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Platelet Inhibitors Reduce Rupture in a Mouse Model of Established Abdominal Aortic Aneurysm

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Abstract

Objective—Rupture of abdominal aortic aneurysms (AAAs) causes a high morbidity and mortality in the elderly population. Platelet-rich thrombi form on the surface of aneurysms and may contribute to disease progression. In this study, we used a pharmacologic approach to

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examine a role of platelets in established aneurysms induced by angiotensin II (AngII) infusion into hypercholesterolemic mice.

Approach and Results—Administration of the platelet inhibitors aspirin (ASA) or clopidogrel bisulfate to established AAAs dramatically reduced rupture. The mechanism of protection appears to be a reduction in abdominal aortic platelet and macrophage recruitment resulting in decreased active matrix metalloproteinases (MMPs) 2 and 9. Platelet inhibitors also resulted in reduced plasma concentrations of platelet factor 4, cytokines, and components of plasminogen activation system in mice. To determine the validity of these findings in human subjects, eligible aneurysm patients were retrospectively analyzed using developed and validated algorithms in the electronic medical record database at Vanderbilt University. Similar to mice, administration of ASA or $P2Y_{12}$ inhibitors was associated with reduced death amongst AAA patients.

Conclusions—These results suggest that platelets contribute to AAA progression and rupture.

Keywords

Abdominal aortic aneurysm; platelets; angiotensin II; clopidogrel; aspirin; mice

Introduction

Abdominal aortic aneurysms (AAA) affect 5-10% of the male and 1% of the female population over the age of 65 and is the 13th leading cause of death (estimated from 15,000 to 30,000 people, both primary and contributing) in the United States (1, 2). AAA is defined as a permanent localized dilation in the arterial wall with a diameter greater than 50% of normal (3). It is an inflammatory disease that is often associated with formation of an intramural clot. Rupture of AAA frequently causes death (4). In spite of a high incidence and catastrophic consequences, there is limited information regarding the sequence of events that lead to initiation, progression, and the eventual rupture of AAAs. Currently, the only treatment for AAA is surgical intervention after the aneurysm has reached a diameter of >5.5 cm (5, 6). Since AAA is categorized as a peripheral artery disease, it is currently recommended that patients with AAA start a regiment of low-dose aspirin (ASA) therapy (7, 8).

Infusion of AngII into hypercholesterolemic mice induces formation of AAA localized to the suprarenal aorta (9, 10). This model is highly reproducible and has been used to define mechanisms of vascular pathology associated with AAA (11-13). AngII infusion promotes elastin fiber destruction, proteolytic destruction of medial connective tissue, inflammation, atherosclerosis within the aneurysm, and rupture, which are all features that occur in human AAA (4, 14). Other mouse models of chemically-induced AAA have been developed that include exposure of the aorta to elastase (15) or calcium chloride (16). In addition, there is a xenograft rat model of AAA, which results in aortic dilatation and the presence of a mural thrombus in ~20% of rats (17). However, AngII infusion is the only consistent mouse model of aortic dilatation and rupture (3, 4, 18).

Platelets are required for hemostasis but also contribute to thrombosis and inflammation (19). Primary hemostasis results from platelet adherence to selected adhesive glycoproteins

in sub-endothelial matrix. Platelet activation, spreading, degranulation, and aggregation leads to formation of a platelet-rich hemostatic plug (reviewed in detail in (20)). Platelet activation occurs through stimulation of a variety of G protein-coupled receptors with soluble agonists, such as thrombin, adenosine diphosphate (ADP), and thromboxane A_2 (T_XA_2) (21). Activation of coagulation and production of thrombin activates platelets by cleavage of protease-activated receptors (Pars). Activated platelets release ADP and T_XA_2 that are required for sustained platelet activation and accumulation in a thrombus (21). ADP stimulates the ADP receptors $P2Y_1$ and $P2Y_{12}$ whereas T_XA_2 activates the thromboxane receptor (TP) (21). The uncontrolled growth of a platelet-rich thrombus can occlude the blood vessel and result in myocardial infarction and stroke. Patients at risk for thrombosis are treated with platelet inhibitors, such as ASA, which blocks TXA generation, and/or clopidogrel bisulfate, which inhibits $P2Y_{12}$ activation. In addition, the Par1 inhibitor, Vorapaxar, has recently been approved for treatment of patients with cardiovascular disease (22, 23).

Studies in a rat model showed that platelet inhibitors reduced abdominal diameter (dilatation) and incidence of experimental aneurysm, suggesting that platelets may enhance AAA (24, 25). Furthermore, patients with AAAs have an activated coagulation system and levels of thrombin generation correlate with the maximum diameter of the aorta in the patients (26-31). Importantly, platelets and platelet-specific secretions (soluble P-selectin, soluble CD40L, soluble glycoprotein V, and platelet-derived microparticles) are present in plasma of AAA patients and are specifically released from the luminal thrombus of an aneurysm (25). Despite these results, several meta-analyses and retrospective clinical trials reported no significant benefit of platelet inhibitors on aneurysm growth and incidence of rupture (32-34).

In this study, we investigated the effects of pharmacologic inhibition of platelet activation on aneurysms that were established by infusion of AngII. In addition, we evaluated the progression and rupture of AAA in patients with or without ASA or platelet inhibitors.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement

Study approvals

All mouse studies were performed with the approval of the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee (IACUC number 13-062.0). All analysis of human data was approved by the Institutional Review Board of Vanderbilt University Medical Center (IRB number 121802). All Clinical data was de-identified and obtained from patients at Vanderbilt University Medical Center Hospital in Nashville, TN.

