Original Research

Alteration in myocardial prostaglandin D synthase expression in pressure overload-induced left ventricular remodeling in rats

Krishna T Nagalla[1], Monica Gole[1], Mario A Claudino[2], Jason D Gardner[2] and David B Murray[1]

[1]Department of Pharmacology, University of Mississippi, University, MS 38677; [2]Department of Physiology, Louisiana State University Health Sciences Center, New Orleans, LA 70112, USA

Corresponding author: David B Murray, Department of Pharmacology, University of Mississippi, School of Pharmacy, 311 Faser Hall, University, MS 38677, USA. Email: dmurray@olemiss.edu

Abstract

We hypothesized that acute pharmacological blockade of cyclooxygenase-2 (COX-2) using nimesulide (Nime) would prevent maladaptive changes in left ventricular (LV) structure and function secondary to abdominal aortic coarctation-induced pressure overload (PO). *In vivo* LV chamber dimension and function were assessed by pressure/volume admittance catheter at 14 days' postsurgery in three groups ($n \geq 6$/group): sham-operated (Sham); untreated PO; and selective COX-2 inhibitor nimesulide-treated PO (PO + Nime; 25 mg/kg/d). Treatment was initiated 24 h prior to surgical induction of PO. Relative to Sham, there was a marked increase in LV mass index in the PO groups (2.2 ± 0.01 mg/g versus 2.9 ± 0.10 mg/g Sham versus PO, PO+Nime: 2.5 ± 0.03 mg/g). End diastolic volume, an indicator of chamber size, was significantly decreased in the PO animals compared with Sham (202 ± 17 μL versus 143 ± 16 μL Sham versus PO, PO + Nime: 226 ± 9 μL). Collagen levels in PO rats assessed by hydroxyproline analysis were significantly elevated relative to Sham values. Nimesulide treatment attenuated: (1) the increase in LV mass index; (2) the reduction in end diastolic volume; and (3) the PO-induced increase in myocardial collagen. In summary, acute COX-2 inhibition with nimesulide attenuated the maladaptive changes in the LV after PO. Acknowledging the clinical failure of chronic COX-2 inhibitor use, we propose that acute treatment with COX-2 inhibition during the initial stages of cardiac remodeling can be beneficial in maintaining the normal cardiac structure and function during PO.

Keywords: nimesulide, COX-2, prostaglandin, hypertension, prostaglandin D synthase

Experimental Biology and Medicine 2012; **237**: 24–30. DOI: 10.1258/ebm.2011.011180

Introduction

Inflammation has been identified as a key component of cardiac remodeling. Consistent with this theme, cyclooxygenase-2 (COX-2) inhibitors are known to prevent adverse left ventricular (LV) remodeling in models of myocardial injury,[1–3] but the precise mechanisms are unknown. COX-2 regulates a large number of downstream prostaglandins (PGs), all with significant bioactivity in multiple systems. Reviews by Moodely, as well as Mukherjee, among others, have documented that the chronic use of certain selective COX-2 inhibitors in patients is limited by significant side-effects, attributed to altered homeostatic balance between certain PGs and thromboxanes.[4–6] Similar to long-term use of selective COX-2 inhibitors, recent clinical studies by several independent groups examining the use of the PPAR-γ agonist Avandia® (GlaxoSmithKline, London, UK) have brought to light cardiovascular complications (ACCORD trial).[7–9] There may indeed be a connection between these two separate pharmacological clinical issues, as COX-2 activity can lead to production of PPAR-γ agonists. COX-2 is responsible for generating a common precursor molecule, which is modified by several tissue-specific isomerases (synthases) that give rise to the various PGs (i.e. PGI, PGF and PGD).[10–12] PGD synthase has two isoforms (hematopoietic: H-PGD and lipocalin: L-PGD) that produce PGD_2 which is the precursor for 15-deoxy 12,14 prostaglandin J_2 (15-d-PGJ_2). It is the PGJ_2 arachidonic acid-derived molecule that has been shown to act in the kidney, lung and vasculature as an endogenous ligand activating PPAR-γ, leading to fibroblast proliferation, collagen deposition and cytokine production.[10,13] We designed this study to investigate chamber dimension and functional changes in the left ventricle secondary to pressure overload (PO) during the initial remodeling phase (first two weeks). We hypothesize that PO will induce changes in myocardial H-PGD and/or L-PGD synthase expression. Further, short-term COX-2 inhibition will alter

the balance between H-PGD and L-PGD synthases promoting beneficial remodeling. Identifying a short-term treatment strategy during initial remodeling could potentially offset the chronic treatment-induced side-effects and represent a significant change in treatment paradigm.

Materials and methods

Animal welfare

All experiments were performed using nine-week-old (200–250 g) male Sprague-Dawley rats housed under standard environmental conditions and maintained on commercial rat chow and tap water *ad libitum*. All studies conformed to the principles of the National Institutes of Health 'Guide for the Care and Use of Laboratory Animals,' and were approved by University of Mississippi's Animal Care and Use Committee. All survival surgeries for induction of PO by abdominal aortic coarctation were performed using 3% isoflorane. The non-survival terminal surgical procedure for placement of the admittance catheter and assessment of LV performance was performed under sodium pentobarbital-induced anesthesia (50 mg/kg, intraperitoneally).

