients for N-terminal proANP versus HW:BrW were calculated by a linear regression model.

RESULTS

Cardiac Pathology: Organ Weights, Gross Observations, and Histopathology

Absolute and relative heart weights were increased in males and females relative to time-matched controls at all doses at both 14 and 28 days in study 1 (Table 1). During blood sampling from the abdomen, distension was evident in the vena cava, suggesting increased blood volume and venous pressure in rats treated with LY510929. Thoracic effusion and edema of the thoracic wall were also evident at necropsy in several rats, with increased prevalence at higher doses and treatment lengths. Edema and thoracic effusion were most prevalent in female rats. Pericardial effusions were not observed.

FIG. 1. Echocardiographic measurements in study 1. (A and B) Mean (average of measurements take at systole and diastole) left ventricular inner diameter (LVIDm), (C and D) mean left ventricular anterior wall thickness (LVAWm), and (E and F) mean left ventricular posterior wall thickness (LVPWm) after 6, 13, and 28 daily administrations of LY510929. Columns represent group mean (n = 4–5) and bars represent SE. *p < 0.05 versus time-matched control.
There were no treatment-related microscopic changes observed in arrangement of myofibers, blood vessels, or interstitium in H&E-stained heart sections in study 1. A circumferential enlargement of ventricular cross sections was observed, and atrial lumena were often expanded in rats administered LY510929 at 50 mg/kg daily for 14 or 28 days.

In study 2, absolute and relative heart weights were increased in male rats administered LY510929 for 2 weeks compared to controls. Absolute heart weights were increased 35% (p < 0.001), heart-to-body weight ratios were increased 30% (p < 0.001), and heart-to-brain weight ratios were increased 39% (p < 0.001). Excised left ventricle weights from rats administered LY510929 at 50 mg/kg were increased 34% (p < 0.002) compared to controls.

In study 3, heart weights were increased in male rats administered LY510929 at 50 mg/kg for 2 weeks compared to rats receiving only vehicle. Absolute heart weights (20%, p < 0.05), HW:BrW (19%, p < 0.05), and heart-to-body weight ratios (14%) were all increased. Brain weights did not vary with administration of LY510929.

**Echocardiography**

Measurements taken from echocardiograms (study 1) revealed increased left ventricular wall thickness and inner diameter (lumen) in rats administered LY510929 compared to time-matched controls receiving vehicle only (Fig. 1). Increases were observed at the earliest time measured after 6 daily administrations at 5 and 50 mg/kg in female rats and at 50 mg/kg in male rats. After 28 daily administrations, increases occurred in wall thickness and inner diameter at all doses in females and at 5 and 50 mg/kg in males.

**Magnetic Resonance Imaging**

Left ventricular wall volume (Fig. 2A), lumen volume (Fig. 2B), wall thickness (Fig. 2C), and CO (Fig. 2D) were increased after administration of LY510929 (study 2). Ejection fraction was not changed by compound treatment.

**Transcript Analysis**

Transcripts for *Nppa* and *Nppb*, the genes encoding ANP and BNP, respectively, were increased in the left ventricles of female rats after 28 daily administrations of LY510929 (Table 2) in study 1 (not collected from male rats). Fold changes (from control) in *Nppa* and *Nppb* were correlated with HW:BrW (r = 0.63 and 0.82, respectively). *Nppa* was correlated with NTproANP concentration in serum (r = 0.84), but *Nppb* was not correlated.

**TABLE 2**

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Nppa</th>
<th>Nppb</th>
<th>Myh7</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>4.2 ± 0.3</td>
<td>4.0 ± 0.6</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>5</td>
<td>17.0 ± 4.2</td>
<td>4.4 ± 0.9</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>50</td>
<td>130.7 ± 96.7*</td>
<td>8.4 ± 1.4*</td>
<td>0.9 ± 0.2</td>
</tr>
</tbody>
</table>

*Nppa, Nppb, and Myh7* transcripts in left ventricles from female rats were analyzed by TaqMan after 28 daily administrations of LY510929 (n = 3–4). Data were normalized to Gapdh and expressed as mean fold change from control ± SE. *p < 0.05 compared to control.
with NTproBNP concentration in serum ($r = 0.14$). In contrast, Myh7 messenger RNA was not increased by treatment.

