Protease-Activated Receptor 2 Deficiency Reduces Cardiac Ischemia/Reperfusion Injury

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Objective—To investigate the effect of protease-activated receptor (PAR) 2 deficiency on ischemia/reperfusion (I/R) injury—induced infarct size, inflammation, heart remodeling, and cardiac function.

Methods and Results—PAR-2 signaling enhances inflammation in different diseases. The effect of PAR-2 deficiency in cardiac I/R injury is unknown. PAR-2−/− mice and wild-type littermates were subjected to 30 minutes of ischemia and up to 4 weeks of reperfusion. Infarct size, oxidative/nitrative stress, phosphorylation of mitogen-activated protein kinases, and inflammatory gene expression were assessed 2 hours after reperfusion. Changes in heart size and function were measured by echocardiography up to 4 weeks after reperfusion. Infarct size was significantly reduced in hearts of PAR-2−/− mice compared with wild-type littermates. In addition, oxidative/nitrative stress, phosphorylation of mitogen-activated protein kinase, and expression of proinflammatory genes were significantly attenuated in injured hearts of PAR-2−/− mice. Finally, PAR-2−/− mice were protected from postinfarction remodeling and showed less impairment in heart function compared with wild-type litters up to 4 weeks after I/R injury.

Conclusion—PAR-2 deficiency reduces myocardial infarction and heart remodeling after I/R injury. (Arterioscler Thromb Vasc Biol. 2010;30:00-00.)

Key Words: cardiac remodeling ■ inflammation ■ myocardial infarction ■ oxidative/nitrative stress ■ protease-activated receptor 2

Protease-activated receptors (PARs) are a family of 7 transmembrane domain G protein–coupled receptors activated by proteolytic cleavage.1 The PAR family consists of 4 members: PAR-1 to PAR-4. PAR-2 is activated by several proteases, including trypsin, mast cell tryptase, matriptase, and the coagulation proteases factor VIIa (FVIIa) and FXa.1–4 PAR-2 can also be activated by synthetic agonist peptides corresponding to the tethered ligand sequence.1 The activation of PAR-2 leads to an elevation of intracellular Ca2+ and stimulation of multiple intracellular signaling pathways, including mitogen-activated protein kinase (MAPK) pathways.1,5,6

PAR-2–dependent signaling plays an important role in many animal models of inflammatory diseases. Recent studies demonstrated a role of PAR-2 in leukocyte rolling, arthritis, inflammatory pain, allergic inflammation, and skin disorders. In contrast, other studies support a protective role of PAR-2 in airway inflammation, ischemic brain injury, and influenza virus infection. Furthermore, in a mouse model of Alzheimer disease, PAR-2 protects neurons from β-amyloid–induced toxicity; however, its activation in microglia cells led to neurotoxicity.16 These studies indicate that PAR-2 activation on different cell types may have detrimental or protective effects in different disease models.

Myocardial infarction (MI) is 1 of the leading causes of mortality and morbidity in the Western world.17 Coronary vessel occlusion and subsequent ischemia result in myocardial cell death.18 Early restoration of blood flow within the coronary vessels is necessary to provide oxygen and nutrients to the ischemic area. However, reperfusion itself contributes to injury of the heart by initiating a local inflammatory response, which leads to further myocardial damage.17,18 This is known as ischemia/reperfusion (I/R) injury. With time, areas of the initial infarct are replaced by collagen-rich scar tissue. The short-term loss of myocardial cells results in abnormal loading conditions that involve the border zone of infarction and the remote myocardium. These abnormal loading conditions lead to hypertrophy, aberrant cardiac remodeling, and, ultimately, heart failure.17,18

PAR-2 is expressed by numerous cell types within the cardiovascular system. Functional PAR-2 expression has been demonstrated on vascular endothelium, smooth muscle...
cells, and cardiomyocytes, but not on cardiac fibroblasts. In addition, PAR-2 is expressed on activated neutrophils. In vitro studies demonstrated that PAR-2 activation on cultured cardiomyocytes results in a series of molecular and morphological changes that lead to hypertrophic growth. Furthermore, several recent articles reported that activation of PAR-2 with a PAR-2 agonist peptide has a beneficial effect in both ex vivo and in vivo models of cardiac I/R injury. The protective mechanism involved vasodilation of coronary vessels, which was mediated by activation of PAR-2 on endothelial cells. However, the effect of global deficiency of PAR-2 on MI has not been investigated.