Results

Effect of platelet inhibition on established AAAs

Most patients are treated with ASA after being diagnosed with an AAA (7, 8). To determine the effect of platelet inhibition on established AAAs, we generated AAAs in mice and then

administered ASA or clopidogrel bisulfate. *Ldlr*$^{-/-}$ mice were fed a HFD for 1 week prior to, and throughout AngII infusion for 28 days. Abdominal aortic diameters were measured by in vivo ultrasound and then mice were implanted with an additional 42 day AngII pump (Figure 1 A and D). Mice were divided into 4 groups: placebo versus ASA, and placebo versus clopidogrel bisulfate. ASA significantly reduced aracadonic acid-mediated integrin activation (data not shown) and completely suppressed plasma T_xB_2 (Supplemental Figure 1 A). Clopidogrel bisulfate administration resulted in attenuation of ADP-mediated integrin activation (Supplemental Figure 1 B). All mice had similar body weights, cholesterol, lipid fractions, and systolic blood pressures (Supplemental Table 1). We observed a decrease in abdominal aortic diameters with clopidogrel bisulfate or ASA versus placebo controls, but this was not significant (Figure 1 A and B, D and E). Similarly, mice treated with ASA or clopidogrel bisulfate had a non-significant reduction in aortic arch area and diameter of the thoracic aorta (data not shown). Importantly, both platelet inhibitors protected mice with established AAA from rupture-induced death versus placebo controls (Figure 1 C and F; ASA 0% versus placebo 50%; clopidogrel bisulfate 0% versus placebo 47%, $P < 0.01$). Further, all deaths were due to rupture of the suprarenal abdominal region of the aorta. Interestingly, both platelet inhibitors also reduced the visible thrombi in mice with aortic arch or thoracic aneurysms (data not shown; $P < 0.048$).

Platelet inhibitors decreased platelet and macrophage accumulation and MMP-2 and 9 activity in abdominal aortas

MMP-2 and 9 have been shown to contribute to the initiation and progression of AAAs (15, 16, 35). Macrophages are a primary source of MMP-9 and platelets contain both MMP-2 and 9 (36-38). Notably, we found that platelet and macrophage accumulation was decreased significantly in mice treated with platelet inhibitors versus placebo controls (Figure 2 A and C). Therefore, we examined whether platelet inhibitors reduced levels of MMP activity in the aorta. Importantly, MMP activity in the aorta was significantly decreased with platelet inhibitors (Figure 2 B and C).

To further characterize the effect of platelet inhibitors on MMP activity in the aorta, abdominal aortas were removed and levels of pro and active MMP2 and 9 were quantified by ELISA and visualized with gelatin zymography. Platelet inhibitors significantly decreased levels of active MMP-2 and 9 versus placebo controls (Figure 3 A-D). Interestingly, platelet inhibitors also decreased abdominal aortic tissue concentrations of total MMP2, with a non-significant decrease in total MMP9, as measured by ELISA (Figure 3 A-B). Importantly, the decrease in both MMP2 and MMP9 were significantly correlated with decreased macrophage ($r^2 = 0.835$) and platelet ($r^2 = 0.913$) counts in the AAAs of ASA or clopidogrel-treated mice (data not shown; $P < 0.001$ for all correlations).

Platelet inhibition decreased plasminogen activators and plasma cytokines in AngII infused mice

Plasmin generation by the plasminogen activators, uPA and tPA, is associated with increased levels of MMP-2 and 9 (39). We determined that total and active uPA and tPA in both plasma and abdominal aorta were significantly decreased by platelet inhibitors (Figure

4 C-F). Further, the endogenous inhibitor of uPA and tPA, PAI-1, was significantly increased by platelet inhibitors (Figure 4 A and B).

Platelets contain and secrete a variety of inflammatory and thrombotic molecules upon activation (40). We ascertained if anti-platelet therapy decreased secretion and circulation of these molecules. Circulating and abdominal aortic PF4 was significantly decreased by anti-platelet therapy (Figure 5 A). In addition, anti-platelet therapy significantly attenuated several platelet-derived cytokines, such as granulocyte colony stimulating factor (G-CSF), interferon gamma (IFN-γ), regulated on activation normal T cell expressed and secreted (RANTES), interleukin-1α and β (IL-1α, β; Figure 5 B-F). Several other plasma inflammatory cytokines were also significantly decreased by anti-platelet therapy, including: IL-4, IL-5, IL-6, IL-7, IL-12(p70), IL-13, IL-17, KC, MCP-1, MIP-1α and β, MIP-2, and TNF-α (data not shown). Importantly, the decreased level of abdominal aortic chemokines, cytokines, and plasminogen activators were all significantly correlated with decreased macrophage and platelet counts in the AAAs of ASA or clopidogrel-treated mice (data not shown; $P < 0.05$).

Treatment with $P2Y_{12}$ inhibitors and ASA significantly reduce rupture and dissection in aneurysm patients

A total of 1,578 eligible participants (non-missing data for all covariates) with aortic aneurysms (AAs; defined as either thoracic, abdominal, or thoracoabdominal) were identified totaling 5,592 years of person time with an average follow-up of 2.28 years per individual. In total, 351 AA dissections (227) or ruptures (124) were recorded. Summaries of drug categories, demographic, and vital characteristics are presented in Table 1.

Following a diagnosis of AAs, $P2Y_{12}$ inhibitors (HR = 0.49, 95% CI: 0.32, 0.74, p-value = 0.001) were significantly associated with decreased dissection or rupture after adjustment for vital (blood pressure, BMI), demographic (age, sex, race), and comorbid factors (diabetes, atrial fibrillation, heart failure, CKD; Table 1). This effect appears to be modified by whether the location of the aneurysm is thoracic versus abdominal, and the effect estimate in thoracic aneurysms is less protective and not statistically significant. ASA also protected against dissection or rupture (HR = 0.50, 95% CI: 0.35, 0.72, p-value = 1×10^{-4}) in adjusted analyses in both the thoracic and abdominal aorta Table 2. Kaplan-Meier plots of survival for each drug exposure are presented in Figure 6 A and B. Participants with at least 30 days of follow-up underwent sensitivity analyses, which did not substantively change event ratios (data not shown).

Discussion

A better understanding of the underlying pathophysiology in aneurysm disease is essential to develop non-surgical therapeutics to reduce the burden of this condition in our aging population. A prominent feature of human AAAs is the accumulation of a laminated mural platelet-rich thrombus that develops along the luminal surface (41, 42). Interestingly, a thrombus is a dynamic biological entity that is balanced between luminal renewal and abluminal fibrinolysis (24, 25, 30, 43). Importantly, clinical studies suggest that thrombus volume or blood displacement caused by presence of mural thrombus may be a predictor of

both AAA expansion and rupture (42, 44). Therefore, it is surprising that the role of platelets in the formation of mural thrombi and their potential contribution to both the progression of AAAs has not been investigated systematically in a mouse model. We found that inhibition of platelets reduced rupture of established AAAs. Furthermore, we found ASA or $P2Y_{12}$ inhibitors administration may protect human AAA patients from rupture. In summary, our results suggest that inhibiting platelet activation slows AAA progression and reduces AAA rupture, which may support the clinical use of ASA and $P2Y_{12}$ inhibitors in AAA patients.

