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Protease-Activated Receptor 1 Contributes to Angiotensin II-Induced Cardiovascular Remodeling and Inflammation

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Keywords
Protease-activated receptor 1 · Pre-hypertension · Angiotensin · Heart failure · Fibrosis

Abstract
Background: Angiotensin II (Ang II) plays an important role in cardiovascular disease. It also leads to the activation of coagulation. The coagulation protease thrombin induces cellular responses by activating protease-activated receptor 1 (PAR-1). We investigated whether PAR-1 contributes to Ang II-induced cardiovascular remodeling and inflammation. ***Methods and Results:*** PAR-1$^{+/+}$ (wild-type; WT) and PAR-1$^{-/-}$ mice were infused with Ang II (600 ng/kg/min) for up to 4 weeks. In WT mice, this dose of Ang II did not cause a significant increase in blood pressure but it did cause pathological changes in both the aorta and the heart. Ang II infusion resulted in vascular remodeling of the aorta, demonstrated by a significant increase in medial wall thickening and perivascular fibrosis. Importantly, both parameters were significantly attenuated by PAR-1 deficiency. Furthermore, perivascular fibrosis around coronary vessels was reduced in Ang II-treated PAR-1$^{-/-}$ mice compared to WT mice. In addition, PAR-1 deficiency significantly attenuated Ang II induction of inflammatory cytokines and profibrotic genes in the aortas compared to WT mice. Finally, PAR-1 deficiency had no effect on Ang II-induced heart hypertrophy. However, the heart function measured by fractional shortening was less impaired in PAR-1$^{-/-}$ mice than in WT mice. ***Conclusion:*** Our data indicate that PAR-1 plays a significant role in cardiovascular remodeling mediated by a blood pressure-independent action of Ang II.

Introduction

The renin-angiotensin system (RAS) is a major contributor to the development and pathophysiology of hypertension (HTN) [1, 2]. The major consequences of HTN are end-organ damage and cardiovascular complications [1, 2]. Angiotensin II (Ang II) is the main mediator of the RAS. Ang II is generated by enzymatic cleavage of angiotensinogen to Ang I by the protease renin, with subsequent conversion of Ang I to Ang II by angiotensin-converting enzyme [1, 2]. In addition, chymase is the primary enzyme leading to this conversion in the heart [3]. Most of the effects of Ang II are mediated by angiotensin type 1 (AT_1) receptors [1, 2]. While the human genome only contains one AT_1 receptor, there are 2 subtypes

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(AT_{1A} and AT_{1B}) in mice [4, 5]. AT_{1A} appears to be the main receptor isoform regulating blood pressure (BP), whereas the specific function of AT_{1B} is unclear [5]. AT_1 receptors are widely distributed and Ang II-dependent activation of this receptor affects the function of virtually all organs, including the vasculature and heart [1, 2]. Long-term exposure to Ang II leads to cardiovascular remodeling, fibrosis, and heart hypertrophy [6, 7]. The mechanism of Ang II-induced heart remodeling may involve the direct action of Ang II on target tissues or be mediated by an Ang II-induced increase in BP [1, 2].

Ang II activation of the AT_1 receptor also leads to upregulation of tissue factor (TF) expression. TF is the primary initiator of the coagulation cascade [8]. It is constitutively expressed in the blood vessel wall, as well as by cardiomyocytes and cardiac fibroblasts in the heart [9, 10]. Several in vitro studies have demonstrated that Ang II induces TF expression in smooth-muscle cells, endothelial cells, and monocytes [7]. TF expression has also been found to be upregulated in the endothelium and media of blood vessels in hypertensive rats [11]. Moreover, blockage of the AT_1 receptor with valsartan inhibits upregulation of TF expression [11]. Importantly, elevated levels of circulating TF have been observed in patients with HTN, and AT_1 receptor blockage significantly reduces TF activity [12, 13]. Recently, it was shown that Ang II infusion accelerates microvascular thrombosis in mice [14]. These data indicate that upregulation of TF expression during HTN is mediated via the AT_1 receptor and may lead to systemic activation of the coagulation cascade.

Thrombin is the central protease of the coagulation cascade [15]. Human HTN patients and animal models of HTN have shown enhanced thrombin generation in plasma as measured by elevated thrombin-antithrombin (TAT) complexes [16, 17]. In addition to its important role in both hemostasis and thrombosis, thrombin can induce multiple cellular responses via the activation of protease-activated receptors, such as protease-activated receptor 1 (PAR-1) [7, 15]. PAR-1 belongs to the family of 7 transmembrane domain G protein-coupled receptors activated by proteolytic cleavage. PAR-1 is widely expressed within the vasculature and heart [7, 18, 19]. Studies by others and us have shown that PAR-1 plays a significant role in the physiology and pathophysiology of the cardiovascular system [7, 20–23]. Several ex vivo studies have documented that activation of PAR-1 induces endothelium-dependent relaxation in the aorta and coronary arteries [20, 22]. However, it has also been shown that activation of PAR-1 can elicit endothelium- or vascular smooth-muscle-dependent vasoconstriction [20, 22]. These studies suggest that PAR-1-dependent vasoregulation may be cell/tissue specific. In mice, activation of PAR-1 with agonist peptide results in a biphasic BP response in which there is a rapid and transient hypotension followed by a sustained HTN, presumably via activation of PAR-1 on smooth-muscle cells [24]. Unstressed PAR-$1^{-/-}$ mice exhibit no obvious abnormalities in baseline BP compared to PAR-$1^{+/+}$ mice [24, 25].

