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Platelets, glycoprotein Ib-IX, and von Willebrand factor are required for FeCl₃-induced occlusive thrombus formation in the inferior vena cava of mice

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Abstract

Venous thromboembolism is a leading cause of death from cardiovascular disease. Despite the importance of the glycoprotein (GP) Ib-IX/von Willebrand factor (vWF) axis in arterial thrombosis, its requirement in venous, not venule thrombosis in response to endothelial injury (not stenosis or stasis) is uncharacterized. GPIb -vWF participation in FeCl₃-induced thrombus formation was evaluated in the inferior vena cava (IVC). Stable, occlusive thrombus formation in response to FeCl₃-induced injury of the IVC was studied. FeCl₃ (20% FeCl₃, 10 minutes)-induced occlusive thrombosis required platelets as confirmed by a lack of occlusion in thrombocytopenic mice, and stable occlusion in control animals. No IVC occlusion was observed using GPIb -deficient animals, a model of the human Bernard-Soulier syndrome (BSS). Transgenic IL-4R/GPIb mice (lack murine GPIb , but express the extracellular domain of the human interleukin (IL)-4 receptor fused to the transmembrane and cytoplasmic domains of human GPIb), were studied to determine if the absence of IVC occlusion in the BSS mouse was caused by GPIb extracellular domain deficiency rather than platelet BSS phenotype associated abnormalities. As with GPIb knock-out (KO) mice, no occlusion was observed in the IVC of IL-4R/GPIb mice. The IVC of vWF-deficient mice also failed to occlude in response to FeCl₃ treatment. The chimeric protein GPIb (2V)-Fc prevented occlusion, demonstrating that GPIb -vWF A1 domain interaction is required for FeCl₃-induced stable thrombus formation in the IVC. Therefore, FeCl₃-induced stable, occlusive thrombus formation in the IVC is platelet, GPIb -vWF interaction-dependent despite the large diameter and low venous flow rate in the IVC.

Keywords

GPIb ; IVC; Platelets; Venous Thrombosis; vWF/GPIb (2V)-Fc

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Declaration of Interest

The authors state that they have no conflicts of interest.

Introduction

Cardiovascular disease is the leading cause of death from disease in the Western world. The majority of those deaths are the result of pulmonary embolism following deep vein thrombosis (DVT) [1]. The combination of DVT and pulmonary embolism is termed venous thromboembolism (VTE) [2]. Approximately 295,000 deaths (data from 2006 the AHA statistics table) result annually from pulmonary embolism associated with VTE, which exceeds the annual number of deaths resulting from either myocardial infarction (approximately 171,000/year) or stroke (approximately 158,000/year) [1]. The incidence of VTE increases steeply at about 55–60 years of age [3]; therefore, the significance of venous thrombosis increases proportionally with the population of older persons.

The mechanism of arterial thrombosis has been studied in detail and is thought to be essentially different from the bases of thrombosis in veins [4]. Although coagulation [5] and inflammation [6] contribute to thrombosis in arteries and arterioles, inflammation is thought to culminate in plaque formation and rupture followed by platelet-dependent thrombus formation [4, 6–8]. Thrombosis in arteries and arterioles is glycoprotein Ib (GPIb)-dependent and therefore platelet-dependent [9–12]. The process primarily [13] – but not exclusively [14] – relies on platelet adhesion to immobilized von Willebrand factor (vWF). Although subendothelial vWF is not the only substrate capable of supporting platelet adhesion in arterioles [14], all thrombus formation in FeCl₃-treated arterial tissue is nonetheless dependent on GPIb [9–12].

In contrast, the mechanism of thrombosis in veins is less clearly understood – and may be more complex – than thrombosis in arteries. Thrombosis in veins is thought to result from a combination of altered blood flow (“stasis”/stenosis), coagulation and fibrinolytic abnormalities, inflammation of the endothelium and tissue factor activity [4]. In fact, venous thrombus formation is thought to occur on intact endothelia [4] at low shear rates; this may explain why venous thrombi are platelet-poor structures rich in red cells trapped in fibrin rather than the platelet-rich white arterial thrombi formed on disrupted endothelia at high shear rates. Accordingly, FeCl₃-induced occlusive thrombus formation in venules has been shown to not be absolutely dependent on GPIb, and therefore possibly not dependent on platelets [15]. This study was initiated to evaluate the GPIb/platelet requirement for FeCl₃-induced occlusive thrombus formation in the inferior vena cava (IVC).