ASA therapy is recommended for patients with AAA from the time of diagnosis until the perioperative period (7, 8). It is currently hypothesized that the benefit of ASA in reducing cardiovascular morbidity and mortality and potentially AAA progression outweigh the risks of bleeding and AAA rupture (7, 8). However, clinical studies examining the effects of platelet inhibitors (clopidogrel bisulfate and/or ASA) on non-genetically categorized AAA ruptures are extremely limited. Among a large meta-analysis of 6 studies, only one reported data with regard to AAA rupture (33). The UKSAT study reported that ‘anti-platelet’ administration resulted in a rupture rate hazard ratio of 0.83, which was not significant (33, 45). The mechanism by which ASA reduces aneurysm expansion is hypothesized to be via a decrease in thrombus formation, a reduction in aortic wall inflammation, and stabilization of the aortic wall (46). A small retrospective study reported patients with medium-sized AAAs had significantly reduced AAA expansion and time to aneurysm repair on low-dose ASA (46). Other studies have demonstrated a reduction in progression of small AAAs, though a definitive association between platelet inhibitors and aneurysm reduction was not established (34). However, a large meta-analysis demonstrated that ‘anti-platelet’ therapy resulted in a non-significant decrease in AAA growth compared to untreated aneurysm patients ($P = 0.241$) after adjusting for confounding variables (33). In our study, we observed protective associations between platelet inhibitors and AA rupture or dissection in 351 patients. We also found an inverse relationship between adverse AA events and non-ASA platelet inhibitor use, as well as an independent effect of ASA use. This finding indicates that addition of ASA or $P2Y_{12}$ inhibitors to standard therapy may be beneficial to AAA patients in addition to effects on other presumed cardiovascular diseases. In support of our findings, a phase 2 clinical trial is examining the efficacy of ticagrelor on patients with small AAAs (Government clinical trial identifier: NCT02070653).

To better mimic this clinical situation, we administered platelet inhibitors to mice with established AAAs. We found that prolonged AngII infusion increased rate of aortic rupture, which was significantly reduced with clopidogrel bisulfate or ASA. These inhibitors also had no effect on abdominal aortic diameter (clopidogrel bisulfate, $P = 0.19$ and ASA, $P = 0.08$). Other studies have shown that a GPIIa/IIIb platelet inhibitor (abciximab) and a P2Y12 receptor antagonist, (AZD6140) prevented aneurysm growth in the rat xenograft model of aneurysm (17, 24, 25). While this model does not exhibit rupture, it does exhibit an intraluminal thrombus similar to human aneurysms in a certain percentage of rats (17). We speculate that our results may be different because of the large amount of ruptures in our placebo groups, resulting in a lack of subsequent measurements of aortic diameters. Alternatively, a previous publication demonstrated a distinct difference between the incidence or maximal diameter of AngII-induced AAAs and increased mortality due to rupture (47).

The continued accumulation of platelets and macrophages may result in proteolytic destruction of the aortic architecture via release of MMPs. Platelets (36, 37) and macrophages (38) are a robust source of MMPs. Further, platelet-derived chemokines can regulate the expression of MMPs from VSMCs and macrophages (48, 49). In addition, plasmin production by uPA or tPA is a critical step in fibrinolysis and MMP activation (39). Here, we demonstrate clopidogrel bisulfate and ASA intervention reduce platelet and macrophage infiltration into the vessel wall, circulating platelet-derived cytokines, plasminogen activators, and ultimately the amount of active MMP-2 and MMP-9 in the abdominal aortas. Importantly, MMP-2 and MMP-9 are correlated with increased aneurysmal disease and rupture (16, 50). Further, several of the attenuated cytokines and chemokines play a significant role in AAA progression (51-54). Additionally, there is a role for the uPA, uPAR, tPA, and PAI-1 plasminogen axis in both the progression and rupture of experimental aneurysm (47, 55-57). While it is uncertain whether these MMPs, cytokines, and plasminogen activators/inhibitors are primarily derived from platelets or macrophages, there are significant correlations between platelets or macrophages amongst all of these inflammatory mediators. However, there are several contradictions in the literature regarding the role of Th1/Th2 chemokines and cytokines and MMPs with regard to the outcome of AAA pathogenesis (58, 59). Indeed, the differences in cytokine and MMP profile only reflect a single stage of AAA development/degeneration thus complicating a proper analyses and interpretation of these correlations.

In conclusion, we show that platelet accumulation and activation is detrimental in a mouse model of established AAAs. The pathological role appears to involve macrophage recruitment and the production of MMPs resulting in vessel instability and rupture. We further show a positive association with platelet inhibitors and ASA in the prevention of human AA rupture or dissection. The results indicate that platelet inhibitors are beneficial in pre-existing aneurysms. Future studies will be directed at dissecting the role of platelet signaling in AAAs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Nonstandard Abbreviations and Acronyms

AA	aortic aneurysm
AAA	abdominal aortic aneurysm
ADP	adenosine diphosphate
AngII	angiotensin II
ASA	acetylsalicylic acid (aspirin)
BMI	body mass index
CKD	chronic kidney disease
G-CSF	granulocyte colony stimulating factor
IFN	interferon
IL	interleukin
Ldlr	low-density lipoprotein receptor
MMP	matrix metalloproteinase
PAI-1	plasminogen activator inhibitor 1
Par	protease-activated receptor
PF4	platelet factor 4
RANTES	regulated on activation, normal T cell expressed and secreted
tPA	tissue plasminogen activator
TP	thromboxane receptor
TxA2	thromboxane A2
uPA	urokinase plasminogen activator

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Significance

Abdominal aortic aneurysm (AAA) is a progressive expansion of the aorta which may result in catastrophic rupture and death. This cardiovascular disease is estimated to affect almost 10% of people over the age of 50 with an estimated 1 out of every 250 people affected. Despite decades of research, there are no clinically approved drug regimens for this disease with surgical intervention as the only approved therapy. Here, we demonstrate commonly used antiplatelet drugs prevents rupture of advanced AAAs in a mouse model. Antiplatelet therapy dramatically reduces the amount of destructive enzymes and tissue/circulating inflammatory proteins. Lastly, we verify this effect in a retrospective analysis of human aneurysm patients. These results identify platelets as a critical component of aneurysm rupture and suggests utilizing antiplatelet therapy may be beneficial in patients with AAA.

