Molecular Cardiology

Microsomal Prostaglandin E₂ Synthase-1 Deletion Leads to Adverse Left Ventricular Remodeling After Myocardial Infarction

Norbert Degousee, PhD*; Shafie Fazel, MD, PhD*; Denis Angoulvant, MD, PhD*; Eva Stefanski, MSc; Sven-Christian Pawelzik, BSc; Marina Korotkova, PhD; Sara Arab, PhD; Peter Liu, MD, PhD; Thomas F. Lindsay, MD, MSc; Sun Zhuo, PhD; Jagdish Butany, MD; Ren-Ke Li, PhD; Laurentaudoly, PhD; Ronald Schmidt, PhD; Carlo Angioni; Gerd Geisslinger, MD, PhD; Per-Johan Jakobsson, MD, PhD; Barry B. Rubin, MD, PhD

Background—Pharmacological inhibition of cyclooxygenase-2 increases the risk of myocardial infarction (MI) and stroke. Microsomal prostaglandin (PG) E₂ synthase-1 (mPGES-1), encoded by the Ptges gene, functions downstream from cyclooxygenase-2 in the inducible PGE₂ biosynthetic pathway. We caused acute MI in Ptges⁻/⁻ and Ptges⁻/⁺ mice to define the role of mPGES-1 in cardiac ischemic injury.

Methods and Results—Twenty-eight days after MI, Ptges⁻/⁻ mice develop more left ventricular (LV) dilation, have worse LV systolic and diastolic function, and have higher LV end-diastolic pressure than Ptges⁺/⁺ mice but have similar pulmonary wet-to-dry weight ratios, cardiac mass, infarct size, and mortality. The length-to-width ratio of individual cardiomyocytes is significantly greater in Ptges⁻/⁻ than Ptges⁺/⁺ mice after MI, a finding consistent with eccentric cardiomyocyte hypertrophy in Ptges⁻/⁻ mice. Expression of atrial natriuretic peptide, brain natriuretic peptide, and α- and β-myosin heavy chain, markers of ventricular hypertrophy, is higher in the LV of Ptges⁻/⁻ than Ptges⁺/⁺ mice after MI. Ptges⁻/⁺ mice express cyclooxygenase-2 and mPGES-1 protein in inflammatory cells adjacent to the infarct after MI but do not express these proteins in cardiomyocytes. Ptges⁻/⁻ mice express cyclooxygenase-2 in inflammatory cells adjacent to the infarct and do not express mPGES-1 in any cells in the heart. Levels of PGE₂ but not PGD₂, thromboxane A₂, PGI₂, or PGF₂α are higher in the infarct and LV remote from the infarct after MI in Ptges⁺/⁺ than Ptges⁻/⁻ mice.

Conclusions—In Ptges⁻/⁻ mice, mPGES-1 in inflammatory cells catalyzes PGE₂ biosynthesis in the LV after MI. Deletion of mPGES-1 leads to eccentric cardiac myocyte hypertrophy, LV dilation, and impaired LV contractile function after acute MI. (Circulation. 2008;117:1701-1710.)

Key Words: hypertrophy • inflammation • myocardial infarction • prostaglandins • remodeling

Prostaglandins (PGs) are biologically active lipid mediators that are synthesized by the sequential action of phospholipase A₂, cyclooxygenase (COX), and PG synthase enzymes. Many aspects of cardiac physiology, including heart rate, coronary blood flow, coronary microvascular permeability, and left ventricular (LV) contractility, are regulated by PGs.¹ ³ PGE₂, the principal PG generated by ventricular cardiomyocytes,⁴ may be synthesized by microsomal PGE₂ synthase-1 (mPGES-1), an inducible enzyme,⁵ ⁶ or by mPGES-2 or cytosolic PGE₂ synthase, which are constitutively expressed.

Clinical Perspective p 1710

Studies with mice harboring a targeted deletion of Ptges (Ptges⁻/⁻ mice) have shown that elimination of mPGES-1 expression decreases pain, fever, and inflammation in vivo.⁷⁻⁹ From these observations, pharmacological inhibition of mPGES-1 has been proposed as an alternative to inhibition of...
COX-2 in the management of patients with pain and inflammatory diseases. Targeted deletion of *Ptges* also attenuates brain ischemia-reperfusion injury and reduces plaque burden in fat-fed *Ptges*−/−/low-density lipoprotein receptor−deficient (LDLR−/−) mice but has no effect on thromboxane biosynthesis, clot formation, or blood pressure. In contrast, inhibition of the PGE2 receptor EP4 attenuates cardiomyocyte hypertrophy in vitro, and deletion of EP4 increases infarct size and compromises LV contractile function after 1 hour of coronary occlusion and 1 day of reperfusion in vivo. Therefore, although deletion of mPGES-1 has potentially beneficial effects in multiple disease processes, deletion of mPGES-1 and attenuated PGE2 biosynthesis may have deleterious effects on the cardiac response to myocardial infarction (MI).

LV remodeling takes place after MI and is characterized by a state of volume overload. This maladaptive state is associated with an increase in LV wall stress that leads to eccentric cardiomyocyte hypertrophy with the addition of sarcornes in series and longitudinal cell growth, infarct zone thinning, and LV dilation. PGE2 induces hypertrophy of ventricular cardiomyocytes in vitro, and mPGES-1 catalyzes the majority of PGE2 biosynthesis by cardiomyocytes and inflammatory cells. In this study, we evaluated the effect of targeted deletion of mPGES-1 on cardiomyocyte hypertrophy and LV remodeling after MI. We found that mice lacking mPGES-1 generate less PGE2 in the LV and develop eccentric cardiomyocyte hypertrophy, LV dilation, impaired LV systolic and diastolic function, and elevated LV end-diastolic pressure (LVEDP) after MI compared with wild-type littermates. In contrast, *Ptges*−/− and *Ptges*−/− mice have similar pulmonary wet-to-dry weight ratios, cardiac mass, infarct size, and mortality after MI.