Shear rate appears to have a profound influence on thrombus formation. The rate of platelet adhesion to immobilized vWF *in vitro* is a direct function of shear rate [16]. Binding of platelets to immobilized vWF tethers the platelets and the tethered platelets – either alone or in combination with shear stress – initiate a signal transduction cascade that results in platelet activation. Activated GPIIb/IIIa binds immobilized vWF thereby arresting tethered platelets at the site of injury [16, 17]. Platelet deposition on the platelets immobilized on the subendothelium is mediated partly by the interaction of platelet vWF with GPIb and activated GPIIb/IIIa on adjacent platelets [10, 17, 18]. The efficiency of this step *in vitro* – and presumably *in vivo* – is enhanced by arterial shear rates [17, 18]. Therefore, the growth of thrombi presumably occurs less extensively and more slowly at venous shear rates than under conditions of arterial shear rates.

The effect of shear rate on thrombus formation is complex. Although solution vWF cannot bind stably to GPIb under static conditions, *in vitro* and *ex vivo* studies have shown that platelets can bind to immobilized vWF at shear rates as low as 50 sec⁻¹ and 200 sec⁻¹ [17–19]; such shear rates are characteristic of veins and venules, respectively [20]. However, under these conditions “thrombus” formation/growth *in vitro* is much slower and less extensive than at high shear rates, presumably due to increased shear force enhancing the

ability of soluble vWF to bind GPIb [21, 22]. Therefore, *in vivo* growth of a platelet thrombus is expected to be slow and possibly insufficient to occlude veins due to the diameter of the vessel relative to the effects of shear rate on thrombus growth. Consequently, a related paradigm states that “under low shear conditions such as those found in larger arteries and veins, platelet adhesion to the vessel wall mainly involves binding to fibrillar collagens, fibronectin, and laminin” [23]. Although this paradigm is generally accepted and has greatly affected our thinking about venous thrombosis, this concept has not been tested *in vivo*; hence, it is not known whether stable, occlusive thrombus formation can be platelet, GPIb and/or vWF dependent in the IVC in response to FeCl₃-induced injury. This is an important consideration since stable thrombus formation in FeCl₃-treated venules is not GPIb -dependent [15]. Due to this lack of information, the previously-stated view that GPIb -vWF interactions are not important under conditions of low shear stress [23] and the increasing importance of venous thrombosis to our aging population, we characterized the requirement of platelets and the GPIb -vWF interaction in FeCl₃-induced total occlusion in the IVC of mice. Concurrent with this study, another group described the role of GPIb and vWF in the IVC in response to stenosis and stasis, but not vascular injury [24].

Materials and methods

Animals

Mice deficient in platelet receptor glycoprotein Ib and IL-4R transgenic mice were derived as described [25, 26]. The IL-4R/GPIb -tg mice lack murine GPIb and express the extracellular domain of the human IL-4 receptor fused with the transmembrane and cytoplasmic domains of human GPIb. The von Willebrand factor KO mice [27] were bred in our animal facility (University of Memphis) from breeding stock obtained from Jackson Laboratory, Maine. Heterozygotes of this KO strain have been backcrossed with C57Bl/6 mice for eight generations. All the mice strains were congenic. The mice were gender and age matched as closely as possible.

Ferric chloride-induced inferior vena cava injury

Mice were anesthetized using a mixture of oxygen and isoflurane (Baxter International Inc., Deerfield IL). The intact IVC was dissected free from surrounding tissue and placed on a paper strip covered with adhesive tape (13×3 mm), for support. Injury was induced as previously described [11, 12] with slight modification by topical application of a longitudinally-folded strip of Whatman No. 1 filter paper (14×3mm) saturated with 20 % ferric chloride [28] (J.T. Baker, NJ) for 10 mins at room temperature. Afterwards the tissue was rinsed with physiological saline to remove FeCl₃. Blood flow was monitored using a laser Doppler system equipped with a pencil probe [11, 12]; monitoring was initiated with the application of the FeCl₃ and lasted for 45 minutes. The definition of occlusion used was that described by Wang et al. [29]: blood flow decrease of 75% or more for at least 3 minutes. Stable occlusion was defined as occlusion that was not reversed by embolus formation subsequent to occlusion and the end of the observation period.