In this study, we investigated the role of PAR-2 in cardiac I/R injury. We found that PAR-2 deficiency resulted in a significant reduction in inflammation and infarct size, as well as attenuation of pathological heart remodeling.

**Methods**

**Human Study**

PAR-1 and PAR-2 mRNA levels were determined in heart samples from the left ventricular (LV) free wall (toward the apex) of 5 male patients in end-stage HF of ischemic origin (mean age, 57 ± 9 years) at LV assist device placement. Nonfailing tissue was obtained from the LV free wall (toward the apex) of 5 male nonfailing organ donor hearts rejected for transplantation because of physical incompatibilities (mean age, 48 ± 7 years). LV tissue obtained from surgery was immediately frozen in liquid nitrogen and stored at −80°C. All surgical procedures and tissue harvesting were performed with informed patient consent and are concordant with National Institutes of Health and University of Rochester institutional review board guidelines.

**Mice**

PAR-2+/− mice were backcrossed onto a C57Bl/6J background and intercrossed to generate PAR-2−/− and wild-type (WT) littermate mice. Male mice, aged 8 to 12 weeks, were used for experiments. All experiments were approved by the Animal Care and Use Committees of the different institutions and complied with National Institutes of Health guidelines.

**Cardiac I/R Injury Model**

For the short-term I/R model (30 minutes of ischemia and 2 hours of reperfusion), the surgical protocol and infarct size determination were performed as previously described, with some modifications. Briefly, intraperitoneal injection of pentobarbital, 100 mg/kg (Abbott Laboratories, Abbott Park, Ill), was used for anesthesia. Mice were intubated orally to provide artificial ventilation (0.3-mL tidal volume, 120 breaths/min). The left anterior descending coronary artery was occluded with a 7-0 silk suture (US Surgical Corp, Norwalk, Conn) passed through PE tubing (US Surgical Corp) to make a Rumel snare. After 30 minutes of ischemia, the snare was released and the heart was reperfused for 2 hours. Finally, the artery was reocluded, and 4% Evans blue dye was injected into the aortic root to delineate the area at risk from not-at-risk myocardium (blue). Hearts were then explanted, rinsed in 0.9% normal saline, and placed in 1% agarose gel (UltraPure agarose; Life Technologies, Gaithersburg, Md) in PBS (pH, 7.4). Hearts were sectioned parallel to the AV groove in approximately 1-mm sections. Viable and necrotic sections were delineated with 2,3,5-triphenyltetrazolium chloride (Sigma-Aldrich, St Louis, Mo) for 10 minutes at 37°C, followed by 10% neutral-buffered formaldehyde for 24 hours. Each section was weighed and photographed. The LV area at risk and infarct areas were traced and calculated by computer planimetry (Image J, version 1.21). Infarct volumes were calculated as follows: \[ \text{Volume} = \pi \times \frac{(A_1 \times W_1) + (A_2 \times W_2) + (A_3 \times W_3) + (A_4 \times W_4) + (A_5 \times W_5)}{2} \], where \( A \) is the area of infarct for the slice denoted by the subscript and \( W \) is the weight of the respective section.

The long-term model (30 minutes of ischemia and 4 weeks of reperfusion) was performed in a similar manner. Ischemia was validated via ECG recordings. After 30 minutes of ischemia, occlusion of the left anterior descending coronary artery was released, the wound was closed, and animals were returned to their cages. Surgery was performed in a blinded fashion.