Experimental design

Animal groups

Sham-operated (Sham, $n = 7$ per time point), untreated PO (PO, $n = 7$ per time point) and nimesulide-treated PO (PO + Nime, $n = 7$ per time point) groups at 14 days' postsurgery were analyzed for LV systolic and diastolic parameters to assess the effects of COX-2 inhibition on PO-induced myocardial structural and functional alterations. Specifically, 14 days after the creation of abdominal aortic coarctation (i.e. PO), cardiac function was assessed by Scisense™ (Scisense Inc, Ontario, Canada) admittance catheterization of the left ventricle. The selective inhibitor of COX-2, nimesulide (25 mg/kg/d; Tocris, Ellisville, MO, USA), was dissolved in 10% ethanol and delivered via a once daily subcutaneous injection as previously described.[14,15] Treatment was initiated 24 h prior to surgery and continued for the duration of the study period. Drug-treated sham-operated and vehicle-treated (10% ethanol) control studies were also performed; however, no differences were observed relative to sham-operated values (data not shown). At this time point, the mortality associated with the surgical model was 1%. Treatment with nimesulide did not alter this mortality rate.

Abdominal aortic coarctation model of PO

Adult male rats approximately ~250 g (9 weeks of age) were anesthetized by 3% isoflorane. Under sterile conditions, a midline skin incision was made with scissors and the linea alba identified. Following incision of the linea alba, the animal's left abdominal wall was retracted and the intestines were evulsed in a moist sterile gauze. Following blunt dissection with sterile Q-tips (Unilever, London, UK), the left and right renal arteries originating from the abdominal aorta were identified (the right renal artery arises from the aorta 2–3 mm superior to the left renal artery). The abdominal aorta was partially constricted between these branches with a size-0 silk suture tied around a 21 gauge needle.[16–18] The abdominal muscles were closed with an absorbable (size 3-0 Chromic) suture followed by closure of the skin with wound clips. Sham animals underwent the same surgical procedures, but without ligation of the abdominal aorta.

Hydroxyproline assessment of total ventricular collagen concentration

A portion of LV tissue (50 mg) was dried in an 80°C oven to constant weight. Dried samples were then hydrolyzed in 6 N HCl at 110°C for 18 h. After hydrolysis, samples were filtered, and the filtrate dried by vacuum rotary evaporator. Hydroxyproline concentration was then determined using the method described by Woessner.[19]

Assessment of LV chamber volume and contractile function

In vivo LV chamber volume and function were assessed in sodium pentobarbital anesthetized rats using a Scisense ADVantage™ Pressure Volume admittance catheter (FTS-1912B; Scisense Inc) inserted into the LV via the right carotid artery.[20] Data were analyzed using iWorx© (Dover, NH, USA) Labscribe Instrument software. Mean arterial pressure was calculated as Diastolic pressure + 1/3(Systolic pressure − Diastolic Pressure). Preload recruited stroke work (PRSW), the slope of stroke work–end diastolic volume relationship, and Max dP/dt, the peak rate of maximal pressure rise, were reported as indicators of contractility. The slope of the end diastolic pressure volume relationship (EDPVR) was reported as an indicator of passive tissue property (i.e. ventricular compliance, thus, the steeper the curve, the greater the ventricular stiffness). Min dP/dt, peak rate of pressure decline over time, and Tau, the isovolumic relaxation constant, provided indices of ventricular relaxation.

Western blot analysis

Sodium dodecyl sulfate-denatured LV protein extract samples were subjected to electrophoresis in 10% (w/v) polyacrylamide gels. Protein was then transferred via semi-dry method to nitrocellulose membranes. Appropriate dilutions of monoclonal or polyclonal antibody (COX-2 and glyceraldehyde 3-phosphate dehydrogenase [GAPDH], Abcam® Cambridge, MA, USA; PAR-2, Santa Cruz Biotechnology, Santa Cruz, CA, USA; H-PGD and L-PGD synthases, Cayman Chemical, Ann Arbor, MI, USA) were applied and incubated overnight at room temperature. Excess antibody was removed by sequentially washing in Tris-buffered saline and then incubated with goat anti-rat IgG at room temperature for two hours. After the second antibody step, membranes were sequentially washed and developed with enhanced chemiluminescence (Pierce, Rockford, IL, USA) and emittance captured in a VersaDoc Imaging Station (Bio-Rad, Hercules, CA, USA). The membrane was then stripped and reprobed for GAPDH. Once normalized to individual GAPDH values,

the differences in protein expression from hearts belonging to each group (i.e. Sham and PO) were averaged.

Enzyme-linked immunosorbent assay

L-PGD synthase myocardial tissue levels were analyzed using a commercially available kit (Cayman Chemical). Briefly, precoated wells were treated with assay buffer and 50 μL of extracted protein sample for one hour, and washed five times with wash buffer. Wells were then incubated with appropriate monoclonal antibody directed against L-PGD synthase followed by horseradish peroxidase-conjugated antibody after washing for colorimetric analysis. Plates were then read by spectrophotometry at 450 nm, according to the manufacturer's specifications.