**Hemodynamics and Cardiac Function**

Systolic and diastolic blood pressures were decreased by treatment with LY510929 at 50 mg/kg (study 3). Decreased pressure occurred by 48 h after the first dose (SYS, 121.5 ± 9.6 mmHg vs. 112.5 ± 3.6 mmHg and DIA: 83.6 ± 6.9 mmHg vs. 72.0 ± 3.2 mmHg) was significant by 7 days (SYS, 179 h, 123.5 ± 7.2 mmHg vs. 103.0 ± 1.7 mmHg, $p < 0.05$ and DIA, 170 h, 83.8 ± 6.5 mmHg vs. 65.3 ± 2.6 mmHg, $p < 0.05$) and persisted for the duration of the study (14 days). Diastolic and systolic blood pressures decreased proportionately, resulting in an equivalent change in mean blood pressure (Fig. 3A) and no net change in pulse pressure (SYS minus DIA). HR increased in rats administered LY510929 at 50 mg/kg by 20–90 beats per minute (bpm; mean change in HR = 49 bpm and median change in HR = 57 bpm) within 30 h of the first administration ($p = 0.01$) and persisted for the duration of the study. Ejection time was not significantly changed by treatment. In study 4, LVSYS decreased (Fig. 3B) within 48 h of the first dose, just prior the second administration. HR and dP/dt$_{max}$ were not changed with treatment in study 4.

**Biomarker Analysis**

NTproANP concentration increased in serum samples from rats administered LY510929 (study 1; Fig. 4) and correlated with relative heart weight (Figs. 5A and 5B). In contrast, NTproBNP was not significantly changed after 28 days (0.19 ± 0.03, 0.30 ± 0.03, 0.27 ± 0.04, 0.22 ± 0.03 ng/ml at 0, 0.5, 5, and 50 mg/kg, respectively, $p = 0.20$) nor was it correlated with HW:BrW ($r = 0.3$).

In study 2, mean NTproANP concentration in plasma was increased relative to vehicle-treated controls (5.95 ± 0.42 nmol/l vs. 2.15 ± 0.33 nmol/l, $p < 0.0002$) after 14 daily administrations of LY510929. Mean NTproBNP concentration in plasma was also increased ($p < 0.003$) in rats administered LY510929 relative to controls (0.72 ± 0.03 ng/ml vs. 0.43 ± 0.05 ng/ml). Both NTproANP and NTproBNP concentrations were correlated with HW:BrW (Figs. 5C and 5D). Cardiac troponin I concentration in serum was increased in individual rats administered LY510929, but the increase was not statistically significant (0.19 ± 0.07 ng/ml vs. 0.03 ± 0.00 ng/ml, $p = 0.07$). Cardiac troponin I concentration was, however, correlated with heart weight to brain weight ratios ($r = 0.74$).

Repeated measures of NTproANP in plasma from vehicle-treated controls were not different across time in study 3 (mean of all three times = 0.74 ± 0.07 nmol/l, $p > 0.09$). N-terminal proANP concentration after 2, 4, and 8 daily administrations of LY510929 was greater than in controls receiving only vehicle after 2 (1.56 ± 0.12 nmol/l), 4 (3.05 ± 0.36 nmol/l, $p < 0.001$), and 8 (4.52 ± 0.35, $p < 0.001$) days and was correlated with HW:BrW measured after 14 days (Fig. 6). Mean NTproANP concentration was also higher in plasma samples from rats receiving LY510929 than vehicle-treated controls collected after 14 days, and concentrations were correlated with HW:BrW (6.75 ± 0.86 vs. 3.44 ± 0.83, $p < 0.04$, $r = 0.92$).

**FIG. 3.** Blood pressure in studies 3 and 4. Administration of LY510929 at 50 mg/kg (open circles and dotted lines) caused decreased systolic blood pressures (*$p < 0.05$) in male rats compared to vehicle-treated controls (closed circles and solid lines) in the descending aorta (A; $n = 5$, study 3) and left ventricle (B; $n = 3$–4, study 4). Circles represent group mean and bars represent SE.