**TagMan Real-Time PCR**

Total RNA was isolated using Trizol reagent and reverse transcribed into cDNA using a commercially available kit (REtroscript Kit; Applied Biosystems, Foster City, Calif). Levels of different mRNA were analyzed by real-time PCR using RealMasterMix and realplex2 Mastercycler (Eppendorf_AG, Hamburg, Germany). TaqMan primer and probe sets (Applied Biosystems) were used to analyze the mRNA expression of the following: mouse interleukin (IL) \( \beta \) (Mm01336189_m1), mouse IL-6 (Mm99999064_m1), mouse tumor necrosis factor (TNF) \( \alpha \) (Mm00443259_g1), mouse KC (Mm00433859_m1), mouse MCP-1 (Mm00441242_m1), mouse maximum intensity projection (MIP) 2 (Mm00436645_m1), mouse PAR-1 (Mm00438851_m1), mouse PAR-2 (Mm00433160_m1), human PAR-1 (Hs01069258_m1), and human PAR-2 (Hs00683434_m1). The mRNA expression of GAPDH (4352339E) was used as an internal control.

**ELISA**

Protein extracts were generated from mouse hearts as previously described. Cytokine and chemokine protein expression in the hearts was analyzed by ELISAs (Quantikine; R&D Systems, Minneapolis, Minn). Levels of 3-nitrotyrosine, a marker of oxidative/nitrative stress, were analyzed using a commercial ELISA assay (Hbt Hycult Biotechnology, Uden, the Netherlands). Phosphorylation of MAPKs (ie, extracellular signal–regulated kinase [ERK]1/2, JNK, and p38) and AKT was analyzed in heart lysates with a kit ( Duo-Set IC Kit; R&D Systems). All ELISAs were performed according to the manufacturers’ instructions. Data were normalized to the protein concentration in respective lysates and compared with noninjured WT hearts. The protein concentration was measured using an assay (Bio Rad Dc Protein Assay; Bio-Rad Laboratories, Hercules, Calif).

**Echocardiography**

Echocardiography was performed using an ultrasonographic system (VisualSonics Vevo 660), as previously described. The diameter of the LV and LV wall thickness at the end of systole and diastole were measured digitally on M-mode tracings and averaged from 4 cardiac cycles. Fractional shortening was then calculated from measured ventricle dimensions.

**Heart Weight to Body Weight Ratio**

Hearts were stopped at diastole by intraventricular injection of 1 mL of a 20-mmol/L potassium chloride solution. The heart weight to body weight ratio was calculated by dividing the HW (in milligrams) by the BW (in grams).

Hearts were fixed with 10% formalin solution and embedded in paraffin. Deparaffined sections were stained with the Masson trichrome stain.

**Statistical Analysis**

All statistical analyses were performed using computer software (GraphPad Prism, version 5.01). Data are represented as mean ± SEM. For 2-group comparison of parametric data, a t test was performed. For multiple-group comparison, 1- or 2-way ANOVA tests were performed, followed by Bonferroni posttest analysis.
injured hearts of PAR-2 was significantly increased in the hearts of WT mice reperfusion, the level of nitrotyrosine in myocardial tissue from patients with ischemic HF (n=5) and compared with mRNA expression in healthy controls (n=5). *P<0.05 vs uninjured or healthy controls.

weeks of reperfusion) mouse models of I/R injury to determine if the expression levels of PAR-1 and PAR-2 mRNA are altered during MI and heart remodeling, respectively. We found that levels of PAR-2, but not of PAR-1, mRNA expression were upregulated in injured hearts 2 hours after reperfusion (Figure 1A and B). In the long-term model, we observed a significant increase in both PAR-1 and PAR-2 mRNA expression in the hearts (Figure 1A and B). Next, we analyzed the levels of PAR-1 and PAR-2 mRNA expression in heart biopsy specimens obtained from patients with ischemic heart failure. PAR-1 and PAR-2 mRNA expression levels were significantly upregulated in failing hearts compared with the levels observed in the biopsy specimens from healthy donors (Figure 1C and D).

PAR-2 Deficiency Reduces Infarct Size
To investigate the role of PAR-2 deficiency in MI, PAR-2−/− and WT littermate mice were subjected to 30 minutes of ischemia and 2 hours of reperfusion. Infarct size was significantly reduced in hearts of PAR-2−/− mice compared with WT littermates (24.3±1.7% versus 35.8±3.4%), with no significant changes in the area at risk (Figure 2A).