Platelet depletion

Fc receptor-independent decrease of mouse platelets was elicited by the injection of a mixture of rat anti-mouse GPIb monoclonal antibodies (mAbs) into the tail veins of mice [30]. Each isoflurane-anesthetized mouse was dissected in order to expose the IVC prior to treatment with anti-mouse GPIb mAbs (1 µg of the anti-GPIb antibodies {Emfret Analytics, Germany, Catalog # R300} per g of body weight was injected into a tail vein) to prevent excess bleeding. Less than 23% of the platelets remained within 15 minutes of injection of the antibodies (data not shown). FeCl₃ treatment of the exposed IVC was

initiated 15 minutes after injection of the anti-GPIb antibodies. As a negative control for platelet depletion, 1 µg of non-immune rat IgG (Emfret Analytics, Germany) per g of mouse was injected in a tail vein after exposing the IVC.

Histological studies

The tissue was fixed in 16 % paraformaldehyde for 24 hours at 4°C and afterwards further processed with increasing concentrations of ethanol (70% for 30 min, 95% 2×30 min, absolute ethanol 3×30 min). The tissue was then cleared with xylene 3×30 min, infiltrated with paraffin for one hour and subsequently embedded in paraffin blocks. Sections of tissues (5 µm thickness) were prepared and stained with Diffquick.

GPIbα(2V)-Fc

GPIb (2V)-Fc is a recombinant fusion between the amino-terminal 290 amino acids of GPIb (with valine substitutions at positions 233 and 239) and a mutated human IgG1 Fc. The mutations in Fc were engineered to minimize and abolish Fc receptor binding. The fusion protein was expressed in Chinese hamster ovary (CHO) cells and purified by Protein A chromatography. Surface plasmon resonance (SPR, BiaCore) demonstrated that the valine substitutions at positions 233 and 239 (originally glycine and methionine, respectively) in the GPIb domain of GPIb (2V)-Fc resulted in a 30-fold increase in affinity ($K_D=10$ nM) for the A1 domain of recombinant vWF compared with wild type (WT) GPIb. While the wild type did not appear to bind to full-length vWF in this experiment, the affinity (K_D) of GPIb (2V)-Fc for vWF was 3 nM. The enhancing effects of the two valine mutations on GPIb-vWF binding were consistent with naturally-occurring gain of function mutations in vWF [12]. Surface plasmon resonance experiments showed that the valine mutations had no impact on the binding of GPIb to -thrombin or Factor XI (data not shown).

Statistical analyses

Statistical analyses were done using Chi-square test from Microsoft Office Excel which involved comparison of two groups. Values of $p < 0.05$ were considered statistically significant.

Box Plots for the murine blood flow analyses

The box plots for the blood flow analyses were done using SPSS (Statistical Package for Social Sciences). SPSS was used for both control and KO mice.

Results

FeCl₃ treatment of the inferior vena cava causes platelet-dependent stable thrombus formation

Stable, occlusive thrombus formation can be induced in a murine carotid artery (CA) by treatment with FeCl₃ [9, 11, 12]. FeCl₃-induced stable thrombus formation in the CA is GPIb -dependent and therefore platelet-dependent [9, 11, 12]. Stable occlusion of blood flow as a consequence of FeCl₃-treatment was the end point of those studies. Thrombosis – not vasospasm – was shown to be the basis of occlusion of blood flow [12, 28]. Although a variety of studies have characterized FeCl₃-induced thrombus formation in mouse IVC [31–33], occlusion of blood flow in response to thrombus formation was not monitored; rather, the weights of thrombi were measured. As a prelude to investigating the role of GPIb in stable thrombus formation in the IVC, the efficacy of FeCl₃-treatment as an inducer of stable, occlusive, platelet-dependent thrombus formation in the IVC of WT mice was characterized. The platelet requirement for thrombus formation was evaluated by monitoring blood flow following FeCl₃-treatment of the IVC in the presence and absence of a mixture