**Methods**

Reagents were from Sigma Chemical Co (St Louis, Mo) unless otherwise stated. The methodology used for real-time quantitative polymerase chain reaction (PCR) and immunoblotting studies has been described. Reagents and experimental procedures were approved by the Animal Care Committee of the University Health Network and were in accordance with the *Guide for the Care and Use of Laboratory Animals* Research Statutes, Ontario (1980).

**Ptges Gene–Targeted Mice**

Construction of the mPGES-1−deficient mouse line (*Ptges*−/−, DBA/1lac background) was carried out as described. PCR-based genotyping of tail DNA extracts, immunoblotting, and assays of mPGES-1 activity from multiple-organ lysates confirmed the absence of mPGES-1 in *Ptges*−/− mice. Wild-type littermates were used as controls for studies with *Ptges*−/− mice; all mice were generated by breeding heterozygous mice. The genetic status of mice (*Ptges*−/− or *Ptges*−/−) remained unknown to investigators carrying out surgical procedures, echocardiography, acquisition of pressure-volume loops, and morphometric assessments.

**MI Model**

Eight- to 12-week–old male *Ptges*−/− mice or their wild-type littermates were sedated with ketamine (50 mg/kg) and xylazine (5 mg/kg), intubated, ventilated, and maintained with 2% isoflurane. Through a left thoracotomy, the left coronary artery was ligated at a proximal location under the left atrial appendage, thereby simulating acute coronary artery thrombosis. Ischemia was confirmed by the appearance of hypokinesis and pallor distal to the occlusion and by ST elevation on ECG.

**Invasive Hemodynamic Assessment**

Under isoflurane anesthesia, a micromanometer and conductance 1.4F catheter (SPR-839, Millar Instruments, Houston, Tex) was introduced into the LV through the right carotid artery. After stabilization, signals were recorded continuously at a sampling rate of 1000/s with a pressure-volume conductance system coupled to a Powerlab/4SP analog-to-digital converter (AD Instruments, Mountain View, Calif). All pressure-volume loops were analyzed with a cardiac pressure-volume analysis program (PVAN 3.3, Millar Instruments).

**Echocardiographic Assessment of LV Function**

Mice were anesthetized with ketamine (25 mg/kg) and xylazine (2.5 mg/kg). Isoflurane was not used for these studies. Recordings were performed with a Sequoia C256 Ultrasound System (Siemens Medical, Mountain View, Calif) with a 13-MHz linear-array transducer (15L8). M-mode and 2-dimensional images were obtained in the parasternal short axis at the level of the papillary muscles. For each measurement, 3 consecutive cardiac cycles were recorded and averaged by a single experienced examiner. M-mode imaging was recorded at a sweep speed of 200 mm/s for offline measurement of LV end-diastolic and LV end-systolic dimensions, and the endocardium was traced by covering the innermost edge of the endocardial surface. The LV end-diastolic area was determined as the largest cavity size and the LV end-systolic area as the smallest cavity size during the cardiac cycle.

**Volumetry, Morphometric Analysis, and Collagen Content**

At baseline and 28 days after MI, hearts were arrested in diastole, perfusion fixed with 10% formalin at an intraventricular pressure of 20 mm Hg in situ, explanted, weighed, cut into 1-mm transverse sections, and photographed for morphometric measurements. LV chamber volume, LV diameter, LV surface area, and scar area were measured by planimetry with image analysis software (Scion Image, National Institutes of Health Software, Bethesda, Md). The percentage of infarcted LV was calculated by dividing the surface area of the scar by the total surface area of the LV transverse sections. After staining with picrosirius red, total collagen content in the interventricular septum, remote from the zone of infarction, was determined by laser scanning confocal microscopy.

**Statistical Analysis**

Data are presented as mean±SEM. Analyses of data recorded at 1 time point were performed by 2-tailed unpaired Student *t* tests that assumed unequal variance. Analyses of data recorded at several time points for 1 group were performed by 1-way ANOVA to evaluate the effect of time; if significant, Bonferroni correction for multiple comparisons was applied when post hoc analysis between different time points was carried out. Analyses of data recorded at several time points for 2 groups (*Ptges*−/− mice, *Ptges*−/− mice) were performed by 2-way ANOVA (to evaluate the effect of group, time, and group–time interactions); if significant, Bonferroni correction for multiple comparisons was applied when post hoc analysis between different time points or between different groups at the same time point was carried out. Survival after coronary ligation was assessed by a log-rank test. A value of *P*<0.05 was accepted as statistically significant. In total, 119 *Ptges*−/− mice and 129 *Ptges*−/− mice were used in this study.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

**Online-Only Data Supplement**

We describe the methodology used for the measurement of PG levels in the expanded Methods and Results sections in the online-only Data Supplement. The ratio of phosphorylated to total AKT, glyco-
gen synapase kinase (GSK), jun N-terminal kinase-1 (JNK1), protein kinase C (PKC), or PKC and the expression of Nab1 in cardiomyocytes from the LV remote from the infarct is presented in online-only Data Supplement Figure I. Immunohistochemical analysis of COX-2 and mPGES-1 in the LV remote from the infarct is presented in online-only Data Supplement Figure II. PG levels and the expression of PG biosynthetic enzymes in the LV remote from the infarct and in the infarct and peri-infarct tissue are presented in online-only Data Supplement Figures III and IV, respectively. The in vivo 2-dimensional echocardiographic assessment of cardiac structure and function of hearts from Ptges/−/− and Ptges/−/− mice before and 7 and 28 days after coronary artery ligation is presented in online-only Data Supplement Table I.