Oxidative/Nitrative Stress Is Attenuated in the Injured Hearts of PAR-2−/− Mice
The formation of peroxynitrite, a product of NO and superoxide interaction, contributes to the MI by inducing oxidative/nitrative stress. Nitrotyrosine is a stable end product of peroxynitrite oxidation and has been used as a marker of oxidative/nitrative stress in a mouse model of cardiac I/R injury. After 30 minutes of ischemia and 2 hours of reperfusion, the level of nitrotyrosine in myocardial tissue was significantly increased in the hearts of WT mice (Figure 2B). More important, the level of nitrotyrosine in injured hearts of PAR-2−/− mice was significantly reduced compared with the level observed in injured hearts of WT mice (Figure 2B).

PAR-2 Deficiency Reduces Activation of MAPK Pathways
Activation of various MAPK pathways contributes to inflammation in the setting of I/R injury. PAR-2 deficiency had no effect on basal phosphorylation levels of ERK1/2, p38, and JNK in mouse hearts. I/R injury (30 minutes of ischemia and 2 hours of reperfusion) resulted in increased phosphorylation of all 3 MAPKs in the hearts of WT mice (Figure 3A–C). More important, the activation of ERK1/2, p38, and JNK was significantly attenuated in the hearts of PAR-2−/− mice (Figure 3A–C). In contrast, PAR-2 deficiency had no effect on the phosphorylation of AKT (Figure 3D).

PAR-2 Deficiency Attenuates Inflammatory Gene Expression in the Heart
Cardiac I/R injury induced the expression of a variety of inflammatory mediators, including IL-1β, IL-6, TNF-α, and KC 2 hours after reperfusion at both mRNA and protein levels (Figure 4A and B). More important, injured hearts of PAR-2−/− mice exhibited significantly less IL-1β, TNF-α, and KC mRNA expression compared with injured hearts of WT mice (Figure 4A). The IL-6 mRNA expression was also attenuated in PAR-2−/− mice but did not reach statistical significance (Figure 4A). Consistent with changes of mRNA expression, protein levels of IL-1β, IL-6, TNF-α, and KC were significantly reduced in injured hearts of PAR-2−/− mice compared with injured hearts of WT mice (Figure 4B–D).
were significantly reduced in the injured hearts of PAR-2−/− mice compared with the injured hearts of WT mice (Figure 4B). PAR-2 deficiency did not affect the expression of MCP-1 and MIP-2 in injured hearts (data not shown).

**PAR-2 Deficiency Reduces Cardiac Remodeling and Heart Dysfunction**

To study the role of PAR-2 in heart remodeling, PAR-2−/− and WT littermate mice were subjected to 30 minutes of ischemia, followed by 4 weeks of reperfusion. Gross morphological analysis of heart cross sections demonstrated that injured hearts of WT mice were larger, with visible dilatation of the LV, compared with injured hearts of PAR-2−/− mice (Figure 5A). I/R injury resulted in a significant increase in the heart weight to body weight ratio in WT mice, indicating hypertrophic remodeling of the hearts (Figure 5B). More important, the increase in the heart weight to body weight ratio was significantly smaller in PAR-2−/− mice compared with WT littermates (Figure 5B).

To analyze heart function, echocardiography was performed before and up to 4 weeks after cardiac I/R injury in PAR-2−/− and WT littermate mice. Before surgery, the morphological and functional parameters of hearts were similar between WT and PAR-2−/− mice (day 0) (Figure 6). After cardiac I/R injury, PAR-2−/− mice showed signifi-
significantly less ventricular dilatation compared with WT littermates, as demonstrated by changes in LV diameter and volume at the end of systole (Figure 6A and B). Moreover, 3 weeks after the I/R injury, thinning of the LV posterior wall was significantly attenuated in the heart of PAR-2−/− mice compared with WT mice at the end of systole (Figure 6C). I/R injury or PAR-2 deficiency had no effect on these 3 parameters at the end of diastole (data not shown). More important, PAR-2−/− mice showed significantly less impairment in heart function after cardiac I/R injury, measured by percentage of fractional shortening (Figure 6D). These data indicate that PAR-2 deficiency protects against systolic heart dysfunction induced by I/R injury.