In mouse and primate models of vascular injury, PAR-1 expression is upregulated in proliferating neointima [26, 27]. PAR-1 deficiency resulted in protection against vascular remodeling and stenosis in an endothelial denudation model of vascular injury [26]. Inhibition of PAR-1 with an anti-PAR-1 antibody or selective PAR-1 antagonist RWJ-59259 in a balloon catheter injury model also reduced smooth-muscle cell proliferation and the intimal area and thickness, as well as the percentage of stenosis [28, 29]. In vitro studies have demonstrated that activation of PAR-1 with either thrombin or agonist peptide leads to a series of molecular and morphological changes that result in hypertrophic growth of cardiomyocytes and proliferation of fibroblasts [30–32]. Recently, we showed that cardiomyocyte-specific overexpression of PAR-1 induced heart hypertrophy and decreased heart function via a TF-dependent mechanism [23]. In addition, others and we have demonstrated that PAR-1 contributes to cardiac remodeling in different heart injury models [7, 23, 33].

There are compelling data showing that Ang II leads to the activation of coagulation which might contribute to disease progression [7]. We therefore analyzed the contribution of PAR-1 signaling to Ang II-induced cardiac and vascular remodeling.

Materials and Methods

Mice and Ang II Infusion

PAR-$1^{+/-}$ mice were backcrossed at least 11 generations onto a C57Bl/6J background and bred to generate a PAR-$1^{-/-}$ and PAR-$1^{+/+}$ littermate cousin line [23, 25, 34]. Male mice with an age between 8 and 12 weeks were used for all experiments. Mice were implanted with Alzet mini-osmotic pumps (Model 2004; DURECT Corporation) subcutaneously on the back of the neck. Infusion of Ang II (600 ng/kg/min; Sigma-Aldrich, St. Louis, MO, USA) was performed for up to 28 days. This study was approved by the Office of Animal Care and Use of the University of North Carolina at Chapel Hill and complied with National Institute of Health guidelines.

Echocardiography and BP Measurements

Heart function was analyzed by echocardiography in conscious mice using a VisualSonics Vevo2100 system (VisualSonics; Toronto, ON, Canada) as previously described [34, 35]. End systolic

and diastolic LV volumes and LV wall dimensions were measured digitally on M-mode tracings and averaged from at least 4 cardiac cycles. Fractional shortening was calculated from the measured LV dimensions [23]. BP were measured via the pressure-volume loop method with a 1.2-Fr admittance pressure-volume catheter (Scisence, Ithaca, NY, USA) in anesthetized and ventilated mice as described elsewhere [36]. All measurements were done in UNC Rodent Advanced Surgical Models Core according to American Society of Echocardiography guidelines.

Coagulation Parameters

Blood was collected from the inferior vena cava into sodium citrate (final concentration 0.38%) and plasma separated by centrifugation (4,500 *g*, 15 min, 4°C). Levels of TATc in plasma were quantified by ELISA (TAT Enzygnost Micro Kit; Dade Behring/Siemens) [34].

Real-Time PCR

Total mRNA from mouse hearts and aortas was isolated via the TriZol method [35, 37]. One microgram of total mRNA was reverse transcribed into cDNA and analyzed by real-time PCR using RealMasterMix and RealPlex^2 MasterCycler (Eppendorf AG, Hamburg, Germany) [38]. To analyze the expression of interleukin (IL)-1β, CXCL1, CXCL2, transforming growth factor (TGF)-β1, TGF-β3, connective tissue growth factor (CTGF), collagen (Coll) IaI, Coll Ia2, and Coll 3 mRNA we used predesigned probe sets (Integrated DNA Technologies, Coralville, IA, USA). Variations in loading were adjusted using hypoxanthine-guanine phosphoribosyltransferase mRNA expression.

Histology

Fibrosis and cellular infiltration were visualized on formalin-fixed, paraffin-embedded heart and aorta sections stained with Masson's trichrome and H&E, respectively [10, 35, 38]. Stained tissue sections were subsequently viewed using an Olympus BX51 microscope (Tokyo, Japan) and photographed using an Olympus DP70 digital camera with a DP controller and DP manager computer software [34, 35, 38].

Statistical Analysis

All statistical analyses were performed using GraphPad Prism (version 5.04; GraphPad Software Inc., La Jolla, CA, USA). Data are presented as means ± SEM. For 2-group comparisons of continuous data, a 2-tailed Student's *t* test was used. For multiple-group comparisons, normally distributed data were analyzed by 1- or 2-way ANOVA and Bonferroni corrected for repeated measures over time. $p \leq 0.05$ was considered statistically significant.

Results

Infusion of Ang II Leads to Systemic Activation of Coagulation and Heart Hypertrophy Associated with Cardiac Fibrosis and Inflammation

To determine whether chronic AT_1 receptor stimulation leads to cardiovascular remodeling and inflammation as well as activation of coagulation, wild-type (WT) mice were infused for up to 14 days with Ang II (600 ng/kg/min). As expected, AT_1 receptor stimulation in WT mice led to a significant increase in heart weight:body weight (HW:BW) ratios after 14 days of Ang II infusion (Fig. 1a). Importantly, we also observed a systemic activation of coagulation measured by plasma TATc levels on day 14 after pump installation (Fig. 1a). The increased heart hypertrophy and procoagulant state were associated with interstitial and perivascular fibrosis within the heart muscle (Fig. 1b) and increased cellular infiltrations (Fig. 1c) after 7 days of Ang II infusion. Our observation suggests that chronic activation of the AT_1 receptor leads to activation of coagulation which was associated with pathologic cardiac fibrosis, inflammation, and remodeling.