Statistical analysis

Statistical analyses were performed with GraphPad Prism 5.0 (GraphPad Software, Inc, San Diego, CA, USA). All grouped data are expressed as means $\pm$ SEM unless otherwise stipulated. Grouped data comparisons were made by one-way analysis of variance with intergroup comparisons analyzed using Bonferroni *post hoc* testing. Statistical significance was taken to be $P \leq 0.05$.

Results

Myocardial expression of COX-2, and H-PGD and L-PGD synthases

Figure 1a depicts a representative Western blot of myocardial COX-2 expression in sham-operated, untreated PO, and PO+Nime groups at 14 days after the induction of PO. COX-2 levels were unchanged in the untreated PO group compared with sham-operated hearts at this time point (Figure 1b). Treatment with nimesulide significantly attenuated COX-2 protein levels relative to untreated PO and sham-operated values. H-PGD synthase (~45 kDa) was significantly increased in the PO group compared with sham-operated hearts (Figures 2a and b). Treatment with nimesulide prevented the PO-induced increase in H-PGD synthase, maintaining levels near sham-operated values. Myocardial protein levels of L-PGD synthase (~45 kDa) trended downward in the PO group, but this trend did not reach significance and results were considered inconclusive as to whether the expression was unchanged or decreased. Accordingly, we analyzed the same samples via enzyme-linked immunosorbent assay and found a significant decrease in L-PGD synthase in untreated PO hearts which was unchanged by nimesulide (Figure 3).

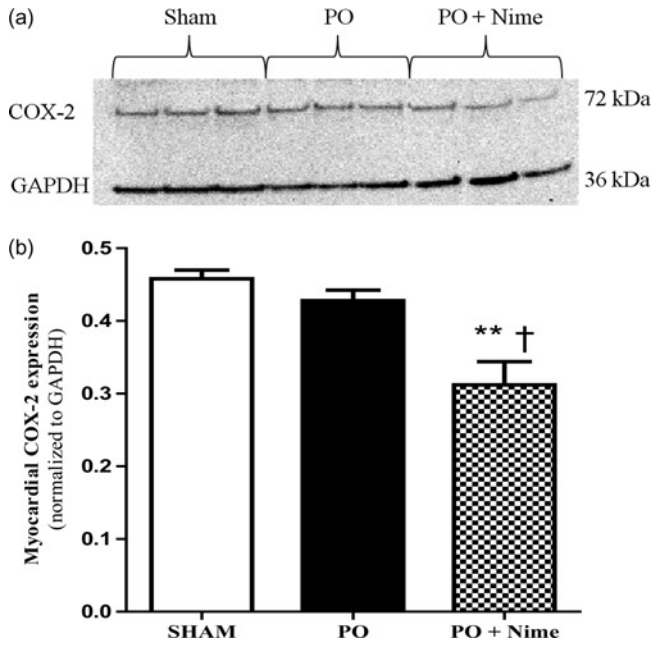

Figure 1 (a) Representative Western blot of myocardial cyclooxygenase-2 (COX-2) expression in sham-operated, untreated pressure overload (PO) and PO + Nimesulide (Nime) groups. (b) Densitometric analysis of myocardial COX-2 expression in sham-operated, PO and PO + Nime groups. Values normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression and expressed as mean $\pm$ SEM. **Denotes $P \leq 0.01$ versus sham-operated. †Denotes $P \leq 0.05$ versus untreated PO

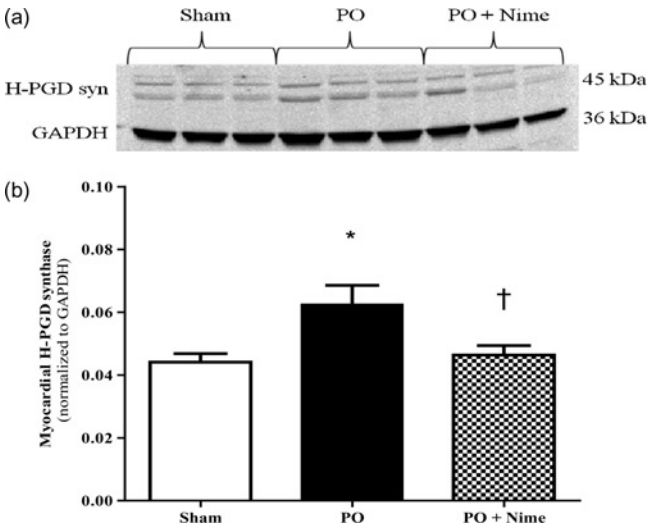

Figure 2 (a) Representative Western blot of myocardial hematopoietic prostaglandin D (H-PGD) synthase expression in sham-operated, untreated pressure overload (PO) and PO + Nimesulide (PO + Nime) groups. (b) Graphical representation of densitometric analysis of myocardial H-PGD synthase expression in sham-operated, PO and PO + Nime groups. Values were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression and expressed as mean $\pm$ SEM. *Denotes $P \leq 0.05$ versus sham-operated. †Denotes $P \leq 0.05$ versus untreated PO