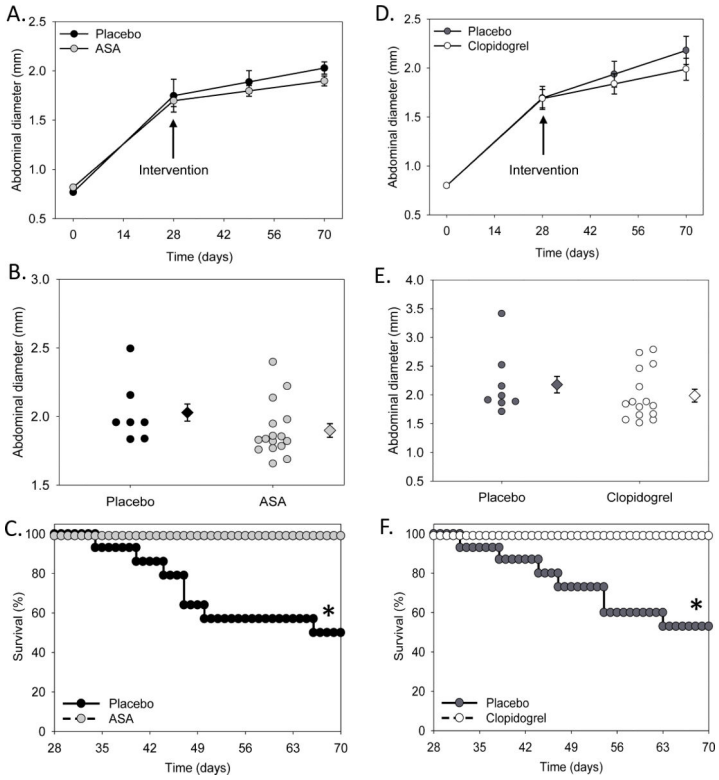
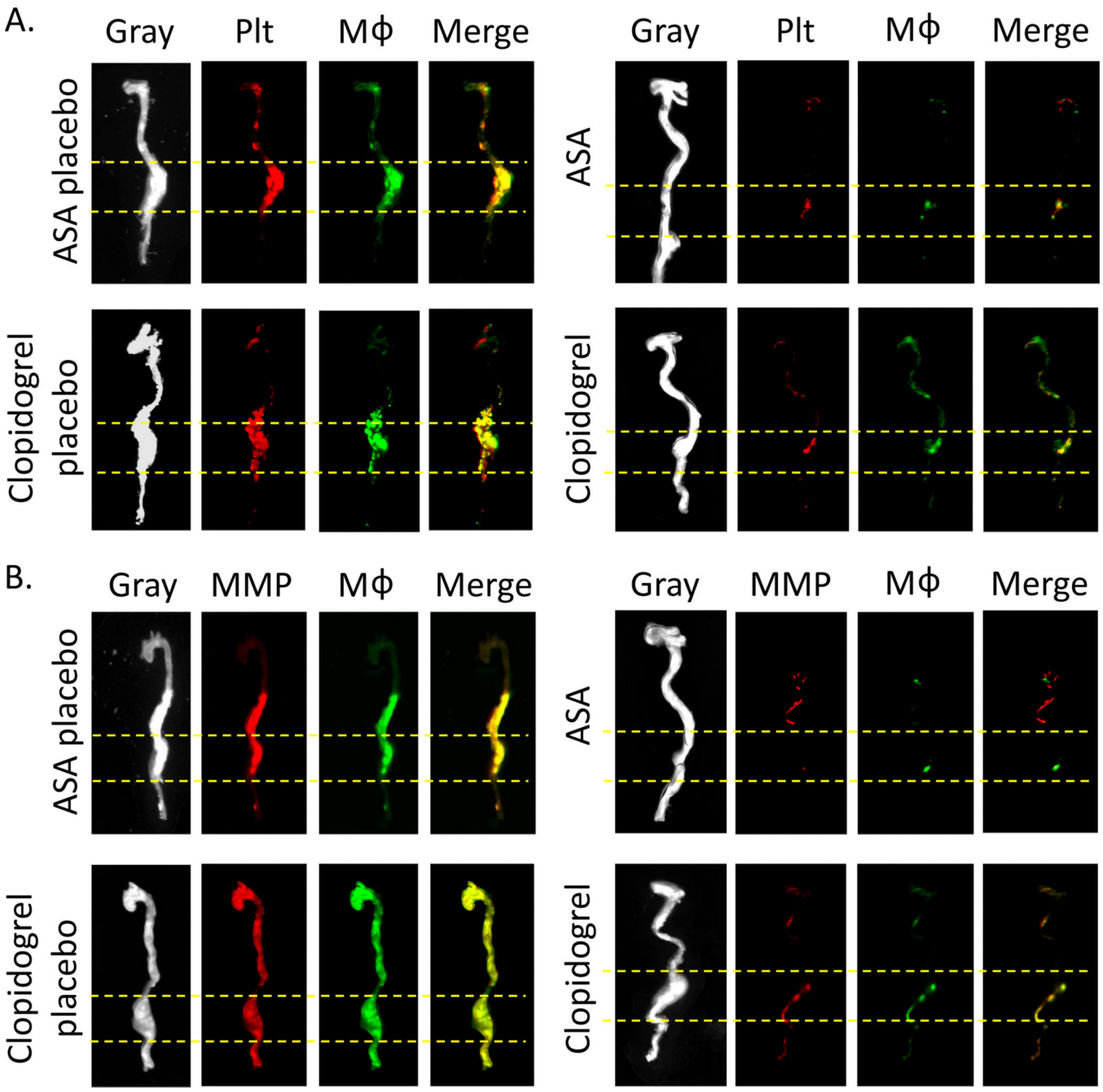


Figure 1. Platelet inhibition was protective against rupture of established AAAs in mice

Ldlr$^{-/-}$ mice were fed a HFD and infused with AngII for 28 days. Mice were then stratified based on in vivo suprarenal abdominal aortic diamters into 4 equal-sized groups and then placed on placebo (n = 14) or ASA (n = 16) and placebo (n = 15) or clopidogrel bisulfate (n = 15) and infused for an additional 42 days. Ultrasonically measured maximal luminal diameters of in vivo suprarenal aortas were measured at days 0, 28, 49, and 70 (A, D luminal diameters over time; B, E luminal diameters at day 70). Survival curves were also determined in these groups between days 28 and 70 (C, F). Circles represent group means ± SEM (A, D). Circles represent individual mice, diamonds represent means ± SEM (B, E). *P < 0.001 treatment groups versus controls. Data was analyzed with a Repeated Measures ANOVA, a Mann-Whitney Rank Sum with Dunn's post hoc, or a Kaplan Meier estimator.