Results

Ptges/−/− Mice Develop LV Dilation and Impaired LV Contractile Function After MI
We did not identify any differences in cardiac mass, dimensions, or function (Figure 1, the Table, and online-only Data Supplement Table I) or cardiomyocyte morphology (Figure 2A through 2G) between noninjured adult Ptges/−/− and Ptges/−/− mice. To evaluate the effect of Ptges gene deletion on the cardiac response to acute MI, we subjected 8- to 12-week-old Ptges/++/++ and Ptges/−/− mice to left anterior descending coronary artery ligation. Survival of Ptges/++/++ and Ptges/−/− mice after MI was similar (Figure 1A). The decreases in LV fractional shortening (Figure 1B) observed in Ptges/++/++ and Ptges/−/− mice 7 days after coronary ligation were comparable and were consistent with the development of a significant MI in these mice. Between 7 and 28 days after MI, fractional shortening (Figure 1B) did not change in Ptges/++/++ mice but decreased significantly in Ptges/−/− mice 28 days after MI. LV volume was 54%...
Eccentric Hypertrophy After MI

similar in these mice 3, 7, and 28 days after coronary ligation but the wet-to-dry weight ratio of pulmonary tissue was attenuated in Ptges−/− mice 28 days after MI. In addition, higher LV volumes were required to generate equivalent LV pressures throughout the cardiac cycle in Ptges−/− versus Ptges+/+ mice after MI (Figure 1H). No differences in the percentage of the LV that was infarcted (Figure 1I and 1J), extent of extracellular matrix degradation remote from the zone of infarction (a feature of ventricular remodeling after MI; Figure 1K and 1L), or fraction of apoptotic versus total cells (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling assay; data not shown) were noted between Ptges+/+ and Ptges−/− mice after MI. After MI, Ptges−/− mice manifested an increase in LVEDP that was not observed in Ptges+/+ mice (Figure 1M), but the wet-to-dry weight ratio of pulmonary tissue was similar in these mice 3, 7, and 28 days after coronary ligation (Figure 1N).

Cardiomyocytes in Ptges−/− Mice Undergo Eccentric Hypertrophy After MI

We found that cardiomyocytes in the LV remote from the zone of infarction underwent hypertrophy in both Ptges+/+ and Ptges−/− mice after MI, but the extent of hypertrophy was attenuated in Ptges−/− mice (Figure 2A and 2B). To study the geometry of individual cardiomyocytes, we enzymatically digested hearts before and 28 days after MI and identified rod-shaped cardiomyocytes by immunostaining with anti-troponin I (Figure 2C). The increase in cardiomyocyte surface area after MI was significantly greater in Ptges+/+ than Ptges−/− mice (Figure 2D). The increases in length of cardiomyocytes from Ptges+/+ and Ptges−/− mice after MI were similar (Figure 2E). In contrast, the width of cardiomyocytes from Ptges+/+ mice did not change after MI, whereas the width of cardiomyocytes from Ptges−/− mice decreased (Figure 2F), thereby resulting in a significantly greater length-to-width ratio of cardiomyocytes from Ptges−/− than Ptges+/+ mice 28 days after MI (Figure 2G). These observations confirm that cardiomyocytes in Ptges−/− mice underwent growth by eccentric hypertrophy after MI.

Expression of Molecular Markers of Cardiomyocyte Hypertrophy in Ptges+/+ and Ptges−/− Mice After MI

The genes encoding atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and β- and α-myosin heavy chain (MHC) are fetal cardiac genes that are re-expressed when cardiomyocytes hypertrophy. Cardiomyocytes in the LV remote from the zone of infarction expressed significantly more ANP, BNP, β-MHC, and α-MHC mRNA (Figure 2H through 2K) in Ptges−/− compared with Ptges+/+ mice 3 and 7 days after coronary ligation. Twenty-eight days after coronary ligation, β-MHC mRNA levels were higher in the LV of Ptges−/− than Ptges+/+ mice, whereas α-MHC mRNA levels remained higher in Ptges−/− than Ptges+/+ mice.

Table. Morphometric and Invasive Hemodynamic Analyses of Hearts From Ptges+/+ and Ptges−/− Mice Before and 28 Days After Left Coronary Artery Ligation