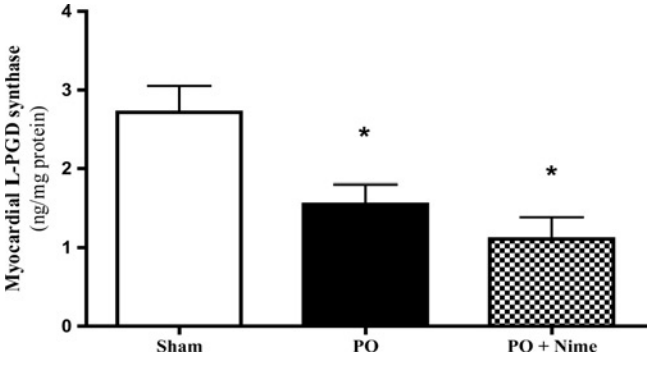

Figure 3 Myocardial L-PGD synthase expression in sham-operated, untreated pressure overload (PO) and PO + Nimesulide (PO + Nime) groups assessed by enzyme-linked immunosorbant assay. Values expressed as mean $\pm$ SEM. *Denotes $P \leq 0.05$ versus sham-operated

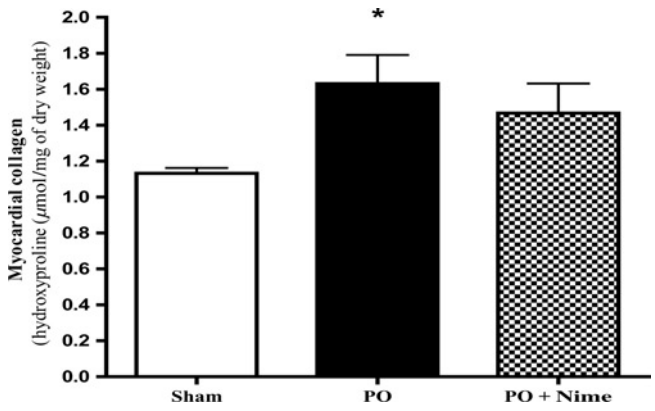

Figure 4 Myocardial collagen levels were assessed by hydroxyproline analysis. Values are presented as mean ± SEM. *Denotes $P \leq 0.05$ versus sham

Myocardial collagen content

Myocardial collagen was assessed by hydroxyproline concentration and was increased by 44% in the untreated PO hearts compared with sham-operated values (Figure 4). Collagen content in the nimesulide-treated hearts was only increased by half that amount (24% respective to Sham) and not significantly different from either Sham or PO values.

LV chamber volumes and functional studies

Relative to age-matched sham-operated animals, LV weights were significantly higher in the untreated PO group (Table 1). Nimesulide treatment markedly attenuated the increase in LV mass. These significant trends were also evident when comparing LV/body weight ratio. Right ventricular weights were not affected by PO. As expected with this model, left kidney weight was significantly reduced in PO groups.[16,18]

The overall functional effects of two weeks of selective COX-2 inhibition with nimesulide were investigated using *in vivo* pressure/volume catheter assessment. Heart rate was significantly elevated in the nimesulide-treated PO group compared with sham-operated animals (Table 2). Mean arterial pressure was increased in the PO groups, regardless of treatment, which was consistent with the elevated LV end systolic pressures in these groups. LV end diastolic pressure was not different between any of the groups. Multiple indices of systolic performance (i.e. max dP/dt, stroke work and PRSW) were significantly increased in the untreated PO group relative to sham-operated control values. Stroke work was increased by 53% in the untreated PO group compared with Sham values. Treatment with nimesulide significantly attenuated stroke work compared with the untreated PO group.

LV end systolic and end diastolic volumes were significantly reduced in the untreated PO group relative to Sham. Treatment with nimesulide prevented the PO-induced changes in these parameters and mirrored values observed in the sham-operated group. Tau, an indicator of LV relaxation rate, was markedly increased in the untreated PO hearts. Preventive treatment with nimesulide significantly attenuated the PO-induced change in Tau. Ventricular stiffness measured by the slope of the EDPVR trended higher in the untreated PO group; this value was unchanged in the PO + Nime animals relative to Sham.

Table 1 Comparison of body weights, LV, RV and kidney wet weights in 14-day postsurgery sham-operated, PO and PO + Nime groups

Parameters *in vivo* conductance catheter	Sham-operated	14-day untreated PO	14-day nimesulide-treated PO
Body weight (g)	335 ± 5	284 ± 8*	296 ± 9*
LV (mg)	752 ± 5	839 ± 27*	739 ± 19†
LV/body weight ratio	2.2 ± 0.01	2.9 ± 0.1*	2.5 ± 0.03*†
RV (mg)	181 ± 6	170 ± 6	155 ± 5*
Rt kidney (mg)	1107 ± 28	1222 ± 117	1131 ± 73
Lt kidney (mg)	1099 ± 22	658 ± 43*	755 ± 67*#

LV, left ventricle; RV, right ventricle; PO, pressure overload
Weight values are reported as average ± SD
*Denotes $P \leq 0.05$ compared with sham
†Denotes $P \leq 0.05$ compared with untreated PO
#Denotes $P \leq 0.07$ compared with untreated PO

Discussion

Initially, studies using COX-2 inhibition detailed beneficial structural and functional effects in animal models of ischemia/reperfusion.[1,3,21] Recent clinical trials of patients with congestive heart failure demonstrated serious complications associated with the chronic use of certain COX-2 selective inhibitors (i.e. Vioxx [Merck, Whitehouse Station, NY, USA] and Celecoxib [Pfizer, New York, NY, USA]) due to their impact on renal vascular tone and thrombolytic events.[5,22] Thus, investigators have struggled with defining the acute role of COX-2 or its arachidonic-derived metabolites in cardiac remodeling.