PAR-1 Deficiency Has No Effect on BP before and after Ang II Infusion

To determine whether PAR-1 activation contributes to basal and Ang II-dependent BP, the BP in WT and PAR-1-deficient mice was measured before and 7 and 28 days after constant Ang II infusion. Consistent with previously published data, PAR-1 deficiency had no significant effect on the baseline diastolic and systolic BP (see online suppl. Fig. 1; for all online suppl. material, see www.karger.com/doi/10.1159/000452269) [24, 25]. Interestingly, infusion of Ang II at a concentration of 600 ng/kg/min caused only a modest but not statistically significant increase in BP in both WT and PAR-1$^{-/-}$ mice, measured on days 7 and 28 after initiation of the infusion (see online suppl. Fig. 1). In addition, we did not find any differences between genotypes with regard to BP on days 7 and 28. This suggests that PAR-1 does not play a major role in baseline BP regulation and that the all of the observations in our study were due to BP-independent AT_1- and PAR-1-mediated signaling events in the setting resembling pre-HTN conditions [39].

PAR-1 Deficiency Attenuates Ang II-Induced Remodeling of the Aorta

Ang II infusion in mice is known to lead to vascular remodeling in the aorta, associated with increased media thickness and enhanced fibrosis [6]. To determine whether PAR-1 affects these parameters, media-to-lumen and fibrosis-to-lumen ratios of aortas were analyzed before and 28 days after Ang II infusion in WT and PAR-1-deficient mice. There were no significant differences with regard to aorta media thickness or the amount of fibrotic tissue around the aorta in mice of both genotypes before Ang II infusion (Fig. 2). As expected, chronic AT_1 stimu-