<table>
<thead>
<tr>
<th></th>
<th>Ptges+/+ (n=9)</th>
<th>Ptges−/− (n=11)</th>
<th>Ptges+/+ (n=9)</th>
<th>Ptges−/− (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass, g</td>
<td>20.73±1.06</td>
<td>18.99±0.59</td>
<td>21.78±0.81</td>
<td>20.11±0.57</td>
</tr>
<tr>
<td>Cardiac mass, g</td>
<td>131±6.2</td>
<td>132±6.0</td>
<td>163±8.8</td>
<td>167±4.5</td>
</tr>
<tr>
<td>LV diameter, cm</td>
<td>0.32±0.033</td>
<td>0.32±0.023</td>
<td>0.38±0.028</td>
<td>0.45±0.015†‡</td>
</tr>
<tr>
<td>Septum diameter, cm</td>
<td>0.13±0.023</td>
<td>0.11±0.013</td>
<td>0.050±0.002†‡</td>
<td>0.046±0.004†‡</td>
</tr>
<tr>
<td>LV volume/body mass</td>
<td>1.08±0.10</td>
<td>1.18±0.05</td>
<td>1.57±0.23†</td>
<td>2.61±0.13†‡</td>
</tr>
<tr>
<td>LV diameter/body mass</td>
<td>0.16±0.02</td>
<td>0.16±0.01</td>
<td>0.18±0.02</td>
<td>0.23±0.01†‡</td>
</tr>
<tr>
<td>Septum diameter/body mass ×10³</td>
<td>6.3±0.1</td>
<td>5.7±0.7</td>
<td>2.3±0.1†</td>
<td>2.3±0.2†</td>
</tr>
<tr>
<td>Cardiac mass/body mass ×10³</td>
<td>6.3±0.2</td>
<td>6.7±0.2</td>
<td>8.2±0.5†</td>
<td>8.8±0.1†</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>358±31</td>
<td>373±21</td>
<td>425±14.8</td>
<td>435±12.8</td>
</tr>
<tr>
<td>LV end-systolic pressure, mm Hg</td>
<td>109±7.3</td>
<td>109±3.3</td>
<td>97.4±2.2</td>
<td>91.6±3.3†‡</td>
</tr>
<tr>
<td>LV end-systolic volume, μL</td>
<td>*</td>
<td>*</td>
<td>27.1±2.4</td>
<td>38.6±3.4‡</td>
</tr>
<tr>
<td>LV end-diastolic volume, μL</td>
<td>*</td>
<td>*</td>
<td>33.8±3.9</td>
<td>45.4±3.8‡</td>
</tr>
<tr>
<td>Stroke volume, μL</td>
<td>*</td>
<td>*</td>
<td>8.4±0.8</td>
<td>8.5±0.9</td>
</tr>
<tr>
<td>Cardiac output, μL/min</td>
<td>*</td>
<td>*</td>
<td>358±346</td>
<td>373±439</td>
</tr>
<tr>
<td>Arterial elastance, mm Hg/μL</td>
<td>*</td>
<td>*</td>
<td>12.5±1.1</td>
<td>11.9±1.5</td>
</tr>
<tr>
<td>Preload-adjusted maximal power, mW/μL²</td>
<td>*</td>
<td>*</td>
<td>52.7±11.7</td>
<td>15.4±1.9†‡</td>
</tr>
</tbody>
</table>

LCA indicates left coronary artery. Hearts were subjected to hyperkalemic arrest, perfusion fixed at systemic pressure in situ, explanted, fixed an additional 24 hours, and then processed for morphometric analysis. Alternatively, hemodynamics were assessed with a 1.4F catheter advanced from the right carotid artery into the LV.

*Before coronary ligation, significant variability in measurements based on calculated volumes was observed, possibly because of the relatively small size of the LV in mice not subjected to MI compared with the size of the micromanometer and conductance catheter. Therefore, these values are not reported. However, morphometric and 2-dimensional echocardiographic analyses documented similar LV volume, cardiac dimensions, and fractional shortening in Ptges+/+ and Ptges−/− mice before LCA ligation.

†P<0.05, day 0 vs 28, Ptges+/+ or Ptges−/− mice; ‡P<0.05, Ptges+/+ vs Ptges−/− mice at 0 or 28 days after LCA ligation.
Degousee et al Deletion of mPGES-1 Impairs LV Remodeling After MI

Differential Activation of Signaling Cascades That Regulate Cardiomyocyte Hypertrophy in Ptges+/+ and Ptges−/− Mice After MI

Next, we evaluated signaling pathways that have been implicated in the regulation of cardiomyocyte hypertrophy. The ratios of activated (phosphorylated) to total of JNK2, extracellular signal-regulated kinase (ERK)-1, ERK2, GSKβ, JNK1, PKCa, or PKCe or in the expression of Nab1, all of which have been implicated in the regulation of cardiomyocyte hypertrophy, were identified between Ptges+/+ and Ptges−/− mice after MI (online-only Data Supplement Figure Ia through If). Thus, mPGES-1 modulates multiple signaling pathways that regulate cardiomyocyte hypertrophy.

mPGES-1 Is Expressed in Inflammatory Cells in the LV After MI

We then evaluated the expression of selected PG biosynthetic enzymes in the heart. In the LV remote from the infarct, COX-2 mRNA increased in Ptges+/+ and Ptges−/− mice, and mPGES-1 mRNA increased transiently in Ptges+/+ mice (Figure 4A and 4B), but no COX-2 or mPGES-1 protein was identified in this part of the heart up to 28 days after MI (online-only Data Supplement Figure II). In the infarct and peri-infarct tissue, the increase in COX-2 mRNA was greater in Ptges−/− than Ptges+/+ mice, whereas mPGES-1 mRNA increased progressively after MI in Ptges+/+ mice (Figure 4C and 4D). COX-2 protein was identified in inflammatory cells in and adjacent to the infarct in Ptges+/+ (Figure 4E) and Ptges−/− mice (Figure 4G) 3 and 7 but not 28 days after MI. mPGES-1 protein also was identified in inflammatory cells in and adjacent to the infarct in Ptges+/+ mice 3 and 7 days and, to a lesser extent, 28 days after MI (Figure 4F). No mPGES-1 protein was identified in the heart of Ptges−/− mice (Figure 4H), and no COX-2 or mPGES-1 protein was identified in cardiomyocytes from Ptges+/+ or Ptges−/− mice at any time point after coronary ligation (Figure 4E through 4H). Therefore, in Ptges−/− mice, coronary ligation leads to the recruitment of inflammatory cells to the infarct and peri-infarct zones that express COX-2 and mPGES-1 protein.