Multiple (tissue specific) isomerases exist which are capable of converting the COX-2-derived precursor molecule PGH_2 to various PGs (i.e. PGI, PGF, PGE and PGD) with each individual PG having their own G-protein-coupled class of receptors. A limited number of studies investigated the effects of PGs and their signaling mechanism in the heart (for review see refs.[23,24]) A similar small quantity studied the contribution of these rapidly developed first-line inflammatory mediators (e.g. PGI, PGE, PGF) to ventricular remodeling secondary to heart failure.[25–27] Even less is known about the role of PGD_2. Additionally, 15-d-PGJ_2, a non-enzymatic conversion product of PGD_2, is documented as an endogenous ligand for PPAR-γ, capable of stimulating fibroproliferation and collagen synthesis.[10,11,13] These effects shown in non-cardiac tissues may shed light on the recent controversial findings regarding the thiazolidinedione class of drug, rosiglitazone, and cardiovascular complications. The myocardial expression of the two PGD synthase isoforms (H-PGD and L-PGD) has been previously reported. Han *et al.*[28] found increased expression of L-PGD synthase in chronic hypoxic myocardium. However, the authors concluded that this was not directly related to the hypoxia, but rather due to a cardiac remodeling response. A clinical study of angina found

Table 2 Systolic and diastolic parameters in 14 days postsurgery sham-operated, PO and PO + Nime groups via *in vivo* conductance catheter.

Parameters *in vivo* conductance catheter	Sham-operated	14-day untreated PO	14-day nimesulide-treated PO
Heart rate (bpm)	324 ± 8	342 ± 15	363 ± 9*
End systolic pressure (mmHg)	121 ± 3	177 ± 9*	170 ± 8*
End diastolic pressure (mmHg)	4 ± 0.3	3 ± 0.5	4 ± 0.6
Mean arterial pressure (mmHg)	115 ± 7	155 ± 4*	141 ± 5*†
End systolic volume (μL)	193 ± 17	127 ± 15*	226 ± 9†
End diastolic volume (μL)	331 ± 14	281 ± 16*	324 ± 15†
Stroke work (mmHg × mL)	14,303 ± 1480	21,900 ± 2203*	13,906 ± 1232†
PRSW (mmHg)	58 ± 9	83 ± 9*	93 ± 10*
Max dp/dt (mmHg/s)	7367 ± 182	10,801 ± 457*	10,064 ± 496*
Min dP/dt (mmHg/s)	−7096 ± 440	−9044 ± 745*	−10,195 ± 247*
EDPVR	0.027 ± 0.01	0.039 ± 0.01	0.028 ± 0.01
Tau (Glantz)	14 ± 0.3	18 ± 1.4*	13 ± 0.4†

PO, pressure overload; Nime, nimesulide

Functional values are reported as average ± SEM. Mean arterial pressure (Diastolic pressure +1/3(Systolic pressure − Diastolic pressure)); preload recruitable stroke work (PRSW), the slope of stroke work–end diastolic volume relationship, and Max dP/dt, the peak rate of maximal pressure rise, were reported as indicators of contractility. The slope of the end diastolic pressure volume relationship (EDPVR) was reported as an indicator of passive tissue property (i.e. ventricular compliance, thus, the steeper the curve, the greater the ventricular stiffness). Min dP/dt, peak rate of pressure decline over time, and Tau, the isovolumic relaxation constant, provided indices of ventricular relaxation

*Denotes $P \leq 0.05$ compared with sham

†Denotes $P \leq 0.05$ compared with untreated PO

increased levels of PGD synthase in coronary effluent due to spillover from the myocardium.[29,30] The authors suggested that PGD synthase may be a useful biomarker of cardiac pathology. Accordingly, we investigated the effect of short-term COX-2 inhibition on myocardial H-PGD and L-PGD synthase expression during the initial phase (first two weeks) of cardiac remodeling in response to PO.

In the setting of non-ischemic-induced ventricular remodeling, our data indicate that expression of COX-2 was not different in Sham or untreated PO hearts 14 days after the onset of PO. The administration of nimesulide significantly attenuated COX-2 protein levels. These findings are consistent with other investigations that demonstrated a positive feedback signaling loop via PGs in favor of the expression of COX-2, but not COX-1.[31] Specifically, only PGE_2 and 15-d-PGJ_2 are shown to induce COX-2 mRNA. Although 15-d-PGJ_2 has been categorized as a member of the PG family, it does not have a specific isomerase and must be derived from PGD_2. Moreover, it should be noted that a recent study by Papanicolaou *et al*.[32] using cardiac specific transgenic mice (COX-2 CKO) demonstrated that the predominant source of COX-2 expression was from the resident non-myocyte cell population.