**Discussion**

In this study, we found that PAR-2 deficiency reduces MI in a mouse model of cardiac I/R injury. Interaction between NO and the superoxide anion, 1 of the ROS generated during cardiac I/R injury, results in the formation of the highly cytotoxic molecule peroxynitrite. Peroxynitrite-mediated toxicity involves direct oxidative damage to lipids, proteins, and DNA; the activation of metalloproteinases; the impairment of mitochondrial respiration; and the nitration of tyrosine residues within proteins. The reduction of nitrotyrosine levels in the myocardium of PAR-2−/− deficient mice suggests that oxidative/nitratve stress is 1 of the mechanisms by which PAR-2 may enhance MI. This concept is supported by recent publications indicating that activation of PAR-2 leads to generation of both NO and ROS, including the superoxide anion.

During I/R injury, release of ROS results in the activation of MAPKs. Furthermore, the activation of PAR-2 also leads to phosphorylation of MAPKs in various cell types, including cardiomyocytes and endothelial cells. Recent studies demonstrated that activation of MAPKs enhances inflammation and contributes to MI. For example, overexpression of a dominant-negative p38 MAPK or inhibition of JNK MAPK results in significant protection from I/R injury. Moreover, reduced activation of MAPK was correlated with smaller infarcts in mice deficient for the lectinlike receptor for oxidized low-density lipoprotein. In our study, we demonstrated that the smaller infarcts observed in the hearts of PAR-2−/− mice after I/R injury were associated with reduced phosphorylation of ERK1/2, p38, and JNK; and attenuated expression of various inflammatory mediators. Therefore, we propose that PAR-2 contributes to MI, in part, by enhancing inflammation in an MAPK-dependent manner.

Many cell types, including endothelial cells, can contribute to a PAR-2–mediated inflammatory response within the injured myocardium. The activation of PAR-2 on endothelial cells induces the expression of IL-1β, IL-6, and IL-8 (a human homolog of mouse KC). The production of chemokines leads to the recruitment of leukocytes, including neutrophils and monocytes, into the injured myocardium. Both cell types express PAR-2; and PAR-2 signaling plays a role in leukocyte adhesion, rolling, and migration. Furthermore, monocytes/macrophages and neutrophils express multiple inflammatory cytokines and may be a source of TNF-α and IL-1β in the injured myocardium. Therefore, PAR-2–dependent signaling in endothelial cells and leukocytes will increase the expression of inflammatory mediators.

There is a growing body of evidence that, in addition to endothelial cells and leukocytes, cardiomyocytes can also contribute to the expression of inflammatory mediators. The activation of toll-like receptors (TLRs) in cardiomyocytes, including TLR4, leads to the expression of IL-6, KC, and MIP-2. More important, TLR4 signaling plays a role in MI and inflammation after I/R injury. Our preliminary data indicate that activation of PAR-2 in cultured neonatal mouse cardiomyocytes also results in the expression of IL-6 and KC but not TNF-α or MIP-2 protein (S.A., N.M., and R.P., unpublished data). Therefore, during I/R injury, the activation of PAR-2 on cardiomyocytes may contribute to the expression of IL-6 and KC. Interestingly, PAR-2–mediated expression of IL-1β was significantly attenuated in TLR4-deficient macrophages. Furthermore, the TLR/IL-1 receptorlike domain of TLR4 interacts with the cytoplasmic C-terminus of PAR-2. A mutation of this domain abolished PAR-2–dependent activation of nuclear factor κB in macrophages. Further studies are required to determine if TLR4 and PAR-2 cooperate in a similar manner in cell types within the injured heart to enhance inflammation.

We also demonstrated that PAR-2 deficiency reduced heart remodeling and heart dysfunction up to 4 weeks after injury. This may be because of a smaller initial infarct size in the hearts of PAR-2−/− mice. The size of the initial infarct affects the extent of heart remodeling after MI. In addition, the activation of PAR-2 induces hypertrophic growth of cardiomyocytes in vitro. Because hypertrophic growth is a major component of post-MI remodeling, it is possible that PAR-2 may also contribute to heart remodeling by increasing the hypertrophic growth of cardiomyocytes.

