Research Article

Role of prostaglandin D_2 receptors in the pathogenesis of abdominal aortic aneurysm formation

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Prostaglandin D_2 (PGD$_2$) released from immune cells or other cell types activates its receptors, D prostanoid receptor (DP)1 and 2 (DP1 and DP2), to promote inflammatory responses in allergic and lung diseases. Prostaglandin-mediated inflammation may also contribute to vascular diseases such as abdominal aortic aneurysm (AAA). However, the role of DP receptors in the pathogenesis of AAA has not been systematically investigated. In the present study, DP1-deficient mice and pharmacological inhibitors of either DP1 or DP2 were tested in two distinct mouse models of AAA formation: angiotensin II (AngII) infusion and calcium chloride (CaCl$_2$) application. DP1-deficient mice [both heterozygous (DP1$^{+/-}$) and homozygous (DP1$^{-/-}$)] were protected against CaCl$_2$-induced AAA formation, in conjunction with decreased matrix metallopeptidase (MMP) activity and adventitial inflammatory cell infiltration. In the AngII infusion model, DP1$^{+/-}$ mice, but not DP1$^{-/-}$ mice, exhibited reduced AAA formation. Interestingly, compensatory up-regulation of the DP2 receptor was detected in DP1$^{-/-}$ mice in response to AngII infusion, suggesting a potential role for DP2 receptors in AAA. Treatment with selective antagonists of DP1 (laropiprant) or DP2 (fevipiprant) protected against AAA formation, in conjunction with reduced elastin degradation and aortic inflammatory responses. In conclusion, PGD$_2$ signaling contributes to AAA formation in mice, suggesting that antagonists of DP receptors, which have been extensively tested in allergic and lung diseases, may be promising candidates to ameliorate AAA.

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Introduction

Abdominal aortic aneurysm (AAA) is an inflammatory vascular disease, characterized by immune cell infiltration and proteolytic degradation of the vascular wall [1]. Immune cells such as macrophages and neutrophils are a major source of proteolytic enzymes and pro-inflammatory cytokines/chemokines, including tumor necrosis factor α (TNFα), interleukin 6 (IL-6) and monocyte chemotactic protein (MCP)-1

in AAA [2]. Targeting these inflammatory signaling pathways is a promising strategy to prevent AAA formation, and development of effective, safe and well-tolerated anti-inflammatory agents is urgently needed to treat AAA in humans. Currently, there are no approved medical therapies available for AAA.

Prostaglandins (PGs), produced by immune and vascular cells, are key mediators of inflammatory responses by amplifying cytokine signaling, inflammatory cell recruitment, vascular permeability etc [3]. PGs are generated from arachidonic acid by cyclooxygenases (COXs), and four major PGs (PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2) and eight receptors [PGD receptors (DP1, DP2), PGE receptors (EP1, EP2, EP3, EP4), PGF receptor (FP), PGI receptor (IP)] have been characterized *in vivo* [3]. PG signaling has been implicated in various inflammatory diseases including arthritis, asthma, atherosclerosis and pulmonary hypertension; moreover, PGE receptors have been implicated experimentally in the pathogenesis of AAA [4,5].

Among the various PGs, prostaglandin D_2 (PGD_2) signaling has been closely associated with inflammation, particularly in the lungs and upper airways [6]. PGD_2 is released by macrophages, which are abundant in AAA tissues [7], and produced in large amounts by activated mast cells following allergen exposure and antigen cross-linking with the high-affinity receptor for immunoglobulin E. Notably, mast cells are prominent in human and mouse models of AAA [8,9], and may contribute to disease pathogenesis [10,11]. Moreover, PGD_2-mediated inflammation in the lung and airways is commonly associated with smoking, the most important risk factor for AAA, suggesting a potential mechanistic link between PGD_2 signaling and AAA pathogenesis.

PGD_2 activates one of the two distinct receptors, DP1 and DP2 [also known as chemoattractant receptor-homologous molecule expressed on T_H2 cells (CRTH2)], which are expressed on a variety of inflammatory cells [e.g., T helper type 2 (T_H2) cells, mast cells, dendritic cells, macrophages and eosinophils] and mediate allergic responses and lung diseases such as rhinitis and asthma [3]. Activation of DP1 or DP2 receptors on human macrophages induced migration and cytokine production and promoted neutrophil inflammatory responses in mice *in vivo* [12], suggesting a pivotal role for PGD_2 receptors in macrophage–neutrophil interactions. Various pharmacological antagonists of DP1 and DP2 have been developed and tested in pre-clinical and clinical trials for the treatment of lung and allergic diseases. Pharmacological blockade of DP2 inhibited neutrophil and lymphocyte trafficking and airway inflammation in murine models of smoke exposure [13]. Furthermore, fevipiprant, an oral DP2 antagonist, reduced airway inflammation in a Phase II clinical trial of patients with asthma [14].

While a large body of experimental data suggest that DP receptors might play a pathogenic role in AAA, a single study unexpectedly showed that global DP1 deletion exacerbated AAA formation in apolipoprotein E knockout (KO) mice in response to angiotensin II (AngII) infusion [15]. The underlying mechanisms whereby deletion of DP1 exacerbated AAA in AngII-infused apolipoprotein E KO mice were not established in that study, and only a single model of AAA formation was employed. In addition, the potential role of DP2 receptors in the pathogenesis of AAA was not investigated.

In the present study, using DP1-deficient mice and pharmacological antagonists of DP1 and DP2, we investigated the role of DP receptors in the pathogenesis of AAA in two distinct animal models of AAA formation: AngII infusion in low-density lipoprotein receptor (LDLR) KO mice and calcium chloride ($CaCl_2$) application in wildtype (WT) mice. Deletion of one or both alleles of DP1 protected against AAA formation induced by $CaCl_2$ application. Interestingly, heterozygous, but not homozygous, deletion of DP1 was protective against AngII-induced AAA formation in LDLR KO mice. Mechanistically, loss of AAA protection in DP1 homozygous KO mice was associated with compensatory up-regulation of pro-inflammatory DP2, which might have confounded the previous findings in the global DP1 KO mouse [15]. Furthermore, our data suggest that selective pharmacological inhibitors of DP1 and DP2, which have been extensively tested in allergic and lung diseases, are promising candidates for AAA therapeutics.