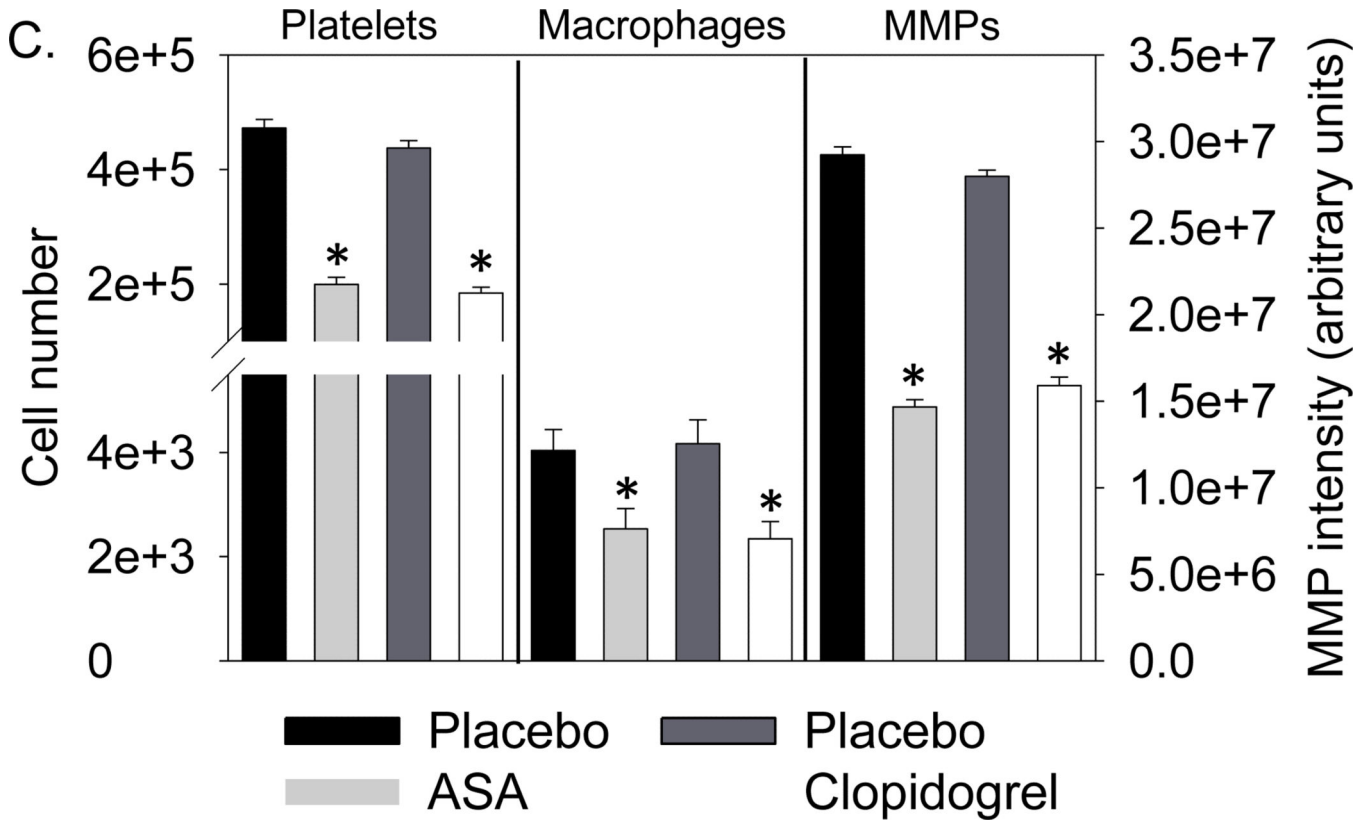


Figure 2. Platelet inhibition decreased platelet and macrophage accumulation and MMP activity in mouse abdominal aortas

Ldlr$^{-/-}$ mice underwent interventional therapy with placebo or ASA and placebo or clopidogrel bisulfate. Platelets (5 days before sacrifice) and macrophages (24 hours before sacrifice) were labelled with anti-GPIX conjugated 700nm fluorophore (red) or MMP 680-sense fluorophore (red) and dextran-coated nanoparticles conjugated to DyLight 800 fluorophore (green), respectively (treatments), or IgG placebo controls (controls). (A) Representative platelet, macrophage, merged, and grayscale images, (B) representative MMP, macrophage, merged, and grayscale images, and (C) subsequent quantification. Histobars represent means ± SEM of 4-8 mice. The abdominal aorta within the dotted yellow lines were analyzed for total fluorescent signal in panel C. *P < 0.001 placebo versus treatment groups. Data were analyzed with a One Way ANOVA on Ranks with Dunn's post hoc.