Herein, we report that changes in the myocardial expression of both L-PGD synthase and H-PGD synthase were evident, as acute PO was associated with a significant decrease in L-PGD synthase protein levels and a marked increase in H-PGD synthase. Previous studies found that changes in PG levels are associated with estrogenic cardioprotection.[33,34] Specifically, L-PGD synthase expression is regulated by the ERβ subclass of estrogen receptors, which are found in both myocytes and cardiac fibroblasts.[35] Strong evidence exists that ovariectomy causes not only the loss of cardioprotection against adverse remodeling (which was ERβ dependent), but abolishes myocardial L-PGD synthase expression as well.[33,35–38] A similar phenomenon was documented by Tokudome *et al*.[39] investigating the beneficial effects of dexamethasone during cardiac ischemia/reperfusion injury. Glucocorticoid receptor activation reduces infarct size; however, this effect was greatly diminished in L-PGD synthase-deficient mice. Combining these studies with our own findings suggests that a reduction in L-PGD synthase may promote adverse cardiac remodeling.

It is documented that the potential for crossover effects between individual PG receptor systems and various PGs exists.[23,24] Moreover, these mediators often work in concerted pairs to bring about agonist and antagonist effector activities even within the cardiovascular system. Further, the response of individual gene expression to decreased feedback from the ultimate product formation may in one instance promote further expression of the target gene or bring about a reduction in gene expression depending upon the promoter system associated with the target gene. This differential pattern of expression may exist within the H-PGD and L-PGD synthases, as these are commonly expressed in separate organ systems within the body yet co-expressed in the heart. While nimesulide has been extensively characterized as a selective COX-2 inhibitor,[14,40] there is no evidence, to date, of any interaction with PGD synthase, as this potential connection has not been investigated directly. Thus, the possibility of a nimesulide-mediated effect on specific PGD synthase as well as altered expression patterns is acknowledged as a potential limitation of the current study.

PO is associated with resultant changes in hemodynamic and mechanical load leading to increased myocardial stress. After two weeks, systolic function was enhanced in the PO group as indicated by maximum dP/dt, preload recruitable stroke work and end systolic pressure. The use of the selective COX-2 inhibitor nimesulide did not affect arterial blood pressure or cardiac contractility. However, nimesulide did attenuate changes in LV mass and chamber morphology. Herein, we report PO-induced changes in Tau, EDPVR and collagen content indicative of adverse changes in

passive tissue properties, which were attenuated by COX-2 inhibition. Previous reviews have documented the role of increased wall stress as a driving factor for ventricular remodeling.[41,42] Our previous work has shown that early treatment to prevent adverse LV remodeling in the initial phase has beneficial effects to extend the compensated state even when the treatment is discontinued.[43]

In conclusion, PO caused an acute increase in myocardial expression of H-PGD synthase, as well as a decrease in L-PGD synthase. Ventricular remodeling due to PO induced by abdominal aortic coarctation was associated with hypertrophy, fibrosis and altered chamber morphology after 14 days. These changes in LV tissue properties were attenuated by selective COX-2 inhibition. Clinically, long-term selective inhibition of COX-2 uncoupled the normal relationship between COX-1 and COX-2-derived PGs, promoting vasoconstriction and thrombosis. Selective targeting of PGD isomerase(s) may lead to more specific and effective therapeutic treatment without harmful side-effects.

Author contributions: DBM conceived of the study, and participated in its design and coordination, and evaluated the statistical analysis and drafted the manuscript. KTN carried out the animal, multiple biochemical studies, and performed statistical analysis. MG and MAC carried out biochemical studies and performed statistical analysis of those studies. JDG was involved in drafting the manuscript or revising it critically for important intellectual content. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

We are grateful to Bindya Patel for her technical assistance. This study was supported by American Heart Association Beginning Grant in Aid #09BGIA2090065 (DBM).