Methods
Mice
DP1 KO mice were obtained from Dr. Shuh Narumiya (Kyoto University, Japan) and bred with LDLR KO mice (Jackson Laboratory) to obtain heterozygotes, which were interbred to produce littermates [WT (+/+), heterozygous (+/−) or homozygous (−/−) for DP1 in the LDLR KO background]. Mice were anesthetized by isoflurane vaporizer (0.5–1.0 l/min for oxygen flowmeter, 4–5% for induction and 1–3% for maintenance, EZ Anesthesia Systems) and killed with intraperitoneal pentobarbital 150 mg/kg, inhaled anesthesia (isoflurane) followed by carbon dioxide (CO_2) narcosis and cervical dislocation or bilateral thoracotomy, in accordance with AVMA Panel 2007 recommendations and institutional IACUC guidelines. All mice were randomized to different treatment groups to minimize the variability of the evaluation and all investigators and surgeons were blinded to group allocation to prevent accidental or selection

bias. All animal experiments were performed at animal facilities of the Medical College of Georgia at Augusta University and the University of Cincinnati. Animal experimental protocols were approved by the Institutional Animal Care and Use Committee at the Medical College of Georgia at Augusta University and the University of Cincinnati and complied with National Institute of Health guidelines.

AngII-induced AAA model

AngII (1000 ng/kg/min, Enzo Life Sciences) was infused into 8–12-week-old male mice via osmotic minipumps (ALZET Model 2004) as described previously [16]. In some experiments, mice were co-treated orally with laropiprant (MK-0524, 3 mg/kg body weight) [17] or ramatroban (0.25 mg/kg body weight) in drinking water [18] to inhibit DP1 or DP2 activity, respectively. Mice were killed at 3 weeks after minipump implantation, and abdominal aortic outer diameter was measured via microscopy. Aortic tissues and blood were collected from mice that survived until the end of the study for further analysis. AAA was defined as dilation of the abdominal aorta that is at least 1.5-times the size of the normal aortic diameter.

CaCl$_2$-induced AAA model

CaCl$_2$ application model of AAA (using 0.5 mol/l of CaCl$_2$, Sigma–Aldrich) was conducted as described previously [16]. In brief, following laparotomy, saline (sham control) or 0.5 mol/l of CaCl$_2$ was applied to the infrarenal aortic adventitial surface for 15 min, followed by rinsing with 0.9% sterile saline and surgical closure. In some experiments, mice were co-treated orally with fevipiprant (5 mg/kg/body weight) to inhibit DP2 activity. This concentration, which was chosen based on safety and tolerability as tested in humans, was converted into dose equivalency in mice [19]. After 3 weeks, mice were anesthetized, abdominal aortic outer diameter was measured via microscopy, and tissues were collected for analysis.

Total cholesterol and triglyceride measurement

Serum cholesterol and triglyceride were quantified using commercial assays (Wako Pure Chemical Industries).

Blood pressure measurement

Blood pressure was measured using a previously validated tail-cuff method (Coda 6, Kent Scientific, Torrington, CT). Mice were conditioned to the instrument and procedure for five consecutive days prior to pump implantation. To insure a more robust estimation of systolic blood pressure (SBP), we used the interquartile mean of SBP measurements achieved through 30 measurement cycles every other day.

Gelatin zymography

Matrix metalloproteinase (MMP)-2 and MMP-9 activity was measured by zymography as previously described [16]. In brief, protein lysate (600 μg) was placed in a non-reducing zymogram buffer and applied without boiling to a 10% zymogram gel (Bio-Rad). Gels were incubated in 2% Triton X-100 at room temperature for 30 min, and then rinsed in distilled water for 5 min. Gels were incubated overnight at 37°C with gentle agitation and proteins were stained with Coomassie Brilliant Blue R-250 solution (Bio-Rad) and de-stained with a solution containing 40% methanol, 10% acetic acid and 50% water.

Immunohistochemistry

Paraffin-embedded aortic tissue sections were stained with Hematoxylin and Eosin (H&E), Verhoeff–van Gieson (VVG), myeloperoxidase (MPO, Abcam), Mac-3 (BD Pharmingen) and MCP-1 (Novus Biologicals), and HistoMouse-SP (Invitrogen) or DAB Substrate (Vector Labs) kits were used for visualization. Quantificational analysis was performed by ImageJ software. The number of elastin breaks in aortas from the AngII model were counted to quantify elastin degradation as previously described [20]. Elastin breakage could not be accurately quantified in the CaCl$_2$ model given the severe degree of elastin destruction associated with this model.