mPGES-1 Regulates PGE2 Biosynthesis in the LV After MI

Ptges+/+ and Ptges−/− mice had similar levels of PGE2 (Figure 4I), PGD2, thromboxane B2 (TxB2; a TxA2 metabolite), PGF2α, and 6k-PGF1a (a PGI2 metabolite) in the LV before MI (online-only Data Supplement Figure IIIa through IIIId). Three and 7 days after MI, PGE2 levels were significantly higher in the LV (Figure 4I) and infarct (which includes the peri-infarct zone; Figure 4J) of Ptges−/− than Ptges+/+ mice. Interestingly, levels of PGE2 in the infarct and peri-infarct zones of Ptges+/+ mice 3 and 7 days after MI were ~8-fold higher than the levels of PGE2 in the LV remote from the infarct. Twenty-eight days after MI, levels of PGE2 remained above baseline levels in the LV and infarct but were not statistically different between Ptges+/+ and Ptges−/− mice. The levels of PGD2, TxB2, PGF2α, and 6k-PGF1a and the expression of PGD2 synthase, TxA2 synthase, and PGI2 synthase mRNA in the LV remote from the infarct (online-only Data Supplement Figure IVa through IVd and IVh through IVj) increased after MI, but no differences were found in the levels of these PGs or the expression of their respective terminal synthases between Ptges+/+ and Ptges−/− mice 3, 7, and 28 days after MI. Therefore, coronary ligation leads to a selective increase in mPGES-1–catalyzed PGE2 biosynthesis in the heart.
Discussion

After acute MI, viable cardiomyocytes remote from the zone of infarction undergo hypertrophy, a critical step in postinfarction LV remodeling. This adaptation is necessary to sustain cardiac function and to prevent heart failure, a leading cause of death after MI. Our findings provide direct evidence that a lack of mPGES-1 leads to eccentric hypertrophy of viable cardiomyocytes, LV dilation, and impaired LV systolic and diastolic function after acute MI. Although Ptges+/− mice also develop higher LVEDP than Ptges+/+ mice 28 days after MI, Ptges+/− and Ptges+/+ mice do not develop pulmonary edema; the wet-to-dry weight ratios of lungs from these mice after MI are similar. This finding and the observation that the percentage of infarcted LV was similar in Ptges+/+ and Ptges+/− mice may explain why no difference was found in the survival of these mice after MI. The failure of Ptges−/− mice to develop pulmonary edema and heart failure after MI may be due to the fact that relatively young mice (8 weeks old) do not develop heart failure even after large (up to 28% of the LV) infarctions.

The relatively low heart rate of mice undergoing echocardiographic assessment (200 bpm to 300 bpm; online-only Data Supplement Table I) may be a manifestation of the anesthesia used for these studies and is below the resting heart rate of these mice (350 bpm to 450 bpm; the Table). Importantly, the measurements of LV dimensions and LV contractile function obtained by 2 independent methods, morphometric analysis of myocardium perfusion fixed in situ and invasive hemodynamic assessment, are consistent with the echocardiographic data. These findings support the conclusion that the echocardiographic measurements are accurate, the relatively low heart rate observed during echocardiographic assessment notwithstanding.

Inflammatory Cells Are the Likely Source of PGE2 Biosynthesis in the LV After MI

The cell type that produces PGE2 in the heart after MI has not been identified. However, although cardiomyocytes, cardiac fibroblasts, and inflammatory cells can express mPGES-1 and synthesize PGE2 in vitro, inflammatory cells are the likely source of PGE2 biosynthesis in the LV because inflammatory cells were the only cells in the heart that express mPGES-1 protein after MI (see Figure 4E through 4H). Because the increase in COX-2 and mPGES-1 protein expression after MI is confined to inflammatory cells in the infarct and peri-infarct regions, we postulate that PGE2 produced by inflammatory cells in adjacent to the infarct diffuses to and regulates the hypertrophy of cardiomyocytes.
remote from the zone of infarction. Eccentric hypertrophy of viable cardiomyocytes in Ptges−/− mice remote from the zone of infarction may be due to a relative deficiency of mPGES-1–catalyzed PGE2 biosynthesis because PGE2 levels in this part of the LV are 4-fold lower in Ptges−/− mice after MI and PGE2 induces cardiomyocyte hypertrophy in vitro.4,14

mPGES-1 Catalyzes PGE2 Biosynthesis in the Heart After MI
mPGES-1 mRNA expression and protein synthesis increase in the infarct zone of Ptges−/− mice after MI. mPGES-2 and cytosolic PGE2 synthase (cPGES) mRNA also are expressed in the infarct and in the LV remote from the infarct after MI (online-only Data Supplement Figures IIIf, IIIf, IVf, and IVg, respectively). Unexpectedly, cPGES mRNA levels in the infarct and LV remote from the infarct were transiently higher in Ptges−/− than in Ptges+/+ mice after coronary ligation. Although mPGES-1, mPGES-2, and cPGES are all expressed in the LV after MI, the observation that PGE2 levels were 4-fold higher in the LV and infarct of Ptges−/− than of Ptges+/+ mice 3 and 7 days after coronary ligation provides direct evidence that mPGES-1 catalyzes the majority of PGE2 biosynthesis in the heart up to 7 days after MI. PGE2 levels remain elevated in Ptges−/− mice 28 days after MI, despite the fact that mPGES-1 protein levels in the infarct are low at this time. Therefore, it is possible that cPGES and/or mPGES-2, both with mRNA species that were identified in the LV and infarct after MI, also could contribute to PGE2 biosynthesis 28 days after MI in Ptges−/− (and Ptges+/+) mice.