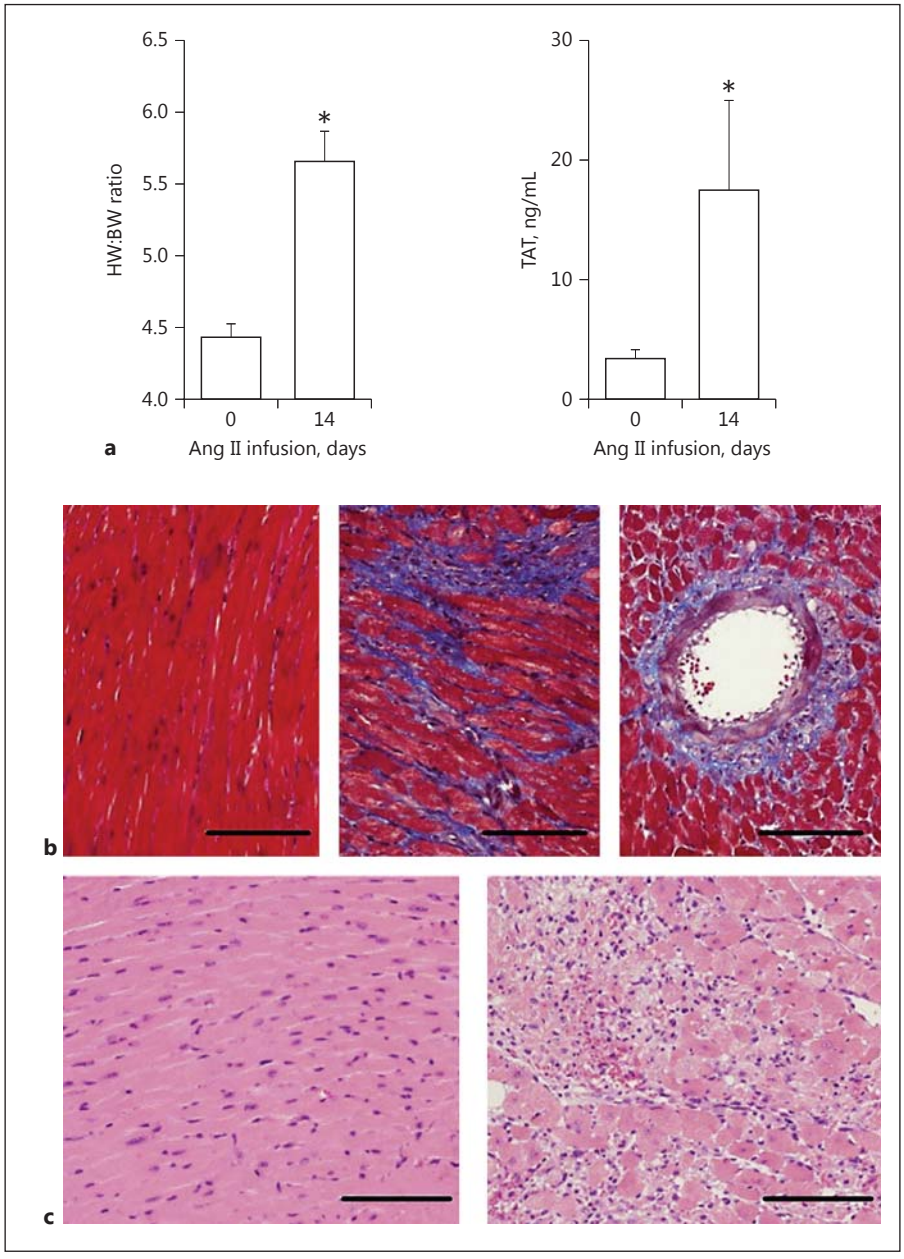

Fig. 1. Infusion of angiotensin (Ang II) leads to cardiac hypertrophy, systemic activation of coagulation associated with cardiac fibrosis and inflammation. **a** Increase in heart weight to body weight (HW:BW) ratios and plasma levels of thrombin-antithrombin complexes (TAT) in Ang II-treated wild-type mice. Data (means ± SEM; $n = 3$ for each time point) were analyzed using Student's t test. Statistical significance is shown as * $p < 0.05$. **b** Masson's trichrome staining of representative heart sections from controls (left) and mice treated with Ang II for 7 days (middle and right). **c** Inflammatory cells are present in H&E-stained heart sections from Ang II-treated mice (right) but not from control mice (left). Scale bars, 200 μm.

lation for 28 days led to an increase in aorta media thickness and increased Coll deposition visualized by Masson's trichrome staining around the aorta in WT mice. Importantly, both parameters were significantly attenuated in Ang II-treated PAR-1$^{-/-}$ mice (Fig. 2). These data suggest that PAR-1-dependent signaling pathways contribute to the vascular remodeling induced by chronic Ang II infusion.

Reduced Expression of Inflammatory and Profibrotic Markers in the Aortas of PAR-1-Deficient Mice

To determine whether changes in vascular remodeling induced by Ang II are associated with changes in the expression profile of inflammatory and profibrotic genes in the aorta, real-time PCR was performed on aorta samples of WT and PAR-1-deficient mice before and 7 and 28 days after Ang II infusion. Importantly, there were no significant differences between genotypes in baseline mRNA expression levels in the aorta of the inflammatory media-

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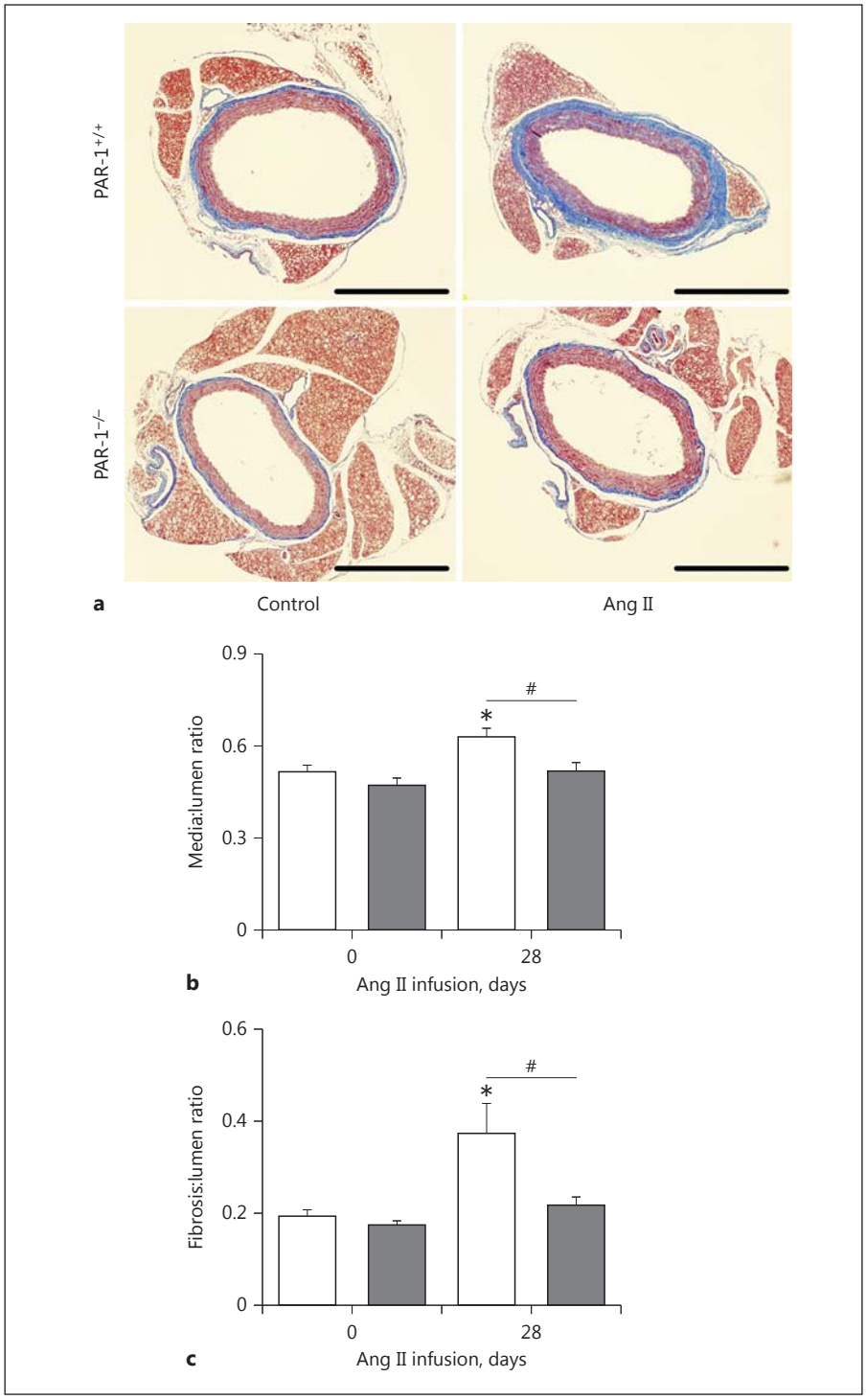