Enzyme-linked immunosorbent assay

Serum levels of MPO were determined by mouse MPO DuoSet enzyme-linked immunosorbent assay (ELISA) kit per manufacturer's instructions (R&D Systems).
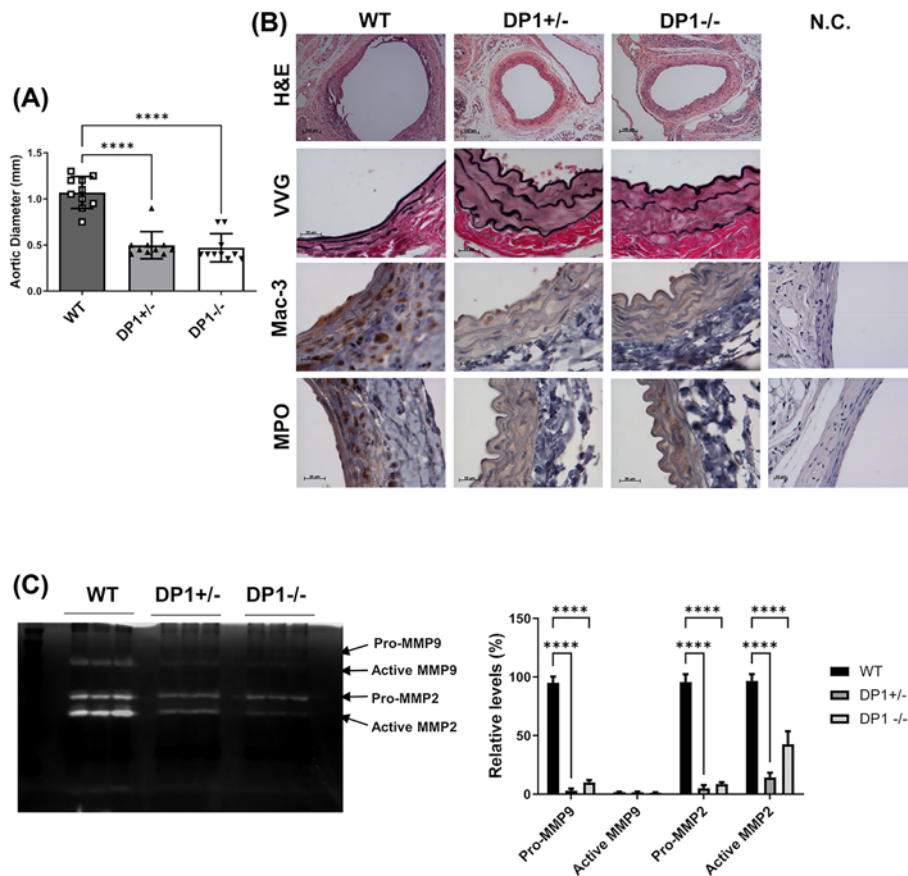
Figure 1. DP1-deficient mice are protected against CaCl$_2$-induced AAA formation
CaCl$_2$ was applied to the infrarenal aorta of C57Bl/6 mice. (**A**) AAA diameter (*n*=10). (**B**) Representative histology; H&E, VVG (elastin fragmentation), Mac-3 (macrophages) and MPO. (**C**) Representative zymogram (left panel) and quantified data (right panel) for aortic MMP-2 and MMP-9 activities (*n*=6). *$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$ vs WT. Data were analyzed using one-way ANOVA followed by Bonferroni's post-hoc analysis. Abbreviations: Mac, macrophage; N.C., negative control (absence of primary antibody).

Western blotting

Protein extraction and Western blotting were performed as described previously [16]. Antibodies for DP1, DP2 and β-actin were purchased from Abcam, Lifespan Biosciences and Santa Cruz Biotechnology, respectively.

Statistical analysis

All statistical analyses were performed using Graphpad Software (GraphPad Software, Inc., U.S.A.). Results are expressed as mean ± SEM. Multiple group datasets were evaluated for normality, and differences were analyzed by one-way ANOVA followed by Bonferroni's post-hoc analysis. *P*-values less than 0.05 were considered to be significant.

Results
DP1-deficient mice are protected against CaCl$_2$-induced AAA formation

We first investigated whether DP1 gene deletion could mitigate AAA formation induced by CaCl$_2$ application, an animal model of AAA which is associated with marked inflammatory cell infiltration and elastin fragmentation. We found that both DP1$^{+/-}$ and DP1$^{-/-}$ mice exhibited significantly reduced abdominal aortic diameter as compared with WT mice (Figure 1A). Prominent elastin fragmentation (H&E and VVG staining), MPO accumulation and macrophage infiltration (Mac-3 staining) were observed in CaCl$_2$-induced AAA tissues in WT mice, which were markedly inhibited in DP1$^{+/-}$ and DP1$^{-/-}$ mice (Figure 1B and Supplementary Figure S1). Furthermore,

MMP-2/MMP-9 activities were significantly decreased in both DP1$^{+/-}$ and DP1$^{-/-}$ mice as examined by zymography (Figure 1C). Notably, deletion of either one or both alleles of DP1 afforded equal protection against AAA formation.

DP1 heterozygous, but not homozygous deletion, protected against AngII-induced AAA formation

Next, we examined the impact of DP1 gene deletion on AAA formation in LDLR KO mice in response to AngII infusion. Interestingly, deletion of one DP1 allele (DP1$^{+/-}$) markedly protected against AngII-induced AAA formation, as evidenced by reduced aneurysm incidence (Figure 2A) and maximal abdominal aortic diameter (Figure 2B), while no thoracic aortic aneurysm (TAA) was observed. Histological analyses demonstrated that thrombus formation, macrophage accumulation and elastase degradation during AAA formation were significantly reduced in DP1$^{+/-}$ mice (Figure 2C,D and Supplementary Figure S1). MMP-2 and MMP-9 activities (evaluated by zymography) were likewise significantly diminished in DP1$^{+/-}$ mice (Figure 2E). We also measured the plasma levels of MPO, which has been reported to be a circulating biomarker of AAA formation [1]. Plasma MPO was likewise reduced in DP1$^{+/-}$ mice (Figure 2F). In contrast with these findings, deletion of both DP1 alleles (DP1$^{-/-}$) resulted in loss of protection against AAA formation, with the mice appearing phenotypically indistinguishable from WT LDLR KO mice. There were no differences in body weight, total cholesterol and triglyceride levels or blood pressure among the various groups (Supplementary Figure S2).

## Compensatory up-regulation of DP2 protein in DP1$^{-/-}$ mice infused with AngII

To explore the mechanisms of the disparate results observed with DP1$^{+/-}$ and DP1$^{-/-}$ mice in the AngII infusion model, we examined expression of DP1 and DP2 in the aortas of these mice, as the two genes are closely related, and compensatory up-regulation of DP2 might occur when DP1 is completely knocked out [21]. As expected, DP1 protein levels were reduced in a gene dose-dependent manner in DP1 KO mice (Figure 3A). Interestingly, we detected marked compensatory up-regulation of DP2 protein in aortas of AngII-infused DP1$^{-/-}$ mice, but not in WT or DP1$^{+/-}$ mice (Figure 3A), while no such compensatory changes were observed in the CaCl$_2$ application model (Figure 3B). These results suggest that loss of protection against AAA in DP1$^{-/-}$ mice infused with AngII may be due to compensatory up-regulation of DP2, which could also contribute to AAA formation.