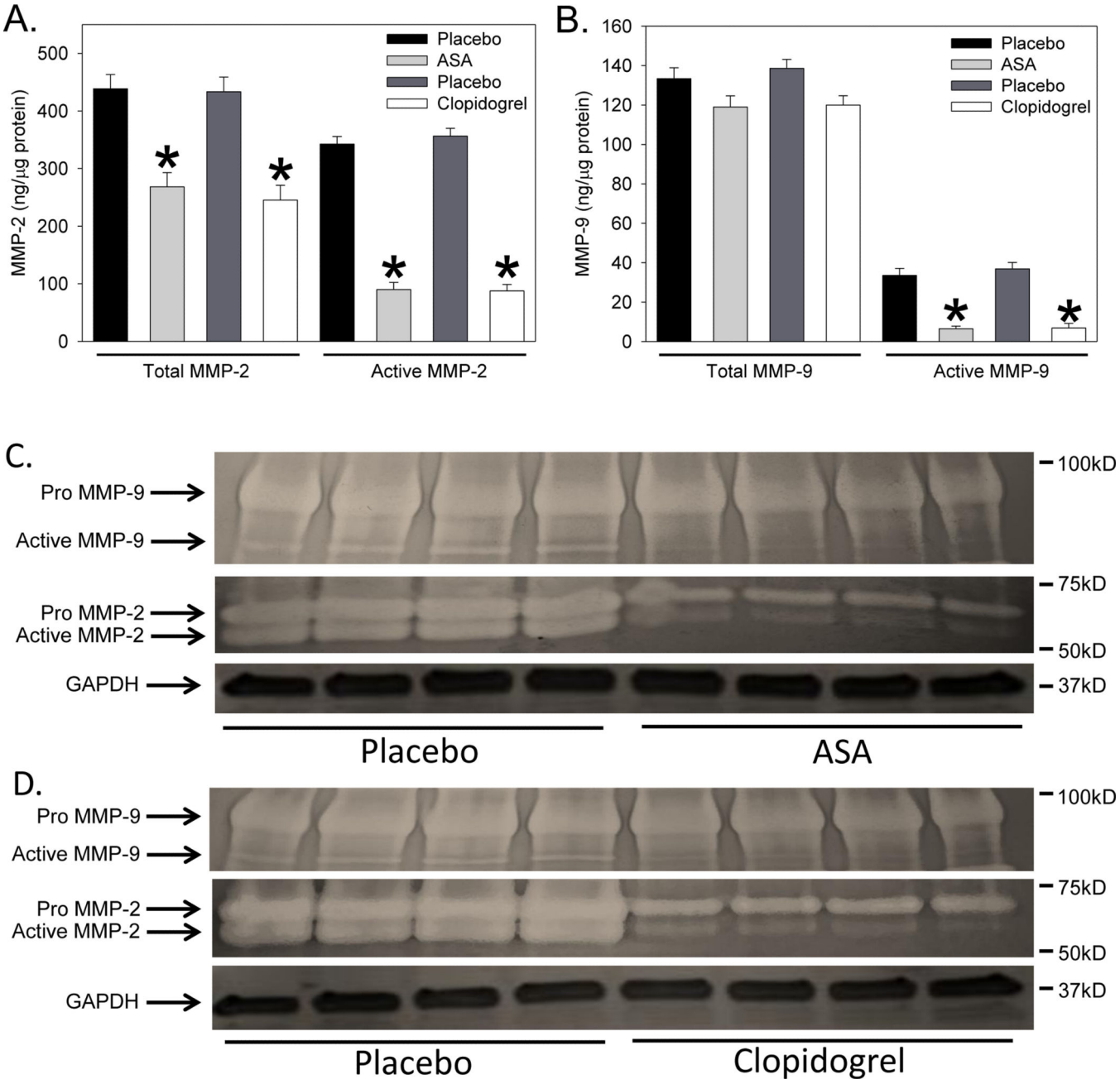


Figure 3. Active MMP-2 and MMP-9 was decreased in the abdominal aorta of mice receiving platelet inhibitors

Protein was harvested from pooled aortas obtained from the intervention study (3 aortas pooled for n =1; total different pools analyzed n = 4). Pooled lysates (1 μg total protein/well) were analyzed by MMP-2 (A) and 9 (B) Biotrak Activity Assay ELISAs. Protein (20 μg/lane) from clopidogrel bisulfate (C) and ASA (D) administered mice was also examined using gelatin zymography. Histobars represent means ± SEM of n = 4 pooled aortas. *P < 0.001 clopidogrel bisulfate and ASA total MMP-2, active MMP-2, and active MMP-9 versus placebo controls. Data were analyzed with a One Way ANOVA with a Holm-Sidak Post Hoc.

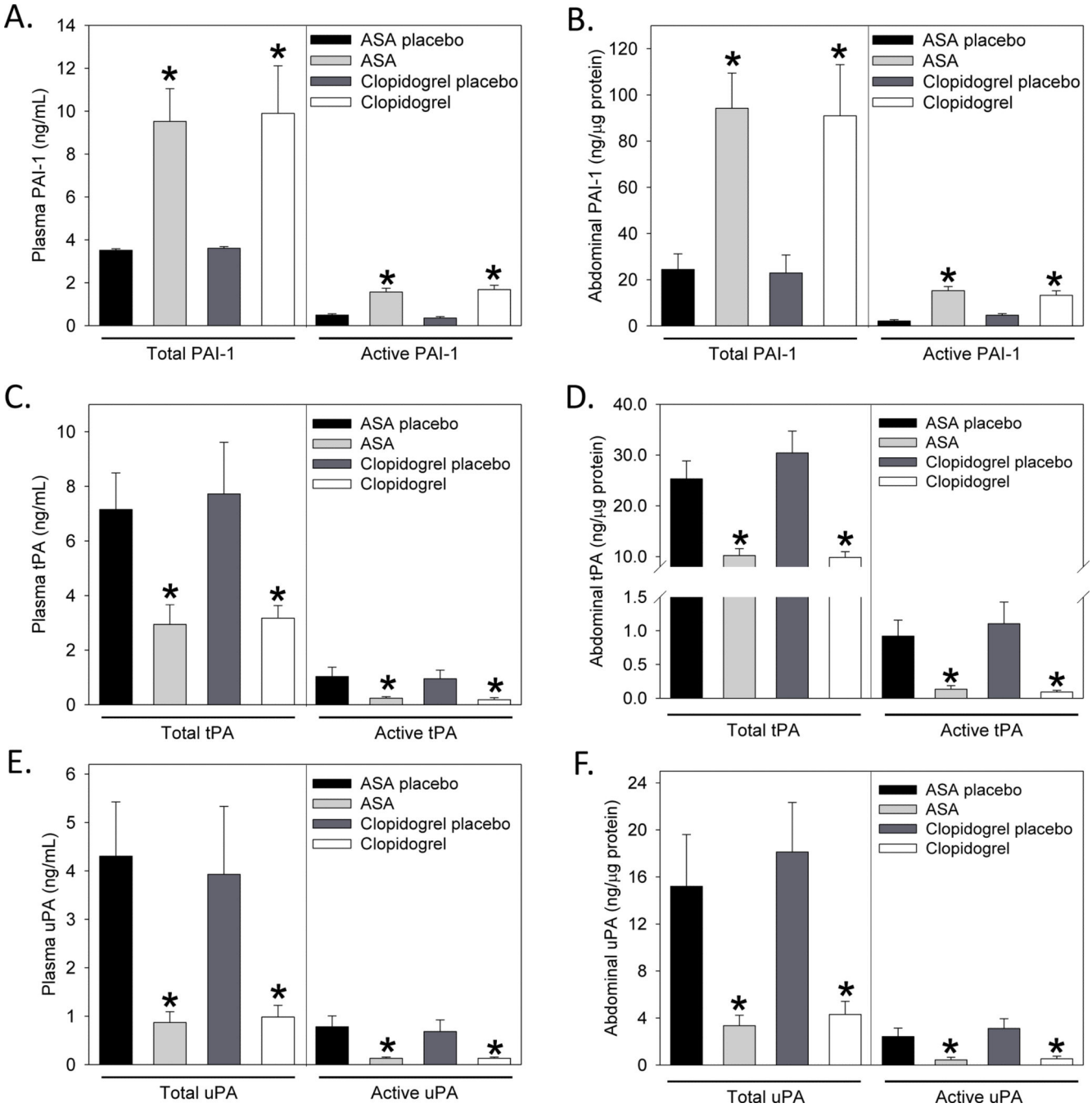


Figure 4. The plasminogen activation system was decreased in plasma and abdominal aorta of mice receiving platelet inhibitors