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Fig. 2. Protease-activated receptor 1 (PAR-1) deficiency attenuates angiotensin II (Ang II)-induced remodeling of the aorta. **a** Representative cross-sections of thoracic parts of aortas stained with Masson's trichrome. Scale bars, 1 mm. Quantification of the media area:lumen area (**b**) and fibrotic area:lumen area (**c**) ratios from PAR-$1^{+/+}$ (white bars) and PAR-$1^{-/-}$ (gray bars) mouse aortas before and 28 days after constant Ang II infusion. Data (means ± SEM; $n = 5$ for day 0 and $n = 10$ for day 28) were analyzed by 2-way ANOVA. Statistical significance is shown as $^{\#}\ p < 0.05$ and $^{*}\ p < 0.05$ vs. day 0 of the respective genotype.

tors IL-1β, IL-6, TNF-α, MCP-1, CXCL1, and CXCL2 or the profibrotic mediators TGF-β1, TGF-β3, and CTGF or the extracellular matrix components Coll Ia1, Coll Ia2, and Coll III (Fig. 3 and data not shown). Except for CTGF, Ang II infusion led to a significant increase in the mRNA expression of all measured genes in WT mice at 7 days. Later, on day 28, the aortic expression levels of the analyzed genes in WT mice returned to baseline, with the exception of CTGF (Fig. 3f). Importantly, PAR-1 deficiency was associated with less proinflammatory and pro-