REFERENCES

1 Abbate A, Salloum FN, Ockaili RA, Fowler AA III, Biondi-Zoccai GG, Straino S, Lipinski MJ, Baldi A, Crea F, Biasucci LM, Vetrovec GW, Kukreja RC. Improvement of cardiac function with parecoxib, a cyclo-oxygenase-2 inhibitor, in a rat model of ischemic heart failure. *J Cardiovasc Pharmacol* 2007;**49**:416–8
2 Abbate A, Limana F, Capogrossi MC, Santini D, Biondi-Zoccai GG, Scarpa S, Germani A, Straino S, Severino A, Vasaturo F, Campioni M, Liuzzo G, Crea F, Vetrovec GW, Biasucci LM, Baldi A. Cyclo-oxygenase-2 (COX-2) inhibition reduces apoptosis in acute myocardial infarction. *Apoptosis* 2006;**11**:1061–3
3 LaPointe MC, Mendez M, Leung A, Tao Z, Yang XP. Inhibition of cyclooxygenase-2 improves cardiac function after myocardial infarction in the mouse. *Am J Physiol Heart Circ Physiol* 2004;**286**:H1416–H1424
4 Moodley I. Review of the cardiovascular safety of COXIBs compared to NSAIDS. *Cardiovasc J Afr* 2008;**19**:102–7
5 Mukherjee D. Nonsteroidal anti-inflammatory drugs and the heart: what is the danger? *Congest Heart Fail* 2008;**14**:75–82
6 Hudson M, Richard H, Pilote L. Differences in outcomes of patients with congestive heart failure prescribed celecoxib, rofecoxib, or non-steroidal anti-inflammatory drugs: population based study. *BMJ* 2005;**330**:1370
7 Lincoff AM, Wolski K, Nicholls SJ, Nissen SE. Pioglitazone and risk of cardiovascular events in patients with type 2 diabetes mellitus: a meta-analysis of randomized trials. *JAMA* 2007;**298**:1180–8
8 Pop-Busui R, Evans GW, Gerstein HC, Fonseca V, Fleg JL, Hoogwerf BJ, Genuth S, Grimm RH, Corson MA, Prineas R. Effects of cardiac autonomic dysfunction on mortality risk in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial. *Diabetes Care* 2010;**33**:1578–84
9 Nissen SE, Wolski K. Rosiglitazone revisited: an updated meta-analysis of risk for myocardial infarction and cardiovascular mortality. *Arch Intern Med* 2010;**170**:1191–201
10 Levi-Schaffer F, Piliponsky AM. Tryptase, a novel link between allergic inflammation and fibrosis. *Trends Immunol* 2003;**24**:158–61
11 Frungieri MB, Albrecht M, Raemsch R, Mayerhofer A. The action of the mast cell product tryptase on cyclooxygenase-2 (COX2) and subsequent fibroblast proliferation involves activation of the extracellular signal-regulated kinase isoforms 1 and 2 (erk1/2). *Cell Signal* 2005;**17**:525–33
12 Gailit J, Marchese MJ, Kew RR, Gruber BL. The differentiation and function of myofibroblasts is regulated by mast cell mediators. *J Invest Dermatol* 2001;**117**:1113–9
13 Frungieri MB, Weidinger S, Meineke V, Kohn FM, Mayerhofer A. Proliferative action of mast-cell tryptase is mediated by PAR2, COX2, prostaglandins, and PPARgamma: possible relevance to human fibrotic disorders. *Proc Natl Acad Sci USA* 2002;**99**:15072–7
14 Famaey JP. *In vitro* and *in vivo* pharmacological evidence of selective cyclooxygenase-2 inhibition by nimesulide: an overview. *Inflamm Res* 1997;**46**:437–46
15 Saeed SA, Ahmed S. Role of cyclooxygenase-2 in myocardial infarction and ischaemia. *J Coll Physicians Surg Pak* 2006;**16**:324–8
16 Stewart JA Jr, Massey EP, Fix C, Zhu J, Goldsmith EC, Carver W. Temporal alterations in cardiac fibroblast function following induction of pressure overload. *Cell Tissue Res* 2010;**340**:117–26
17 Berni R, Cacciani F, Zaniboni M, Savi M, Bocchi L, Lapucci S, Razzetti R, Pastore F, Musso E, Stilli D. Effects of the alpha2-adrenergic/DA2-dopaminergic agonist CHF-1024 in preventing ventricular arrhythmogenesis and myocyte electrical remodeling, in a rat model of pressure-overload cardiac hypertrophy. *J Cardiovasc Pharmacol* 2006;**47**:295–302
18 Ni M, Yang ZW, Li DJ, Li Q, Zhang SH, Su DF, Xie HH, Shen FM. A potential role of alpha-7 nicotinic acetylcholine receptor in cardiac angiogenesis in a pressure-overload rat model. *J Pharmacol Sci* 2010;**114**:311–9
19 Woessner JF Jr. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. *Arch Biochem Biophys* 1961;**93**:440–7
20 Murray DB, Gardner JD, Brower GL, Janicki JS. Effects of nonselective endothelin-1 receptor antagonism on cardiac mast cell-mediated ventricular remodeling in rats. *Am J Physiol Heart Circ Physiol* 2008;**294**:H1251–7
21 Saeed SA, Ahmed S. Anti-ischemic effects of nimesulide, a cyclooxygenase-2 inhibitor on the ischemic model of rabbit induced by isoproterenol. *Arch Pharm Res* 2006;**29**:977–83
22 Hudson M, Rahme E, Richard H, Pilote L. Risk of congestive heart failure with nonsteroidal antiinflammatory drugs and selective cyclooxygenase 2 inhibitors: a class effect? *Arthritis Rheum* 2007;**57**:516–23
23 Jenkins CM, Cedars A, Gross RW. Eicosanoid signalling pathways in the heart. *Cardiovasc Res* 2009;**82**:240–9
24 Harding P, Murray DB. The contribution of prostaglandins versus prostacyclin in ventricular remodeling during heart failure. *Life Sci* 2011;**89**:671–6
25 Martin M, Meyer-Kirchrath J, Kaber G, Jacoby C, Flogel U, Schrader J, Ruther U, Schror K, Hohlfeld T. Cardiospecific overexpression of the prostaglandin EP3 receptor attenuates ischemia-induced myocardial injury. *Circulation* 2005;**112**:400–6
26 Hohlfeld T, Meyer-Kirchrath J, Vogel YC, Schror K. Reduction of infarct size by selective stimulation of prostaglandin EP(3)receptors in the reperfused ischemic pig heart. *J Mol Cell Cardiol* 2000;**32**:285–96
27 Qian JY, Harding P, Liu Y, Shesely E, Yang XP, Lapointe MC. Reduced cardiac remodeling and function in cardiac-specific EP4 receptor knockout mice with myocardial infarction. *Hypertension* 2008;**51**:560–6
28 Han F, Takeda K, Ishikawa K, Ono M, Date F, Yokoyama S, Furuyama K, Shinozawa Y, Urade Y, Shibahara S. Induction of lipocalin-type prostaglandin D synthase in mouse heart under hypoxemia. *Biochem Biophys Res Commun* 2009;**385**:449–53