mPGES-1 Modulates Signaling Cascades That Regulate Cardiomyocyte Hypertrophy After MI
Multiple signaling molecules have been implicated in the pathophysiology of eccentric myocardial hypertrophy. Thus, the absence of telomerase,27 Kruppel-like factor 15,28 the transcription factor GATA4,29 or focal adhesion kinase (in the ventricles)30 leads to eccentric cardiomyocyte hypertrophy. Because PGE2 increases GATA-4 binding activity,31 de-
creased PGE2 levels in the LV of Ptges−/− mice could attenuate GATA4 activation and promote eccentric cardiomyocyte hypertrophy after MI. Activation of the mitogen-activated protein kinase kinase 5 (MEK5)-ERK5 signaling cascade also leads to eccentric hypertrophy of rat neonatal ventricular cardiomyocytes, and transgenic expression of MEK5 results in eccentric cardiomyocyte hypertrophy in the absence of apoptosis or fibrosis,32 a finding reminiscent of the eccentric cardiomyocyte hypertrophy and lack of fibrosis or apoptosis observed in Ptges−/− mice after MI. The observation that the activity of multiple signaling molecules, including JNK2, ERK1, ERK2, GSKα, and PKC8, and the activity of the calcineurin-NFAT pathway are increased in cardiomyocytes in Ptges−/− compared with Ptges+/+ mice suggests that multiple signaling cascades may contribute to the eccentric cardiac myocyte hypertrophy observed in Ptges−/− mice after MI. Because Jnk2−/− mice and transgenic mice expressing dominant-negative JNK1 and JNK2 exhibit enhanced myocardial growth,33 JNK2 activation may attenuate cardiomyocyte hypertrophy in Ptges−/− mice after MI.

mPGES-1 Attenuates Prohypertrophic Fetal Cardiac Gene Expression After MI
Expression of the genes encoding α-MHC, β-MHC, ANP, and BNP is increased when ventricular myocytes hypertrophy.20 α-MHC, which is upregulated in the heart after birth, has high ATPase activity, whereas β-MHC has low ATPase activity. Three days after MI, β-MHC expression increases 5-fold after MI in Ptges−/− mice but does not change in Ptges+/+ mice, whereas α-MHC expression does not change in Ptges−/− mice but decreases 50% in Ptges+/+ mice. The relative increase in β-MHC compared with α-MHC expression in the LV of Ptges−/− mice after MI, a molecular signature of pathologic cardiac remodeling, may lead to a reduction in myofibrillar ATPase activity, reduced shortening velocity of cardiac myofibers, and eventual contractile dysfunction.24 Because alterations in fetal gene re-expression occur before the onset of eccentric cardiomyocyte hypertrophy,30 the increased ratio of β-MHC to α-MHC expression may be predictive of subsequent LV dysfunction in Ptges−/− mice after MI. Expression of 2 other markers of ventricular hypertrophy, the natriuretic peptides ANP and BNP, was higher in the LV of Ptges−/− than Ptges+/+ mice 3 and 7 days after MI. Because ANP attenuates cardiomyocyte hypertrophy in vitro25 and BNP inhibits cell growth in the heart,36 ANP and BNP may function as antihypertrophic factors37 that inhibit cardiomyocyte hypertrophy and attenuate adaptive LV remodeling after MI in Ptges−/− mice. Calcineurin activation,38 ERK1/ERK2-mediated activation of the transcription factor GATA-4,39 and/or increased mechanical strain in the wall of the dilated LV40 could account for the increased BNP mRNA expression in Ptges−/− mice after MI.

Possible Effect of Pharmacological Inhibition of mPGES-1 on Postinfarction LV Remodeling
The increased risk of MI and stroke41,42 and increased mortality after MI43 in patients taking selective COX-2 inhibitors are proposed to be due to an imbalance of prothrombotic eicosanoids (increased TxA2) and anti-thrombotic eicosanoids (decreased PGI2).44 We noted that targeted deletion of the gene encoding mPGES-1 does not alter the levels of TxA2 or PGI2 in the heart after MI. Therefore, pharmacological inhibition of mPGES-1 may not be associated with the perturbations in TxA2 and PGI2 metabolism that increase the risk of arterial thrombosis in patients taking COX-2 inhibitors but may lead to eccentric cardiomyocyte hypertrophy and compromise LV function and LV remodeling after acute MI. This hypothesis should be interpreted with caution because the effect of individual gene deletions on cardiac physiology in mice may not be observed in patients taking pharmacological inhibitors of the corresponding gene product. For example, the diffuse cardiac fibrosis noted in 50% of mice lacking COX-245 has not been identified in patients taking selective COX-2 inhibitors. In addition, COX-2 inhibition was shown to be cardioprotective in mice46,47 but led to adverse LV remodeling and LV rupture in a porcine MI model.48 These observations underscore the need for caution in extrapolating findings in mice to humans without studies in larger animals first.