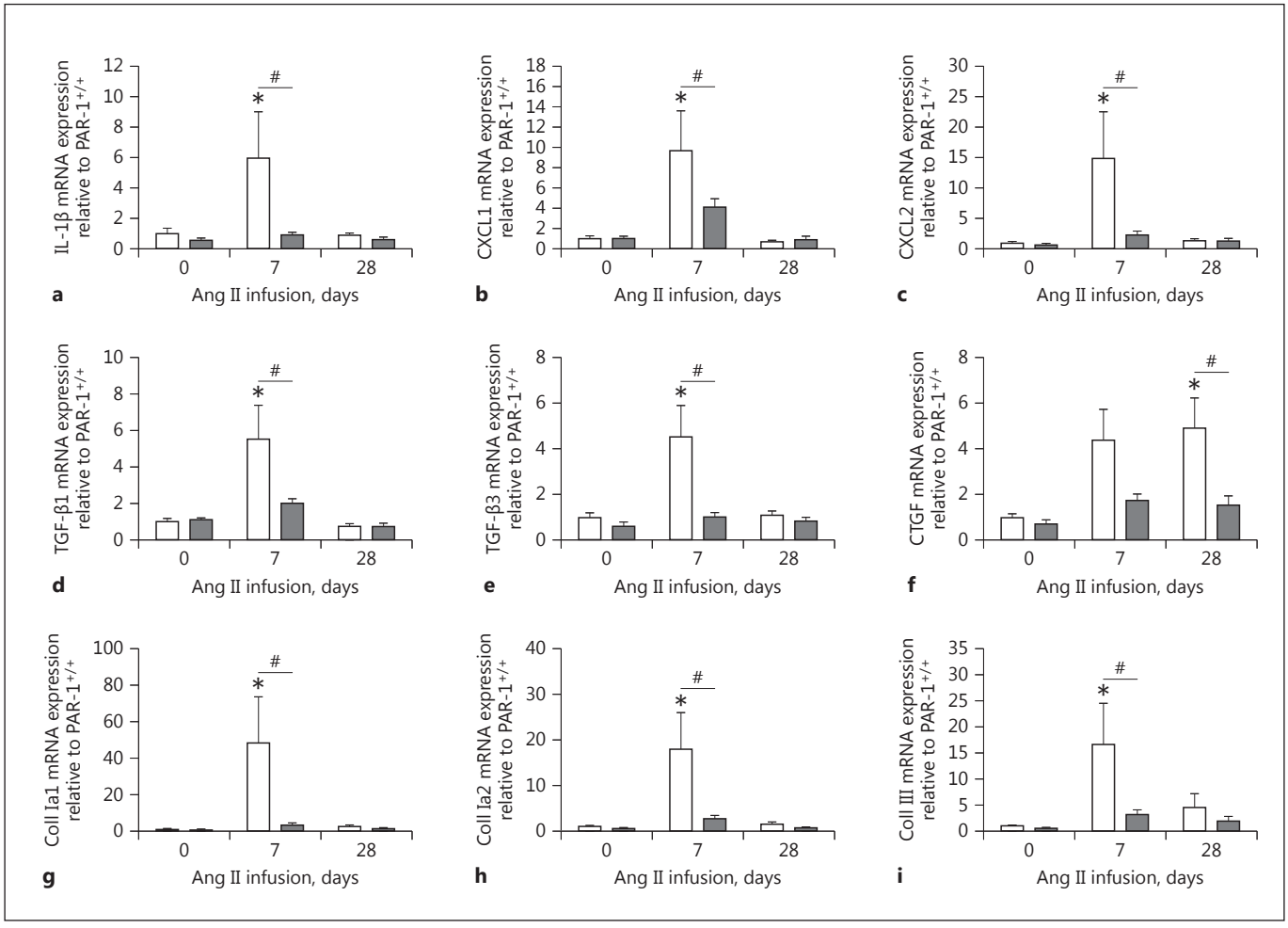

Fig. 3. Reduced expression of inflammatory and profibrotic markers in the aortas of protease-activated receptor 1 (PAR-1)-deficient mice. Real-time PCR analysis of mRNA expression of the inflammatory mediators interleukin (IL)-1β (**a**), CXCL1 (KC) (**b**), and CXCL2 (inflammatory protein 2-α; MIP-2α) (**c**) and the profibrotic markers transforming growth factor (TGF)-β1 (**d**), TGF-β3 (**e**), and connective tissue growth factor (CTGF) (**f**), as well as the extracellular matrix proteins collagen (Coll) Ia1 (**g**), Coll Ia2 (**h**), and Coll III (**i**) in the aortas of PAR-$1^{+/+}$ (white bars) and PAR-$1^{-/-}$ (gray bars) mice before and after 7 and 28 days of constant Ang II infusion. Data (means ± SEM; $n = 6–10$) were analyzed by 2-way ANOVA. Statistical significance is shown as $^{\#}\ p < 0.05$ and $^{*}\ p < 0.05$ vs. day 0 of the respective genotype.

fibrotic gene expression compared to day 7, as well as CTGF on day 28 (Fig. 3). These data indicate that PAR-1 is involved in regulation of a transient proinflammatory and profibrotic phenotype leading to vascular inflammation and remodeling in the aorta during chronic Ang II infusion.

Ang II-Induced Perivascular Fibrosis of Coronary Arteries Is Reduced in Ang II-Treated PAR-1-Deficient Mice

Besides its effect on the aorta, Ang II leads to perivascular fibrosis of coronary vessels and heart hypertrophy [6]. To analyze Ang II-dependent changes in perivascular fibrosis and cardiac remodeling, hearts of WT and PAR-1-deficient mice were analyzed before and 7 and 28 days after Ang II infusion. We did not observe any differences in the level of perivascular fibrosis around the cardiac arteries before Ang II infusion, measured based on the fibrosis area:lumen area ratio (Fig. 4a, b). Chronic AT_1 stimulation led to an increase in perivascular fibrosis around the coronary arteries in both WT and PAR-1-deficient mice, visualized by Masson's trichrome staining of heart sections (Fig. 4a). However, this parameter was significantly increased only in WT but not in PAR-$1^{-/-}$ mice