Pharmacological inhibition of DP1 reduced AngII-induced AAA formation

Since data from DP1$^{+/-}$ mice showed potent protection against AAA formation in both animal models, we next investigated whether pharmacological inhibition of DP1 can also reduce AAA formation. LDLR KO mice were orally treated with laropiprant (3 mg/kg body weight), a selective DP1 inhibitor that has been used in clinical trials in humans [21,22] during AngII infusion for 3 weeks. Similar to that observed in DP1$^{+/-}$ mice, pharmacological inhibition of DP1 by laropiprant supplementation significantly decreased AAA incidence (Figure 4A) and abdominal aortic diameter (Figure 4B). Aortic elastin degradation, macrophage infiltration, MPO accumulation and MCP-1 expression were also markedly reduced by laropiprant treatment (Figure 4C,D and Supplementary Figure S1). Furthermore, laropiprant treatment dramatically reduced aortic MMP-2 and MMP-9 activities (Figure 4D). In contrast, Liropiprant treatment did not affect body weight, total cholesterol and triglyceride levels or blood pressure (Supplementary Figure S3).

Pharmacological inhibition of DP2 reduced CaCl$_2$- and AngII-induced AAA formation

Since we detected compensatory up-regulation of DP2 in DP1$^{-/-}$ mice, along with loss of protection against AAA formation, we next tested the effects of pharmacological inhibition of DP2 using fevipiprant, a selective DP2 inhibitor that was tested in phase three clinical trials for asthma, in the CaCl$_2$-induced AAA model. Treatment with fevipipirant efficiently reduced CaCl$_2$-induced AAA formation (Figure 5A), in association with diminished elastin degradation, aortic macrophage infiltration, MPO accumulation and MCP-1 expression (Figure 5B*). Furthermore, we also tested ramatroban, a dual antagonist for DP2 and thromboxane that has been used in Japan for treating allergic rhinitis, in AngII-induced AAA formation. Ramatroban therapy likewise showed a strong trend towards reducing the incidence of AAA and aortic diameter, in conjunction with adventitial inflammatory cell infiltration and elastin degradation (Supplementary Figure S4). These results suggest that DP2 also plays a role in AAA formation, consistent with the notion that antagonism of DP receptors is a promising therapeutic strategy for AAA.
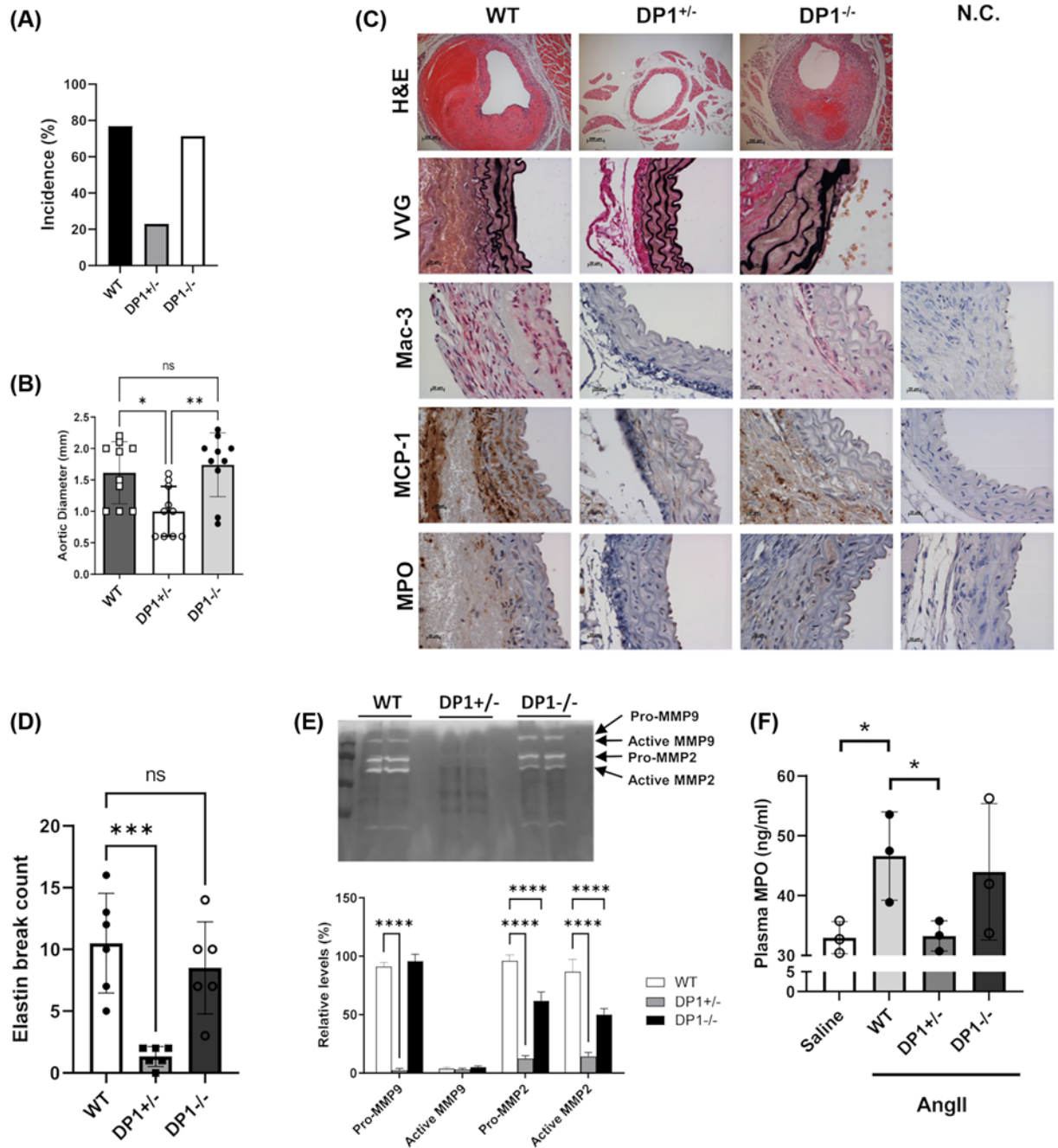
Figure 2. Heterozygous, but not homozygous, DP1 gene deletion protected against AngII-induced AAA formation