Protein was harvested from pooled aortas obtained from the intervention study (3 aortas pooled for n =1; total n = 4). Plasma (n = 14-16 each treatment group) was analyzed on total or active (A) PAI-1, (C) tPA, or (E) uPA ELISA plates. Protein (1 μg protein/well) was run on total or active (B) PAI-1, (D) tPA, or (F) uPA ELISA plates. Histobars represent means ± SEM of n = 14-16 plasma samples (A, C, and E) or n = 4 pooled aortas (n = B, D, or F). *P < 0.001 clopidogrel and ASA total and active PAI-1, uPA, and tPA versus placebo controls. Data were analyzed with a Two Way ANOVA with a Holm-Sidak Post Hoc.

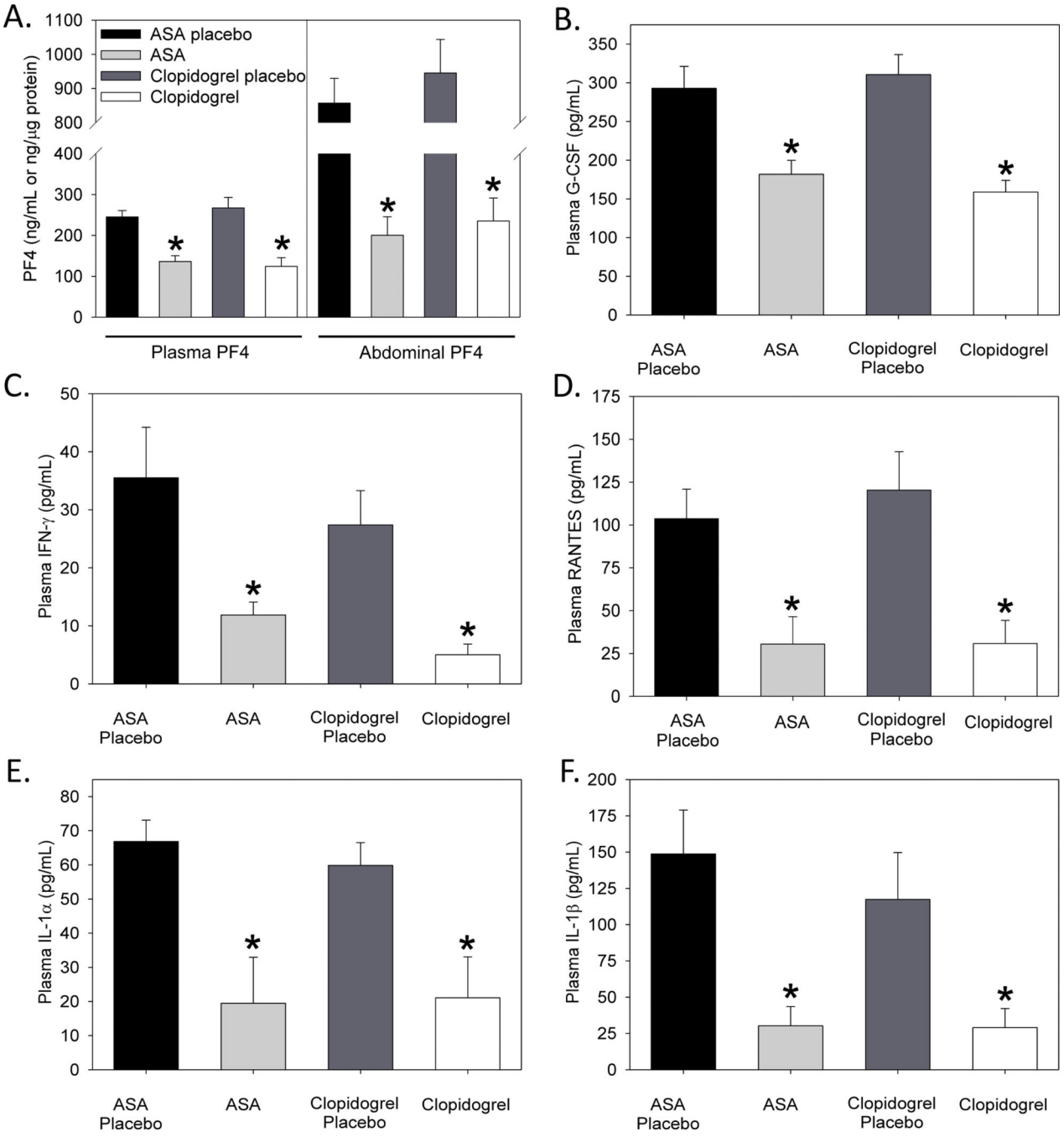


Figure 5. Platelet inhibition reduced plasma and tissue cytokines in established AAAs in mice

Protein was harvested from pooled aortas obtained from the intervention study (3 aortas pooled for n =1; total n = 4). Plasma (n = 14-16 each treatment group) or protein (1μg protein/well) was run on a PF4 ELISA (A). Plasma samples (n = 10 each treatment group) were analyzed on a chemokine/cytokine luminex array and (B) G-CSF, (C) IFN-γ, (D) RANTES, (E) IL-1α, or (F) IL-1β were quantitated. Histobars represent means ± SEM of n = 10 plasma samples (A-F) or n = 4 pooled aortas (A). *P < 0.001 clopidogrel and ASA

versus placebo controls. Data were analyzed with a One Way ANOVA on Ranks with Dunn's Post Hoc (B-F) or Two Way ANOVA with a Holm-Sidak Post Hoc (A).