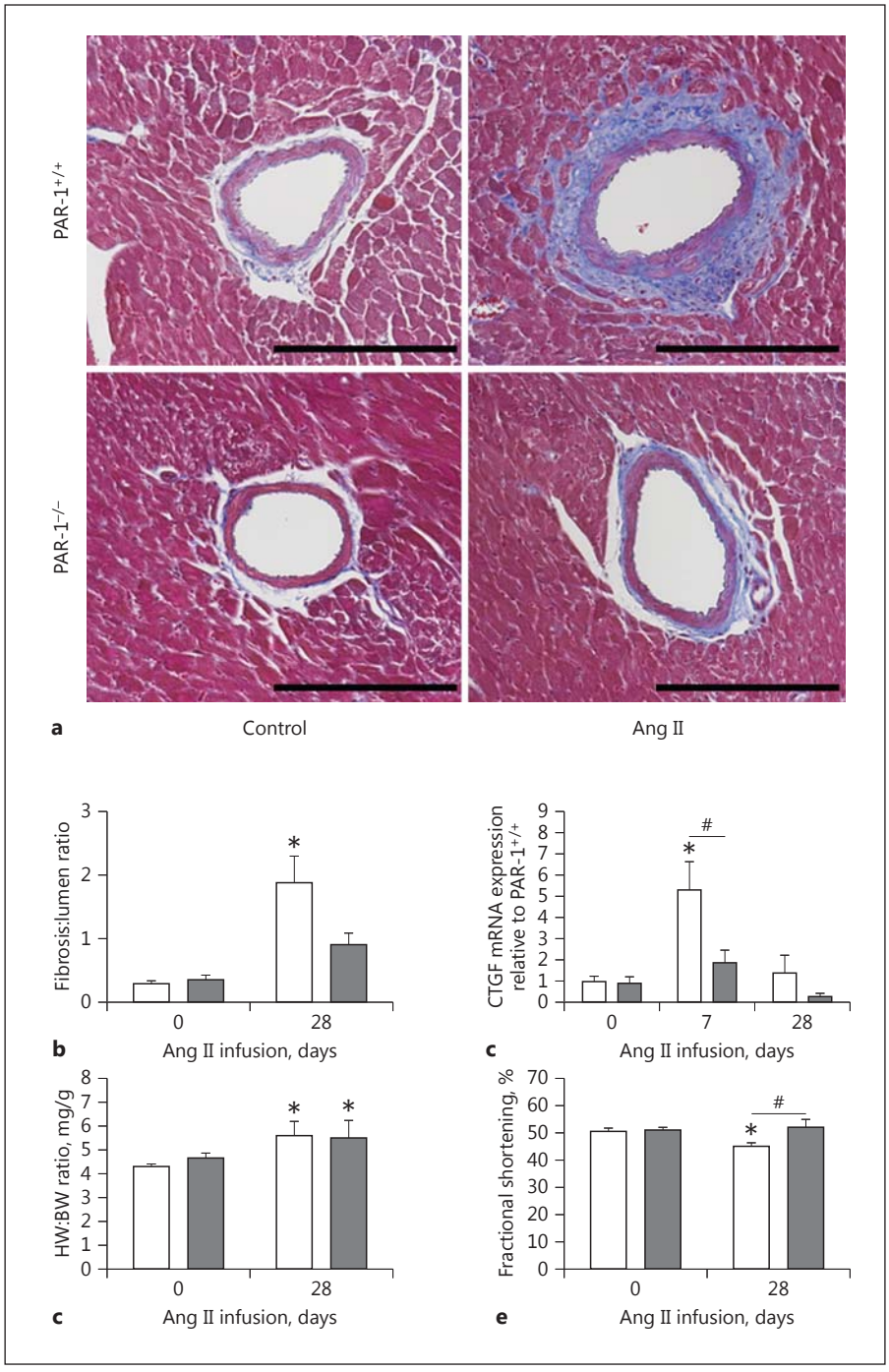

Fig. 4. Protease-activated receptor 1 (PAR-1)-deficient mice are protected against angiotensin II (Ang II)-induced cardiac fibrosis and heart dysfunction. **a** Representative sections of heart coronary vessels from PAR-$1^{+/+}$ and PAR-$1^{-/-}$ mice after 28 days of constant Ang II infusion stained with Masson's trichrome. Scale bars, 200 μm. **b** Quantification of the fibrotic area:lumen area ratio of heart coronary vessels from PAR-$1^{+/+}$ (white bars) and PAR-$1^{-/-}$ (gray bars) mice before and after 28 days of constant Ang II infusion. **c** Real-time PCR analysis of connective tissue growth factor (CTGF) mRNA expression in the hearts of PAR-$1^{+/+}$ and PAR-$1^{-/-}$ mice before and after 7 and 28 days of continuous Ang II infusion. Heart weight:body weight ratios (HW:BW) (**d**) and fractional shortening (**e**) measured by echocardiography before and after 28 days of Ang II infusion. Data (means ± SEM; n = 6–10) were analyzed by 2-way ANOVA. Statistical significance is shown as $^{\#}p < 0.05$ and $^{*}p < 0.05$ vs. day 0 of the respective genotype.

on day 28 (Fig. 4b). Next, we analyzed the expression of CTGF in the heart, which was shown to be associated with pathologic cardiac fibrosis and remodeling [38]. CTGF expression was significantly increased in the hearts of WT mice 7 days after Ang II infusion and returned to baseline (Fig. 4c) on day 28. Importantly, 7 days after Ang II infusion, CTGF mRNA expression was significantly lower in the hearts of PAR-1-deficient mice compared to WT controls (Fig. 4c).

PAR-1-Deficient Mice Are Protected against Ang II-Induced Heart Dysfunction

Chronic AT_1 stimulation is known to induce heart hypertrophy leading to heart dysfunction [40]. To analyze

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changes in heart hypertrophy, HW:BW ratios were calculated in WT and PAR-1-deficient mice before and 28 days after Ang II infusion. PAR-1 deficiency did not affect this parameter at baseline (Fig. 4d). HW:BW ratios were significantly increased 28 days after Ang II infusion. However, PAR-1 deficiency did not affect heart hypertrophy. Despite that, PAR-1-deficient mice had a better preserved heart function compared to WT mice 28 days after Ang II infusion (Fig. 4e). This observation suggests that a lack of PAR-1-dependent signaling protects against Ang II-induced heart dysfunction independently of heart hypertrophy.

Discussion

In this study, we demonstrated that PAR-1 deficiency attenuates pathologic Ang II-induced remodeling of the cardiovascular system. PAR-1 deficiency was associated with a reduced mRNA expression of proinflammatory and profibrotic markers, which correlated with attenuation of fibrosis and remodeling in the aortas of Ang II-treated mice. In addition, we also observed reduced perivascular fibrosis of coronary vessels in the hearts of PAR-1-deficient mice after Ang II treatment. The reduced cardiac fibrosis in PAR-1-deficient mice led to a preserved heart function compared to Ang II-treated WT mice. Interestingly, our observation was independent of a change in BP as well as heart hypertrophy since PAR-1 deficiency had no effect on these parameters. Our data indicate that PAR-1 plays a significant role in cardiovascular remodeling mediated by the BP-independent action of Ang II.

In our study, we were not able to detect increased BP in anesthetized mice after 600 ng/kg/min Ang II infusion. This was surprising since even a lower Ang II dose (490 ng/kg/min) was shown to increase BP in telemetric observed animals [41]. However, the BP discrepancy might be due to the measurement used. Anesthesia has cardiodepressive effects leading to hypotension, which might have masked the real BP elevation in our experimental mice [42]. Thus, our study mimicked a murine pre-HTN model with BP below levels defined as HTN. Pre-HTN is described as BP between 120 and 139 mm Hg systolic or 80 and 89 mm Hg diastolic [39]. It is thought that a pre-HTN status adds a moderate-to-high risk to the total cardiovascular risk [39]. Importantly, the Framingham Heart Study showed that patients with pre-HTN have an increased risk of developing HTN [43]. Furthermore, the ATTICA study reported an association between pre-HTN and increased inflammation, which was linked to the atherosclerotic process [39]. It was concluded that increased inflammation might be a mechanism in the initiation and/or progression of pre-HTN [44, 45].