AngII was infused via osmotic minipump in DP1-deficient mice in the LDLR KO background. (**A**) AAA incidence (n=10–12). (**B**) Aortic diameter (n=10). (**C**) Representative histology; H&E, VVG (elastin fragmentation), Mac-3 (macrophages), MCP-1 and MPO. (**D**) Aortic elastin break count in AngII-infused mice (n=7). (**E**) Representative zymogram (upper panel) and quantified data (lower panel) for aortic MMP-2 and MMP-9 activities (n=6). (**F**) Plasma MCP-1 levels (ELISA, n=4–6). *$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$ vs WT. Data were analyzed using one-way ANOVA followed by Bonferroni's post-hoc analysis. Abbreviations: Mac, macrophage; N.C., negative control (absence of primary antibody).
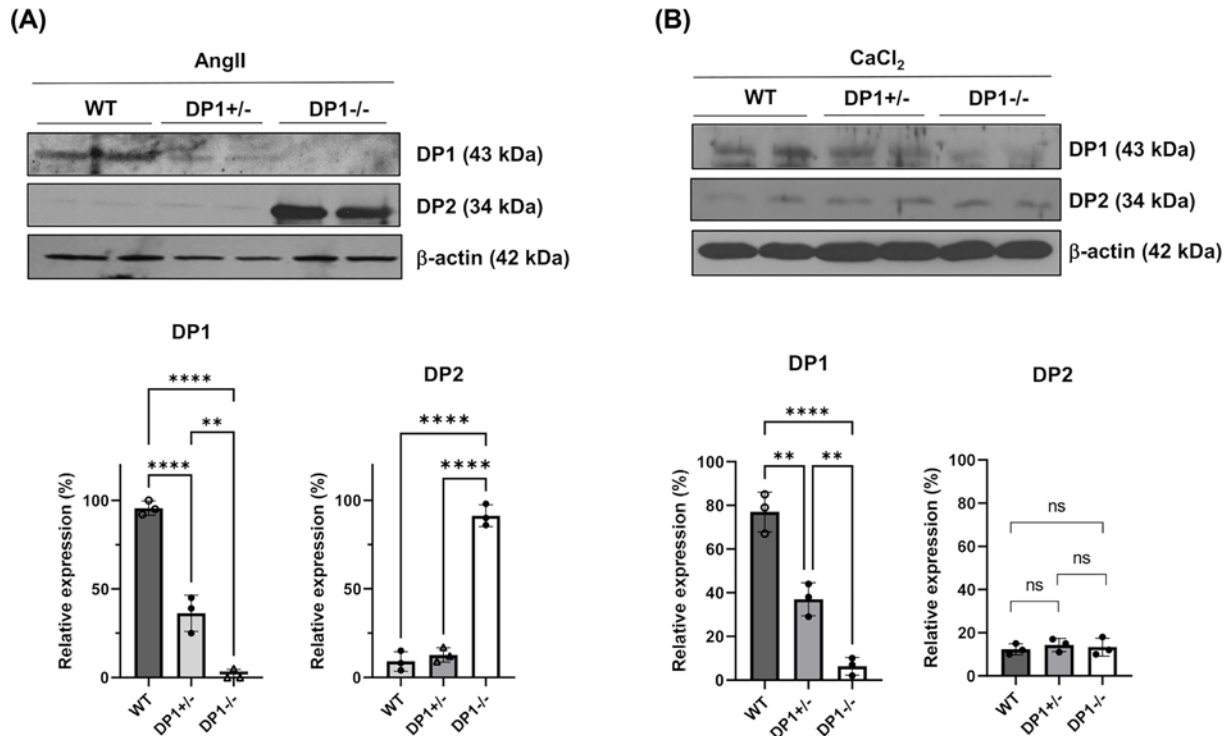
Figure 3. Compensatory up-regulation of DP2 protein in DP1$^{-/-}$ mice in the AngII infusion model, but not the CaCl$_2$ model, of AAA

(**A**) Representative Western blot images (upper panel) and quantification (lower panel, $n=3$) of aortic DP1 and DP2 protein levels in the AngII-induced AAA model. (**B**) Representative Western blot images (upper panel) and quantification (lower panel, $n=3$) of aortic DP1 and DP2 protein levels in the CaCl$_2$-induced AAA model. $*P<0.05$, $**P<0.01$, $***P<0.001$, $****P<0.0001$ vs WT. Data were analyzed using one-way ANOVA followed by Bonferroni's post-hoc analysis. Abbreviation: ns, not significant.

Discussion

Blocking selective PG receptors has been reported to abrogate pro-inflammatory responses in various tissues and organs, thus ameliorating inflammatory diseases. In the present study, using genetic and pharmacologic approaches in two distinct animal models of AAA, we report that blocking DP1 receptors prevents AAA formation, concomitant with reduced vascular inflammation. Interestingly, heterozygous, but not homozygous, deletion of DP1 prevented AngII-induced AAA formation. Deletion of both alleles of DP1 led to up-regulation of aortic DP2 and loss of protection against AngII-induced AAA. In contrast, in the CaCl$_2$ application model, loss of either one or both DP1 alleles strongly protected against AAA formation, and no compensatory up-regulation of DP2 expression was observed. Taken together, these findings suggest that DP2 up-regulation may compensate for loss of DP1 to promote AAA. Indeed, a selective pharmacologic inhibitor of DP2 prevented AAA formation. Thus, our data suggest that a nonselective DP1/DP2 antagonist could be an attractive agent to test for efficacy in pre-clinical trials of AAA.