Sources of Funding
This work was supported by grants from the Canadian Institutes of Health Research (53297 and 37778 to Drs Rubin and Liu, respectively), Heart and Stroke Foundation of Canada (NA-4387 to Drs Rubin and Degousee; Tx-5294 to Dr Li), Canadian Heart Failure Network (CHFNET) and Tailored Advanced Collaborative Training In Cardiovascular Science for Research Fellows (TACTICS) Partnership Program grants (Dr Liu), Physicians of Ontario through the PSI Foundation (01-12 and 98-049 to Drs Rubin and Lindsay, respectively), German Research Association (GE 695/2-2 and Excellence Cluster Cardio-Pulmonary System to Dr Geisslinger), Swedish Medical Research Council (2004–5259 to Dr Jakobsson), King Gustaf V 80 Years Foundation (Dr Jakobsson), Swedish Rheumatism Association (Dr Jakobsson), Erik och Edith Fernstroms Foundation for Medical Research (Dr Jakobsson), Börje Dahlin Foundation (S.C. Pawelzik), and the Karolinska Institutet (Dr Jakobsson). Dr Li is a career investigator of the Heart and Stroke Foundation of Canada. Dr Rubin is a Wylie Scholar in Academic Vascular Surgery, Pacific Vascular Research Foundation, San Francisco.

Disclosures
Dr Audoly has stock ownership or options in Pfizer Inc and has received fees (or fees are pending) for patents, licenses, or licensing. Dr Jakobsson has received consultancies from Pfizer Inc. The other authors report no conflicts.

References
Degousee et al. Deletion of mPGES-1 Impairs LV Remodeling After MI


CLINICAL PERSPECTIVE
Pharmacological inhibition of microsomal prostaglandin E2 synthase-1 (mPGES-1) has been proposed as an alternative to inhibition of cyclooxygenase-2 in the management of patients with pain and inflammatory diseases. Here, we examined the role of prostaglandin E2 (PGE2) in postinfarction cardiac remodeling by taking advantage of homozygote mPGES-1 knockout mice, which have low basal and induced PGE2 synthesis. Contrary to our original hypothesis, we found that deletion of mPGES-1 did not affect the size of the infarct after coronary ligation. However, the mPGES-1 knockout animals had worse left ventricular systolic and left ventricular diastolic function, more ventricular dilation, and markedly attenuated cardiomyocyte hypertrophy in the region remote from the infarction compared with wild-type mice. Coupled with the observation that the bulk of PGE2 biosynthesis in the infarct was carried out by inflammatory cells, these findings suggest that diffusion of PGE2 from the infarct and peri-infarct regions influences the hypertrophy of cardiomyocytes remote from the infarction. These data imply that hypertrophy in the region remote from the infarct is not regulated purely by mechanical forces but also by inflammatory mediators such as PGE2. The potential clinical importance of these observations is significant because the millions of patients who previously took cyclooxygenase-2 inhibitors are potential candidates to take pharmacological inhibitors of mPGES-1, which are currently in development. Our findings emphasize the importance of carefully evaluating cardiac function in patients at risk for myocardial infarction who are treated with agents that selectively block PGE2 biosynthesis, which have been proposed to have less cardiovascular toxicity than inhibitors of cyclooxygenase-2.
Microsomal Prostaglandin E2 Synthase-1 Deletion Leads to Adverse Left Ventricular Remodeling After Myocardial Infarction
Norbert Degousee, Shafie Fazel, Denis Angoulvant, Eva Stefanski, Sven-Christiant Pawelzik, Marina Korotkova, Sara Arab, Peter Liu, Thomas F. Lindsay, Sun Zhuo, Jagdish Butany, Ren-Ke Li, Laurent Audoly, Ronald Schmidt, Carlo Angioni, Gerd Geisslinger, Per-Johan Jakobsson and Barry B. Rubin

Circulation. 2008;117:1701-1710; originally published online March 17, 2008;
doi: 10.1161/CIRCULATIONAHA.107.749739

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/117/13/1701

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2008/03/21/CIRCULATIONAHA.107.749739.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/
Online Supplemental Figure 2.
Online Supplemental Methods

**Microsomal Prostaglandin E₂ Synthase-1 Deletion leads to Adverse Left Ventricular Remodeling After Myocardial Infarction.**

Norbert Degousee et al.

**Detection and Quantification of Prostaglandin Metabolites in Cardiac Samples**

**Prostanoid Extraction from Cardiac Samples**

Eicosanoids were extracted from the cardiac tissue samples by liquid–liquid extraction. 125 µl PBS, 20 µl methanol and 20 µl internal standard (in methanol) were added per 25 mg tissue each. Deuterated isomers of the analytes PGE₂, PGD₂, PGF₂α, TXB₂ and 6-keto-PGF₁α were used as internal standard. Samples were homogenised on wet ice, and 600 µl ethyl acetate was subsequently added to the tissue homogenate and thoroughly mixed for 30 s. After centrifugation at 10,000 × g, the organic phase was collected and extraction with ethyl acetate was repeated once. Samples for standard curves of all analytes were extracted similarly from PBS. The solvent was then evaporated under a gentle stream of nitrogen at a temperature of 45 °C. The residue was finally dissolved in 50 µl acetonitrile/water/formic acid (20:80:0.0025, v/v/v).