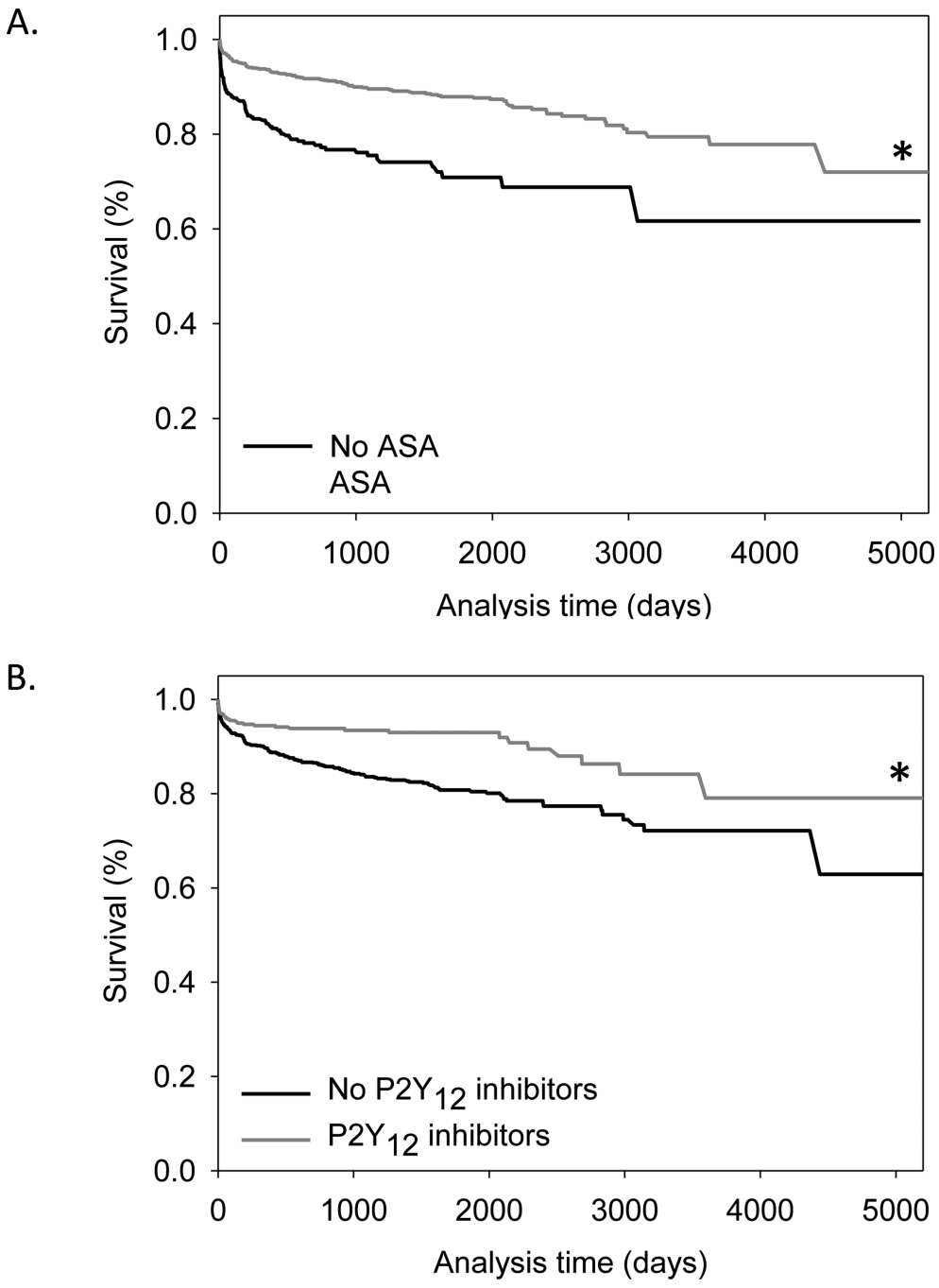


Figure 6. The effects of ASA and $P2Y_{12}$ inhibitors on AA rupture in a human cohort

Kaplan-Meier plot of adverse event-free rates stratified by (A) ASA and (B) platelet inhibitors and adjusted for age, sex, race, BMI, smoking, diabetes, CKD, dialysis, heart failure, and atrial fibrillation. *P < 0.001 drug therapy versus control. Data was evaluated with the proportional hazards test (PHtest) within the parameters of a kaplan-meier estimator in STATA and were found to be satisfied. All statistical tests assumed two-tailed distributions.

Table 1

Demographic, vital, and medication characteristics for AA individuals stratified by outcome

Characteristic	Events n = 351	Non-Events n = 1524	p-value*
European ancestry (%)	82.1	75.5	0.009
Female (%)	68.7	69.9	0.635
Age (mean [SD])	66.5(11.1)	67.6(10.9)	0.125
BMI (mean [SD])	28.0(5.81)	27.9(5.8)	0.667
Type II diabetes (%)	2.85	4.72	0.121
Smoking (%)	89.8	85.5	0.045
Atrial Fibrillation (%)	10.5	15.8	0.012
Heart Failure (%)	12.3	21.0	<0.001
Chronic Kidney Disease	8.83	13.5	0.017
Dialysis	3.42	2.10	0.141
AA Dissection (%)	64.6	NA	NA
AA Rupture (%)	35.4	NA	NA
Abdominal AA (%)	22.7	NA	NA
Thoracic AA (%)	17.7	NA	NA
Thoracoabdominal AA (%)	59.5	NA	NA
Anti-platelet drugs (%)			
$P2Y_{12}$ Inhibitors	11.7	25.9	<0.001
ASA	49.9	74.9	<0.001

*Categorical variables assessed with exact tests, continuous variables with student's t-test

Table 2

Cox proportional hazards regression analysis of drugs by class.

Term	HR All	95% CI	HR Abd.	95% CI	HR Thor.	95% CI
P2Y12 Inhibitors (crude)	0.36	(0.24-0.53)	0.21	(0.10-0.42)	0.42	(0.15-1.15)
ASA (crude)	0.35	(0.26-0.45)	0.28	(0.18-0.43)	0.27	(0.17-0.45)
P2Y12 Inhibitors (adjusted)	0.49	(0.32-0.74)	0.24	(0.12-0.51)	0.81	(0.27-2.41)
ASA (adjusted)	0.50	(0.35-0.72)	0.47	(0.22-1.00)	0.30	(0.15-0.60)

Adjusted models are fit with terms for age, sex, race, BMI, smoking, diabetes, heart failure, atrial fibrillation, chronic kidney disease, and dialysis.

Abd.— Abdominal; Thor.— Thoracic; HR — Hazard Ratio; CI — Confidence Interval