The ongoing inflammation might be mediated by both innate and adaptive immune processes. The innate immune pathway not only responds to exogenous pathogens but can be also activated by damage-associated molecular patterns released endogenous by stressed, damaged, or necrotic cells [46]. Damage-associated molecular patterns are present in cardiovascular diseases during HTN [47]. AT_1 activation has been shown to induce p53-dependent apoptosis of cultured rat cardiomyocytes, which might lead the release of damage-associated molecular patterns [48]. Interestingly, Singh et al. [49] reported that Ang II-mediated cardiac hypertrophy and proinflammatory gene expression were mainly caused by TRIF pathway activation. Those authors further showed that the expression of the TRIF-dependent cytokine CXCL10 was increased in $MyD88^{-/-}$ mouse hearts but reduced in mice lacking TRIF signaling [49]. We found that PAR-1 stimulation enhanced Toll-like receptor (TLR)-3/TRIF-dependent CXCL10 expression in cardiac fibroblasts and immune cells [34, 37]. This suggests that PAR-1 possibly enhanced pathologic TRIF signaling during Ang II infusion. Besides its effect on TLR signaling, PAR-1 activation induces the classic hallmarks of inflammation, including enhanced vascular permeability and upregulation of proinflammatory mediators and adhesion molecules [21]. In our study, we observed a PAR-1-dependent proinflammatory state in mice after Ang II infusion measured by increased IL-1β, IL-6, TNF-α, MCP-1, CXCL1, and CXCL2 expression in the aorta. Consistent with this observation, a study showed that increased plasma levels of IL-1β, a cytokine involved in monocyte activation, precede changes in BP in HTN [50]. Furthermore, IL-6, MCP-1, and especially TNF-α, have been shown to be essential for the pathologic effects of Ang II on the vasculature by increasing oxidative stress, activating fibroblasts, and attracting T cells [47, 51].

Expression of proinflammatory cytokines is further linked to induction of a profibrotic phenotype. In addition, a mild-to-moderate increase in BP has been reported to stimulate fibroblasts and increase Coll formation leading to fibrotic remodeling of the myocardium with a normal LV mass [52]. TGF-β1 mRNA and protein expression are increased in HTN patients [53]. CTGF has a role as a downstream mediator of the chronic fibrotic effects of TGF-β. Activated TGF-β induces CTGF expression in fibroblasts and cardiomyocytes [54]. During this

process fibroblasts differentiate into myofibroblasts, the major cells that synthesize Coll in cardiac remodeling [55, 56]. Activation of PAR-1 on fibroblasts induced cell proliferation and expression of profibrotic genes [32, 57]. Here, we found increased expression of CTGF and TGF-β, as well as Coll, in Ang II-infused WT mice but not in PAR-$1^{-/-}$ mice. This PAR-1-dependent profibrotic phenotype resulted in increased vascular and perivascular remodeling associated with fibrosis and a possible reduced elasticity of the aorta and coronary arteries. This is consistent with the finding of Celik et al. [44, 58] that pre-HTN was associated with impairment of aortic elasticity and inflammation compared to healthy controls. These changes may lead to a reduced coronary flow very early in pre-HTN before hypertrophy is apparent and thus may cause subsequent ischemia and fibrosis [59, 60]. Structural abnormalities are already seen in pre-HTN patients, though in a milder manner compared to newly diagnosed HTN patients, which might lead to cardiac remodeling [33, 61]. In our study, we did not observe any differences in cardiac hypertrophy between the two genotypes. This was surprising since others and we have shown that PAR-1 contributes to cardiomyocyte hypertrophy and cardiac remodeling [23, 30]. However, the significant differences in fibrosis independently of hypertrophy might be due to the AT receptor distribution on cardiac fibroblasts and cardiomyocytes. Cardiomyocytes express comparable amounts of AT_1 and AT_2 receptors, whereas fibroblasts express predominantly AT_1 [62]. In addition, AT_2 has been shown to counteract AT_1 activation [62]. Importantly, increased vascular inflammation, as well as arterial stiffness, has been shown to be predictive of cardiovascular events [63, 64].

The remaining topic for further studies is the physiologic activator of PAR-1 in the setting of chronic Ang II infusion, AT_1 stimulation, and HTN. The most likely candidate is thrombin. In our study, we observed increased TAT levels after chronic Ang II infusion. Consistent with our findings, it has been shown that AT_1 receptor stimulation and HTN are linked to increased activation of coagulation due to increased TF expression and thrombin generation [11–13, 16, 17], which lead to a prothrombotic phenotype [14]. Another possible activator is the non-canonical activation of PAR-1 by matrix-metalloproteinases (MMP)-1 or MMP-13 [7]. Recently, it was shown that MMP-1 enhanced Ang II-induced vasoconstriction in endothelium-intact omental arteries in a PAR-1-dependent way ex vivo [65]. Furthermore, MMP-13 is expressed and active during Ang II-induced HTN [66]. We showed that the MMP-13/PAR-1 pathways is active in cardiovascular disease models [7, 18, 33, 34].

Importantly, besides its proven pathologic role, TF expressed by extravascular cells in the brain, lung, and heart, such as astrocytes, epithelial cells, smooth-muscle cells, fibroblasts, and cardiomyocytes maintains organ hemostasis [10, 11, 23, 67–71]. Reduced TF-dependent initiation of coagulation can result in hemorrhages, increased tissue fibrosis, and a reduced overall survival under healthy as well as pathologic conditions [10, 68, 71]. This dual role makes is difficult to use TF blockage as a viable therapy option [70]. Based on our data, further studies are warranted to investigate the effect of the PAR-1 inhibitor vorapaxar in Ang II-induced cardiovascular remodeling.

Conclusion

Taken together, our data suggest that the PAR-1 signaling pathway contributes to Ang II-induced cardiac fibrosis and heart dysfunction.

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Conflict of Interest

There are no financial interests.

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