In contrast to the reduced infarct size observed in PAR-2−/− mice, we previously demonstrated that a deficiency of PAR-1 has no effect on infarct size in the same short-term model of cardiac I/R injury. This indicates that there are differences between PAR-1– and PAR-2–dependent signaling in MI. In the present study, we showed that short-term cardiac I/R injury led to the significant increase of PAR-2, but not PAR-1, mRNA expression. This may explain, in part, the different effects of PAR-1 and PAR-2 deficiency on MI. Inducible expression of PAR-2 is likely to play a pathological role in the inflammatory response during I/R injury. We also showed that PAR-1 and PAR-2 mRNA expression in the heart is significantly increased in a long-term model of I/R injury and in heart samples from patients who developed ischemic heart failure. It is uncertain if the increased expression of these 2 genes contributes to the development of heart failure or is just an effect of heart remodeling. Previous data indicate that an increase PAR-1 signaling in cardiomyocytes leads to the development of eccentric heart hypertrophy and a reduction of heart function. We also found that cardiomyocyte-specific overexpression of PAR-2 led to heart hypertrophy (S.A., N.M., and R.P., unpublished data). These data, together with the observation that PAR-2 and PAR-1 deficiencies significantly reduced long-term heart remodeling, suggest that both PAR-1 and PAR-2 may contribute to ischemic heart failure in humans.
Our study raises the question of what protease(s) activate PAR-2 during cardiac I/R injury. Researchers have previously demonstrated that tissue factor (TF), the primary initiator of the coagulation cascade, contributes to MI in rabbit and mouse models of cardiac I/R injury. Subsequently, TF-dependent thrombin generation and fibrin deposition enhanced MI. More important, the TF:FVIIa complex can activate PAR-2, which is a possible mechanism of PAR-2 activation during I/R injury. Further studies are required to determine if TF:FVIIa and/or FXa activate PAR-2 in injured hearts. In addition, PAR-2 can be activated by other proteases, such as mast cell tryptase. Mast cells have been found between muscle fibers in the heart, and mast cell–deficient mice exhibit reduced infarct size and inflammation after cardiac I/R injury. This may be due, in part, to a reduction in PAR-2 activation.

In contrast to our results, several recent publications have reported that activation of PAR-2 with a synthetic PAR-2 agonist peptide has a beneficial effect in both ex vivo and in vivo rat models of cardiac I/R injury. How can the administration of PAR-2 agonist peptide be protective while a global deficiency of PAR-2 results in a smaller infarct? Depending on the disease model, activation of PAR-2 may have either protective or detrimental effects. Furthermore, recent studies in a mouse model of Alzheimer disease suggest that PAR-2–dependent signaling may have opposite effects in different cell types within the brain. It is possible that a similar scenario may occur in the heart after I/R injury. In the short-term, I/R injury model activation of PAR-2 on endothelial cells may be protective, whereas PAR-2 signaling on other cell types, such as cardiomyocytes or infiltrating leukocytes, may lead to detrimental effects. Another possible explanation for these different results comes from a recent article demonstrating that PAR-2 is differentially activated by tethered versus soluble ligands, such as an agonist peptide. These 2 types of ligands differentially bind and stabilize different conformations of the receptor, resulting in activation of distinct subsets of signaling cascades. A better understanding of the cell type–specific responses of PAR-2 during cardiac I/R injury is needed. Interestingly, PAR-2 deficiency and treatment with PAR-2 agonist peptide had beneficial effects not only in heart I/R injury but also in the mouse model of colitis induced by intrarectal injection of trinitrobenzene sulfonic acid.

In summary, we demonstrated that PAR-2 deficiency reduces inflammation, MI, and cardiac remodeling after I/R injury. Further studies are required to determine the effect of inhibition of PAR-2 in WT mice before considering PAR-2 as a potential target for therapy to prevent myocardial damage.

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Disclosures
None.

References
reperfusion salvages myocardium via an ERK1/2 pathway in vivo.


41. Johansson U, Lawson C, Dahare M, Syndercombe-Court D, Newland AK, Howells GL, Macey MG. Human peripheral blood monocytes express protease receptor-2 and respond to receptor activation by production of IL-6, IL-8, and IL-1[beta]. J Leukoc Biol. 2005;78:967–975.


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