29 Eguchi Y, Eguchi N, Oda H, Seiki K, Kijima Y, Matsu-ura Y, Urade Y, Hayaishi O. Expression of lipocalin-type prostaglandin D synthase (beta-trace) in human heart and its accumulation in the coronary circulation of angina patients. *Proc Natl Acad Sci USA* 1997;**94**:14689–94
30 Urade Y, Eguchi Y, Eguchi N, Kijima Y, Matsuura Y, Oda H, Seiki K, Hayaishi O. Secretion of lipocalin-type prostaglandin D synthase (beta-trace) from human heart to plasma during coronary circulation. *Adv Exp Med Biol* 1999;**469**:49–54
31 Vichai V, Suyarnsesthakorn C, Pittayakhajonwut D, Sriklung K, Kirtikara K. Positive feedback regulation of COX-2 expression by prostaglandin metabolites. *Inflamm Res* 2005;**54**:163–72
32 Papanicolaou KN, Streicher JM, Ishikawa TO, Herschman H, Wang Y, Walsh K. Preserved heart function and maintained response to cardiac stresses in a genetic model of cardiomyocyte-targeted deficiency of cyclooxygenase-2. *J Mol Cell Cardiol* 2010;**49**:196–209
33 Gabel SA, Walker VR, London RE, Steenbergen C, Korach KS, Murphy E. Estrogen receptor beta mediates gender differences in ischemia/reperfusion injury. *J Mol Cell Cardiol* 2005;**38**:289–97
34 Booth EA, Flint RR, Lucas KL, Knittel AK, Lucchesi BR. Estrogen protects the heart from ischemia-reperfusion injury via COX-2-derived PGI2. *J Cardiovasc Pharmacol* 2008;**52**:228–35
35 Otsuki M, Gao H, Dahlman-Wright K, Ohlsson C, Eguchi N, Urade Y, Gustafsson JA. Specific regulation of lipocalin-type prostaglandin D synthase in mouse heart by estrogen receptor beta. *Mol Endocrinol* 2003;**17**:1844–55
36 Gardner JD, Brower GL, Janicki JS. Gender differences in cardiac remodeling secondary to chronic volume overload. *J Card Fail* 2002;**8**:101–7
37 Gardner JD, Brower GL, Voloshenyuk TG, Janicki JS. Cardioprotection in female rats subjected to chronic volume overload: synergistic interaction of estrogen and phytoestrogens. *Am J Physiol Heart Circ Physiol* 2008;**294**:H198–204
38 Gardner JD, Murray DB, Voloshenyuk TG, Brower GL, Bradley JM, Janicki JS. Estrogen attenuates chronic volume overload induced structural and functional remodeling in male rat hearts. *Am J Physiol Heart Circ Physiol* 2010;**298**:H497–504
39 Tokudome S, Sano M, Shinmura K, Matsuhashi T, Morizane S, Moriyama H, Tamaki K, Hayashida K, Nakanishi H, Yoshikawa N, Shimizu N, Endo J, Katayama T, Murata M, Yuasa S, Kaneda R, Tomita K, Eguchi N, Urade Y, Asano K, Utsunomiya Y, Suzuki T, Taguchi R, Tanaka H, Fukuda K. Glucocorticoid protects rodent hearts from ischemia/reperfusion injury by activating lipocalin-type prostaglandin D synthase-derived PGD2 biosynthesis. *J Clin Invest* 2009;**119**:1477–88
40 Vago T, Bevilacqua M, Norbiato G. Effect of nimesulide action time dependence on selectivity towards prostaglandin G/H synthase/cyclooxygenase activity. *Arzneimittelforschung* 1995;**45**:1096–8
41 Hutchinson KR, Stewart JA Jr, Lucchesi PA. Extracellular matrix remodeling during the progression of volume overload-induced heart failure. *J Mol Cell Cardiol* 2010;**48**:564–9
42 Brower GL, Gardner JD, Forman MF, Murray DB, Voloshenyuk T, Levick SP, Janicki JS. The relationship between myocardial extracellular matrix remodeling and ventricular function. *Eur J Cardiothorac Surg* 2006;**30**:604–10
43 Murray DB, McMillan R, Brower GL, Janicki JS. ETA selective receptor antagonism prevents ventricular remodeling in volume-overloaded rats. *Am J Physiol Heart Circ Physiol* 2009;**297**:H109–116

(Received May 25, 2011, Accepted October 10, 2011)