Emerging evidence suggest that various PG signaling pathways are associated with the pathogenesis of AAA. Whole genome-expression profiling study demonstrated that COX-2, a PG-producing enzyme, is one most highly expressed genes in human AAA tissues [23]. Moreover, COX-2 pharmacological inhibitors attenuated AAA progression in hyperlipidemic mice [24,25] and blocked secretion of inflammatory PGs and cytokines from human AAA tissue explants [26,27], suggesting that PG signaling plays a role in AAA. However, PGs can have opposing pro- and anti-inflammatory effects, and PG receptor cross-reactivity has been reported, suggesting that targeting specific pro-inflammatory PG receptors is a more promising strategy than COX inhibition to treat AAA. Among various PGs, PGE$_2$ and its receptors are abundantly expressed [3], and their role in inflammatory disease has been well characterized. PGE$_2$ has also been implicated in vascular wall remodeling via regulating MMP activities in human AAA, and anti-inflammatory drugs that inhibit PGE$_2$ synthesis prevented aortic fibrosis, thereby protecting the aorta from expanding [28]. Furthermore, expression of EP4, one of the PGE$_2$ receptors, is associated with human and mouse AAA [4], and pharmacological inhibition or gene deletion of EP4 inhibited AAA formation in mice [29,30]. Moreover,
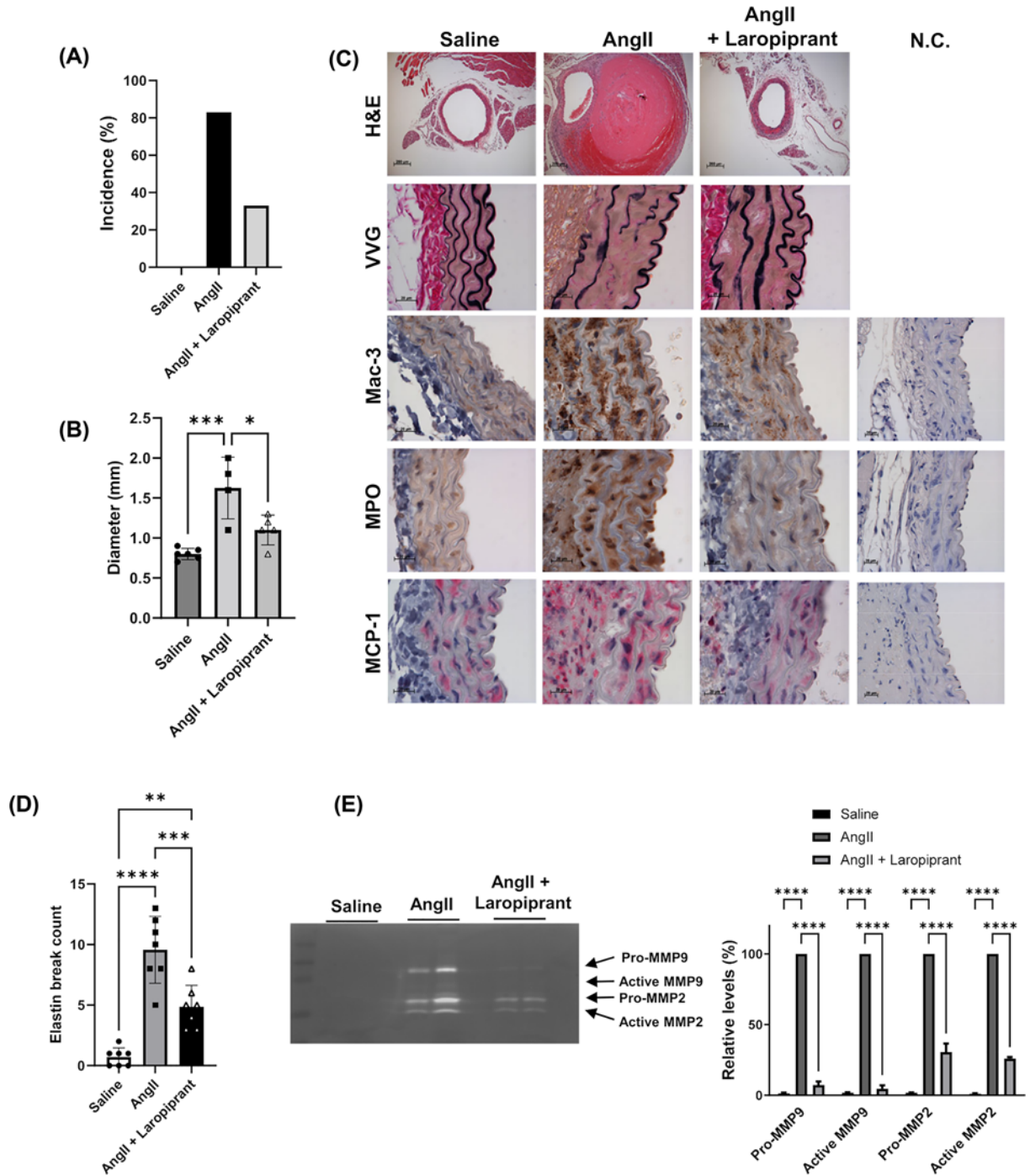
Figure 4. Pharmacological inhibition of DP1 reduced AngII-induced AAA formation