**FIG. 4.** Increased NTproANP concentration in serum from study 1. Treatment with LY510929 caused increased mean ($n = 5$, except in 5 mg/kg female groups, $n = 4$) NTproANP concentrations in study 1. (A) 14 days, female; (B) 28 days, female; (C) 14 days, male; and (D) 28 days, male. Columns represent group mean and bars represent SE. *$p < 0.01$, **$p < 0.01$, and ***$p < 0.001$ in time-matched controls.
LY510929 Concentration in Plasma

Dose proportional increases in exposure were observed from 0.5 to 5 mg/kg/day in male rats and at all doses in females after 28 daily administrations of LY510929. At 0.5 and 5 mg/kg/day, exposures (2980, 27,800 ng/C1h/ml) in females were approximately threefold higher than in males (1150, 9250 ng/C1h/ml) but were not significantly different at 50 mg/kg (268,000 ng/C1h/ml in males and 284,000 ng/C1h/ml in females). Changes in heart weight and NTproANP concentrations were correlated with compound concentration after 28 days (Fig. 7).

DISCUSSION

In these studies, we compared NTproANP, NTproBNP, cTnI, transcripts, cine mode MRI, echocardiography, and hemodynamic measurements as biomarkers of LVH induced by a PPARα/γ dual agonist. Each end point was able to detect or predict altered cardiovascular parameters and the development of LVH while offering insights into the physiological processes through which it was caused.

Treatment with suprapharmacologic doses of LY510929 caused dose-dependent increases in heart weight in male and female rats after 14 or 28 daily administrations, characterized by increased left ventricular lumen volume and wall thickness. Increased NTproANP concentrations in plasma and serum correlated with HW:BrW at both times. When sampled after 2, 4, or 8 days of treatment, NTproANP concentrations were already increased in samples from compound-treated rats. Moreover, NTproANP concentrations measured at these early time points were well correlated with HW:BrW measured after 14 days, demonstrating that NTproANP was predictive of changes in heart weight developed over 2 weeks of daily compound administrations. Increased NTproANP concentrations at these early time points may also reflect changes in blood pressure measured in the left ventricle and descending aorta. Increased NTproBNP concentrations in serum correlated with HW:BrW after 14 days, but not after 28 days, suggesting NTproBNP secretion was affected by additional, as yet unidentified, factors. Thus, NTproANP appears to be a better biomarker of the progression of PPARα/γ agonist–induced LVH in rats.

Increased left ventricular transcription of Nppa correlated with HW:BrW after 28 days as well as with NTproANP concentration in serum. Increased transcription of Nppb also correlated with HW:BrW after 28 days, but not with NTproBNP concentration in serum, suggesting possible pharmacologic suppression of secretion (Makino et al., 2006), posttranslational modifications, or differential regulation of transcription and secretion. Similar increases in expression of Nppb transcripts without corresponding increases in circulating BNP have been observed during mineralocorticoid escape in rats following treatment with deoxycorticosterone acetate (Yokota et al., 1994). Brain natriuretic peptide is known to reduce cardiac preload and

FIG. 5. Relationship of N-terminal pro-natriuretic peptides and heart-to-brain weight ratios after treatment with LY510929 in studies 1 and 2. N-terminal proANP (nmol/l) versus heart-to-brain weight ratios (HW:BrW, g/g) from study 1 after (A) 14-day and (B) 28-day treatment, and in study 2, (C) NTproANP, and (D) NTproBNP after 14-day treatment. Correlation analyzed by linear regression, (A) $r = 0.72$, (B) $r = 0.65$, (C) $r = 0.89$, and (D) $r = 0.75$. Open symbols represent females, solid symbols represent males, squares = 0 mg/kg, triangles = 0.5 mg/kg, circles = 5 mg/kg, and diamonds = 50 mg/kg.
afterload via diuresis and natriuresis though renal effects and inhibits cardiac remodeling by blockade of protein synthesis and promotion of matrix metalloproteinase production (Tsuruda et al., 2002). Suppression of BNP secretion may have contributed to LVH in our studies or exacerbated hypertrophy caused by volume overload, metabolic changes, or direct effects of PPARα/γ agonism in the heart.