**LC-MS/MS analysis**

Prostanoid analytes were separated on a Synergi Hydro-RP column and precolumn (150mm × 2mm i.d., 4 µm particle size and 80 Å pore size from Phenomenex, Aschaffenburg, Germany) during a 40 min gradient run at a flow rate of 0.4 ml/min (see table). Mobile phase was acetonitrile/formic acid (100:0.1, v/v) as solvent A and water/formic acid (100:0.1, v/v) as solvent B. After injection of 45 µl of the sample, the gradient started from 20% solvent B
to reach 38% solvent B during 27 min. Within two steps of 2 min each, the percentage of solvent B was raised first to 45% and finally to 90%, where it was held for 3 min. Within the next 2 min, it was then shifted back to 20% solvent B and held there for an additional 4 min in order to equilibrate the column for the next sample.

The API 4000 triple quadrupole tandem mass spectrometer was operated in negative ion mode. Collision gas thickness was set at 4 instrument units. Multiple reaction monitoring (MRM) was used for quantification with Analyst Software V1.4 (Applied Biosystems, Darmstadt, Germany). Mass transitions used for quantification of the respective substances were m/z 351.2 → m/z 271.2 for PGE$_2$ and for PGD$_2$, m/z 353.2 → m/z 309.1 for PGF$_{2\alpha}$, m/z 369.2 → m/z 168.9 for TXB$_2$, and m/z 369.3 → m/z 163.2 for 6-keto-PGF$_{1\alpha}$. 
## LC-MS/MS Mobile Phase Gradient

<table>
<thead>
<tr>
<th>Step</th>
<th>total time (min)</th>
<th>flow rate (µl/min)</th>
<th>solvent A (%)</th>
<th>solvent B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>400</td>
<td>80.0</td>
<td>20.0</td>
</tr>
<tr>
<td>1</td>
<td>27</td>
<td>400</td>
<td>62.0</td>
<td>38.0</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>400</td>
<td>55.0</td>
<td>45.0</td>
</tr>
<tr>
<td>3</td>
<td>31</td>
<td>400</td>
<td>10.0</td>
<td>90.0</td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>400</td>
<td>10.0</td>
<td>90.0</td>
</tr>
<tr>
<td>5</td>
<td>36</td>
<td>400</td>
<td>80.0</td>
<td>20.0</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>400</td>
<td>80.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Days post left coronary ligation</td>
<td>In vivo 2-D echocardiography</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-----------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ptges&lt;sup&gt;++&lt;/sup&gt; (n = 8)</td>
<td>Ptges&lt;sup&gt;−/−&lt;/sup&gt; (n = 9)</td>
<td>Ptges&lt;sup&gt;++&lt;/sup&gt; (n = 8)</td>
<td>Ptges&lt;sup&gt;−/−&lt;/sup&gt; (n = 9)</td>
</tr>
<tr>
<td>LV area, end systole (cm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>0.033 ± 0.001</td>
<td>0.033 ± 0.001</td>
<td>0.050 ± 0.004&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.058 ± 0.004&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LV area, end diastole (cm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>0.092 ± 0.003</td>
<td>0.091 ± 0.002</td>
<td>0.11 ± 0.004&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.12 ± 0.004&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LVESD (cm)</td>
<td>0.131 ± 0.004</td>
<td>0.129 ± 0.003</td>
<td>0.218 ± 0.013&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.238 ± 0.006&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LVEDD (cm)</td>
<td>0.26 ± 0.006</td>
<td>0.26 ± 0.003</td>
<td>0.36 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.37 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Anterior wall (cm)</td>
<td>0.077 ± 0.002</td>
<td>0.079 ± 0.002</td>
<td>0.075 ± 0.002</td>
<td>0.074 ± 0.001</td>
</tr>
<tr>
<td>Posterior wall (cm)</td>
<td>0.075 ± 0.002</td>
<td>0.078 ± 0.002</td>
<td>0.073 ± 0.003</td>
<td>0.073 ± 0.003</td>
</tr>
<tr>
<td>Heart Rate</td>
<td>232 ± 11</td>
<td>223 ± 21</td>
<td>264 ± 28</td>
<td>225 ± 15</td>
</tr>
<tr>
<td>LV mass (g)</td>
<td>0.058 ± 0.004</td>
<td>0.056 ± 0.002</td>
<td>0.088 ± 0.003&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.093 ± 0.005&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LV mass / body mass (×10&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>2.8 ± 0.1</td>
<td>3.0 ± 0.1</td>
<td>4.4 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.2 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LVEDD / body mass</td>
<td>0.13 ± 0.006</td>
<td>0.14 ± 0.005</td>
<td>0.18 ± 0.009&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.21 ± 0.005&lt;sup&gt;a,d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Supplemental Table 1. *In vivo* 2-dimensional echocardiographic assessment of cardiac structure and function of hearts from Ptges<sup>++</sup> and Ptges<sup>−/−</sup> mice before and 7 and 28 days after left coronary artery ligation. LVEDD, left ventricular end diastolic diameter; LVESD, left ventricular end systolic diameter.<sup>a</sup>p < 0.05, t = 0 vs. t = 7, Ptges<sup>++</sup> or Ptges<sup>−/−</sup>, <sup>b</sup>p < 0.05, t = 7 vs. t = 28, Ptges<sup>++</sup> or Ptges<sup>−/−</sup>, <sup>c</sup>p < 0.05, t = 0 vs. t = 28, Ptges<sup>++</sup> or Ptges<sup>−/−</sup>, <sup>d</sup>p < 0.05, Ptges<sup>++</sup> vs. Ptges<sup>−/−</sup> at t = 0, 7 or 28 days. 2-way ANOVA, followed by Bonferroni’s correction for multiple comparisons.