AngII was infused via osmotic minipump in LDLR KO mice treated with or without laropiprant (3 mg/kg body weight) in the drinking water. (**A**) AAA incidence (*n*=7), (**B**) aortic diameter (*n*=4–6). (**C**) Representative histology; H&E, VVG (elastin fragmentation), Mac-3 (macrophages), MPO and MCP-1. (**D**) Aortic elastin break count in AngII-infused mice (*n*=7). (**E**) Representative zymogram (left panel) and quantified data (right panel) for aortic MMP-2 and MMP-9 activities (*n*=6). *$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$ vs WT. Data were analyzed using one-way ANOVA followed by Bonferroni's post-hoc analysis. Abbreviations: Mac, macrophage; N.C., negative control (absence of primary antibody).
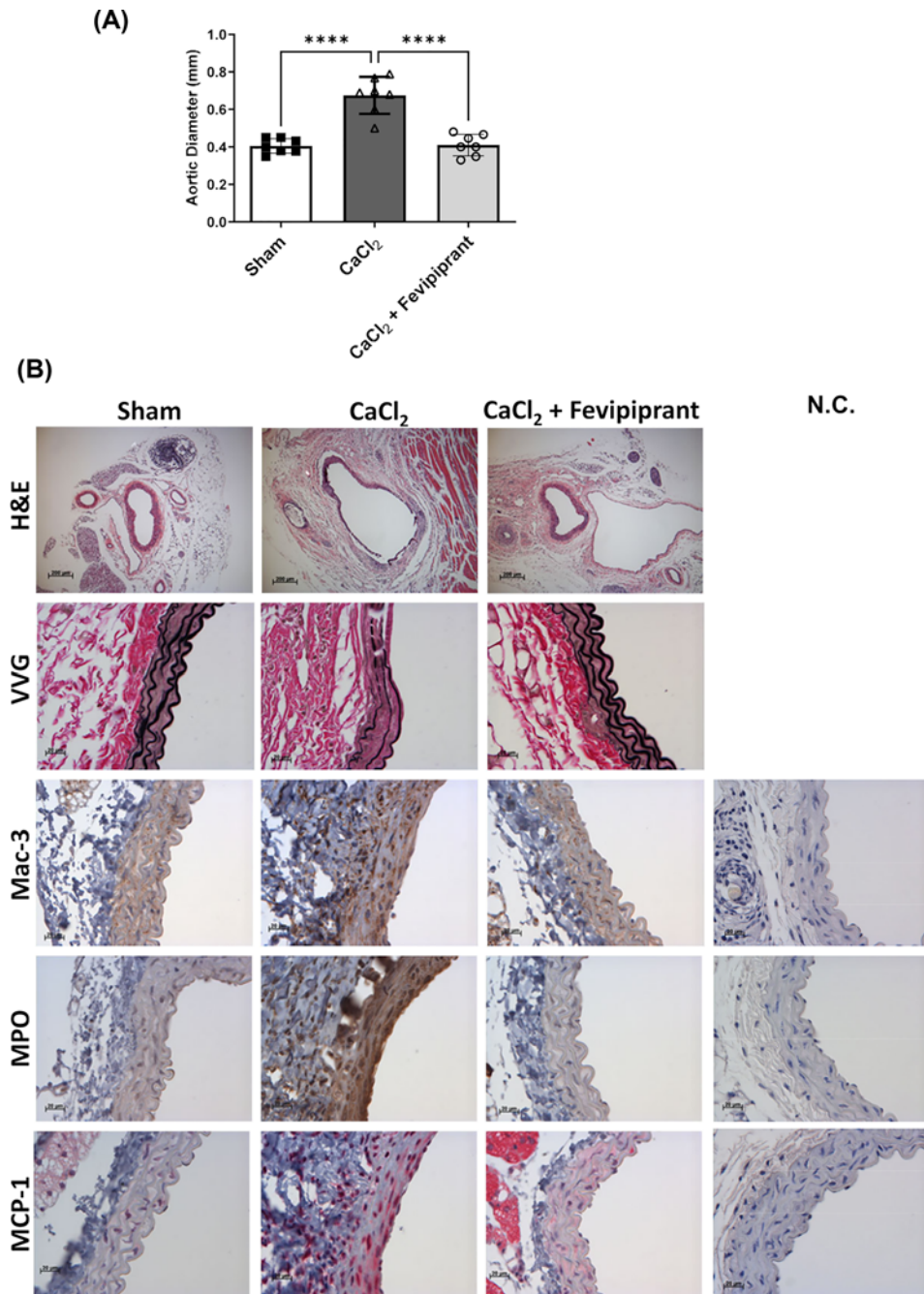
Figure 5. Pharmacological inhibition of DP2 reduced CaCl$_2$-induced AAA formation

CaCl$_2$ was applied to the infrarenal aortae of C57Bl/6 mice treated with or without fevipiprant (10 mg/kg body weight) in the drinking water. (**A**) AAA diameter ($n=7$). (**B**) Representative histology; H&E, VVG (elastin fragmentation), Mac-3 (macrophages) and MPO. *$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$ vs WT. Data were analyzed using one-way ANOVA followed by Bonferroni's post-hoc analysis. Abbreviations: Mac, macrophage; N.C., negative control (absence of primary antibody).

VSMC-specific deletion of EP4 was reported to reduce AAA formation in response to AngII infusion [31]. Interestingly, deletion of one EP4 allele in VSMCs protected against AAA formation in response to AngII infusion, while deletion of both alleles exacerbated it [31,32], which is similar to our findings with DP1. Moreover, homozygous deletion of EP4 in bone marrow-derived cells also increased AngII-induced AAA formation [33]. However, the underlying mechanisms of these discrepancies were not investigated in prior studies. Based on our results, we speculate

that homozygous EP4 gene deletion might have induced compensatory up-regulation of other PGE_2 receptors, such as EP1, EP2 and/or EP3, which in turn might promote AAA formation. Data from previous and the present studies emphasize the importance of testing heterozygous mice and exercising caution when interpreting results obtained in mice deficient in both alleles of PG receptors.