Diastolic and systolic blood pressures in the descending aorta decreased with treatment. This was consistent with increased NTproANP concentrations at all times measured, given the role of ANP in regulation of arterial blood pressure through vasodilation, natriuresis, and diuresis (Sabrane et al., 2005). Decreased blood pressure was accompanied by increased HR and increased CO after 14 daily administrations. LVH may result in this model as a compensatory mechanism to maintain SYS by increasing CO, which has been identified as a significant positive correlate with increased left ventricular mass in human patients (Weaver et al., 2009). Increased CO may contribute to increased venous pressure, increasing cardiac preload, causing atrial stretch, and stimulating increased secretion of NTproANP (Dietz, 2005).

Decreased LV/SYS and SYS were statistically significant within 2 and 7 days after initiation of treatment, respectively. Decreased SYS may be evidence of decreased afterload, the pressure against which the heart must pump, consistent with vasodilation and decreased SYS. Increased afterload is a factor involved in stimulation of BNP secretion (Toischer et al., 2008), and its absence is another possible reason that NTproBNP concentrations in serum and plasma were not increased after 28 days.

Increased left ventricular lumen and wall dimensions measured by MRI were detected at the earliest time measured, 4 days after initiation of treatment. These findings were consistent with eccentric LVH and chamber dilation during volume overload, during which the ratio of chamber dimension and wall thickness are preserved in order to accommodate increased preload and maintain CO (Grossman et al., 1975).

FIG. 6. Predictive value of NTproANP concentration in plasma from study 3. N-terminal pro-atrial natriuretic peptide concentrations in plasma collected via tail vein puncture after 2 (A), 4 (B), 8 (C), and 14 (D) days are correlated with heart-to-brain weight ratios (HW:BrW, g/g) measured after 14 daily administrations of LYS10929 at 0 mg/kg (squares) or 50 mg/kg (diamonds) in study 3. Correlation analyzed by linear regression: (A) $r = 0.76$, (B) $r = 0.84$, (C) $r = 0.74$, and (D) $r = 0.92$.

FIG. 7. Relationship of compound concentration in plasma to NTproANP and change in heart weight in study 1. Group mean percent change in heart weight (HW; closed symbols, left axis) and NTproANP concentration (open symbols, right axis) for males (squares) and females (triangles) were related to LYS10929 exposure (AUC, area under curve). Linear regression for combined sexes: $r = 0.83$ (HW); 0.89 (NTproANP).
In an experimental model of compensated volume overload versus overt heart failure, differential expression of ANP and BNP were reported, with BNP expressed only during overt heart failure (Langenickel et al., 2000). This suggests that for the duration of our studies, overt heart failure did not occur and that NTproBNP may prove useful in monitoring its development in studies of longer duration. Additionally, a panel of NTproANP and NTproBNP may offer useful insights when used as toxicological biomarkers, including differential monitoring of increased preload and afterload and compensated versus decompensated heart failure.

Small changes in cTnI concentration, which were not statistically significant, occurred in rats treated with LY510929. In spite of this, myocardial degeneration was not detected by histological observation. Nevertheless, cTnI concentrations were well correlated with HW:BrW, suggesting that the process of cardiac remodeling, or increased HR, caused reversible loss of membrane integrity or lesions which may be detectable through electron microscopy. Small nonsignificant changes in cTnI concentration make its value as a predictive or monitory biomarker of LVH in this model questionable.

SUMMARY

Imaging, biomarker analysis, and blood pressure measurement all offer the opportunity for early detection of altered cardiovascular parameters indicative of drug-induced toxicity and are fully translational to the clinic. MRI or echocardiography and continuous hemodynamic measurements assess cardiac morphology and function, revealing the form of cardiac hypertrophy and its progression toward overt heart failure. Taken together, they offer insights into the complex development of LVH during PPARα/γ dual agonism. While MRI and echocardiography present higher technical challenges and expense, the functional and anatomical details they provide cannot be discerned from blood borne biomarker concentrations. Measurement of NTproANP offers the advantages of relatively low-cost and low-technical requirements, rapid diagnosis, and available assays are likely to be cross-reactive with many mammalian species due to the highly conserved sequence of Nppa.

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