Specific variants of DP1 have been found to be associated with lower risk of asthma in genetic studies [34]. Moreover, mice deficient in DP1 were found to develop significantly decreased asthmatic responses in an ovalbumin-induced asthma model [6]. In a guinea pig model, a selective DP1 antagonist, S-5751, reduced antigen-induced nasal blockage, plasma exudation in the conjunctiva, and inflammatory cell infiltration into the upper and lower airways [35]. TM30089, a selective DP2 antagonist, also inhibited several typical asthma pathologies, including peribronchial eosinophilia and mucus cell hyperplasia [36]. Various pharmacological inhibitors of PGD_2 signaling have been developed and tested in pre-clinical and clinical studies for allergic inflammation and asthma. For example, pretreatment with the DP1 antagonist laropiprant at a dose of 25 or 100 mg daily for 3 days inhibited nasal congestion induced by installation of PGD_2 [22]. However, Philip et al. reported that laropiprant did not demonstrate efficacy in patients with asthma or allergic rhinitis [23], suggesting that targeting DP1 for airway disease may not be clinically useful. Fevipiprant, a selective DP2 inhibitor, has also been tested in clinical trials and showed acceptable safety and tolerability in Phase II studies [14]; however, the drug recently failed to achieve clinically relevant endpoint reductions in patients receiving current standard-of-care treatment for moderate-to-severe asthma [37]. Moreover, AMG853, a selective dual antagonist of DP1 and DP2, also failed to improve asthma symptoms or lung function in patients [38], further suggesting the futility of targeting DP receptors in airway inflammation and asthma. Despite these disappointing clinical results, these particular drugs have been proven safe and well-tolerated in humans. Additionally, ramatroban is commercially available and currently used to treat allergic rhinitis in Japan [39]. Repurposing these agents to test for efficacy against AAA in pre-clinical studies thus appears to be an attractive and feasible goal.

Our findings demonstrate for the first time that antagonizing either DP1 or DP2 receptors prevents AAA formation in two distinct animal models of AAA, in association with reduced aortic inflammation and matrix metalloproteinase activity, and independent of effects on blood pressure or serum lipid levels. Blocking either DP1 or DP2 appears sufficient to prevent AAA formation, suggesting that the signaling pathways lack redundancy. Nevertheless, under some circumstances, DP receptor subtypes may be able to compensate for each other, which can complicate interpretation of experimental data. Moreover, functional DP receptor compensation should be taken into consideration when designing pre-clinical or clinical studies of DP inhibitors for inflammatory and allergic diseases.

Study limitations

In the present study, we tested gene deletion and pharmacological inhibition of DP receptors in AAA formation using two different mouse models. However, these animal models do not perfectly mimic all pathological aspects of AAA in humans. Furthermore, while we demonstrated that the pharmacological inhibitors could prevent AAA formation, we did not test their efficacy against AAA growth/rupture when applied to established models of AAA formation. Thus, further investigations will be required to establish pre-clinical efficacy for AAA treatment in humans.

Conclusions

We provide novel evidence that inhibition of DP receptors can protect against AAA formation in mouse models. Since pharmacological inhibitors of DP receptors have been developed and extensively tested in allergic disease and asthma, there is a large body of existing safety data surrounding these drugs in humans. It may be possible to repurpose DP inhibitors to test their efficacy in patients with AAA. Based on our own data and data from other studies demonstrating compensatory up-regulation of PG receptors, a non-selective DP1/DP2 antagonist may be the most promising strategy for future pre-clinical and clinical investigations.

Clinical perspectives

- DP1 heterozygous- and homozygous-deficient mice are protected against $CaCl_2$-induced AAA formation.

- DP1 heterozygous mice, but not homozygous mice, are protected against AngII-induced AAA formation; this loss of protection against AAA formation when both DP1 alleles are deleted is associated with compensatory up-regulation of DP2.

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- Blocking activation of either DP1 or DP2 by selective pharmacological inhibitors significantly reduces AAA formation and PGD_2 receptor antagonists that have been tested in late-stage clinical trials for allergic diseases and asthma could potentially be repurposed to treat AAA.

Data Availability

The data associated with the present study will be available upon request to the corresponding authors.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Funding

CRediT Author Contribution

Neal L. Weintraub: Conceptualization, Resources, Data curation, Formal analysis, Supervision, Funding acquisition, Investigation, Methodology, Writing—original draft, Project administration, Writing—review & editing. **Andra L. Blomkalns:** Conceptualization, Data curation, Validation, Investigation, Methodology, Writing—review & editing. **Mourad Ogbi:** Formal analysis, Validation, Investigation, Methodology. **Tetsuo Horimatsu:** Formal analysis, Validation, Investigation, Visualization, Methodology. **Tyler W. Benson:** Formal analysis, Validation, Investigation, Methodology. **Yuqing Huo:** Validation, Writing—review & editing. **David J. Fulton:** Resources, Validation, Writing—review & editing. **Gautam Agarwal:** Methodology, Writing—review & editing. **Richard Lee:** Validation, Methodology, Writing—review & editing. **Michael A. Winkler:** Formal analysis, Visualization, Writing—review & editing. **Lufei Young:** Resources, Validation, Writing—review & editing. **Ken Fujise:** Validation, Methodology, Writing—review & editing. **Avirup Guha:** Formal analysis, Validation, Writing—review & editing. **Tohru Fukai:** Resources, Validation, Writing—review & editing. **Masuko Ushio-Fukai:** Resources, Validation, Writing—review & editing. **Xiaochun Long:** Resources, Validation, Writing—review & editing. **Brian Annex:** Resources, Supervision, Project administration, Writing—review & editing. **Ha Won Kim:** Conceptualization, Resources, Data curation, Formal analysis, Supervision, Validation, Investigation, Methodology, Writing—original draft, Project administration, Writing—review & editing.

Abbreviations

AAA, abdominal aortic aneurysm; AngII, angiotensin II; $CaCl_2$, calcium chloride; COX, cyclooxygenase; DP, D prostanoid receptor; H&E, Hematoxylin and Eosin; KO, knockout; LDLR, low-density lipoprotein receptor; MCP, monocyte chemotactic protein; MMP, matrix metallopeptidase/metalloproteinase; MPO, myeloperoxidase; PG, prostaglandin; PGD_2, prostaglandin D2; VVG, Verhoeff–van Gieson; WT, wildtype.

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