of purified rat anti-mouse GPIb mAbs [30]. The control treatment was an injection of nonimmune IgG into the tail veins of mice subsequently treated with FeCl₃. Compared with the control, treatment of the mice with anti-GPIb mAbs resulted in depletion of at least 77% of the platelets (data not shown). FeCl₃-treatment of the IVC resulted in occlusion of blood flow in all control animals. Five of the 7 controls treated with nonimmune IgG underwent stable, total occlusion, 2 of the thrombi that caused total occlusion subsequently embolized prior to the end of the observation period. Two of the 7 FeCl₃-treated control veins underwent stable, partial occlusion; in other words, total occlusion and embolus formation did not occur during the monitoring period. In contrast, the FeCl₃-treated IVCs (n=7) of the mice containing the rat anti-GPIb mAbs did not occlude (Figure 1A). Occlusion in the control animals was caused by platelet-containing thrombi (Figure 1C) – not vasospasm – which was consistent with the platelet requirement for thrombus formation.

Platelet receptor GPIb α is required for stable, occlusive thrombus formation in FeCl₃-induced injury in mouse inferior vena cava

The data in Figure 1 demonstrate that FeCl₃-induced occlusion of the IVC is platelet dependent; consequently, the role of the platelet specific receptor GPIb in FeCl₃-induced stable thrombus formation in the IVC was also investigated. In order to evaluate the requirement of GPIb in venous (not venule) thrombus formation in the IVC, GPIb⁻ deficient [25] mice were included in the study. In agreement with the data reported in Figure 1, the average time to occlusion for WT mice in response to FeCl₃ treatment was 27 minutes (Figure 2). In contrast, no occlusion was observed in any of the FeCl₃-treated GPIb⁻ KO mice (n=6, Figure 2), (p = 0.00006). Interpretation of these results was complicated by the fact that GPIb⁻ KO mice exhibit platelet deficiencies similar to those of Bernard-Soulier patients [25]. The platelets of GPIb⁻ deficient mice are significantly lower in count (28% of normal) and larger (~3.6X) than normal platelets (macrothrombocytopenia) [25]. In order to evaluate the possibility that the lack of occlusion in GPIb⁻ KO mice was due to macrothrombocytopenia rather than GPIb⁻ deficiency, the response of mice lacking murine GPIb and instead expressing the extracellular domain of the human interleukin-4 receptor fused with the transmembrane and cytoplasmic domains of human GPIb (IL-4R/GPIb⁻tg mice) was characterized [26]. While these platelets lack the extracellular domain of GPIb, they contain the normal transmembrane and cytoplasmic domains of human GPIb and therefore are more normal in count (65%) and size (2X) than GPIb⁻ deficient platelets. Despite the respective increase and decrease in the number and size of platelets, the IVCs of the IL-4R/GPIb⁻tg mice failed to occlude (Figure 3) (P = 0.00006), thereby demonstrating that the extracellular domain of GPIb is required for stable, occlusive thrombus formation in response to FeCl₃-induced injury of the IVC and possibly in veins in general.

VWF is required for GPIb α -dependent thrombus formation in the mouse IVC

The paradigm for FeCl₃-induced arterial thrombosis is that GPIb mediates occlusive thrombus formation by typically – although not exclusively – binding to vWF on the subendothelium exposed by damage to the endothelium [10, 13, 14]. The data presented in Figures 2 and 3 demonstrate that stable, occlusive thrombus formation in the IVC resulting from FeCl₃-treatment is GPIb⁻ dependent; however, the requirement for vWF in this process has not been reported. Accordingly, vWF-deficient mice were evaluated in this system. The effect of the absence of vWF was unambiguous – none of the 7 vWF KO mice formed occlusive thrombi in response to FeCl₃-induced injury (Figure 4). Of the 7 vWF heterozygous (+/-) mice used as controls, five showed total occlusion while the remaining two showed a decrease in blood velocity but only partial occlusion. The lack of thrombus formation in all vWF KO mice demonstrated that vWF is required for FeCl₃-induced thrombus formation under venous shear rates. The prolonged time to occlusion for the vWF

heterozygous mice may suggest that both wild type alleles are required to support a shorter occlusion time. More work is required to resolve this issue.

VWF/GPIIb interaction is required for occlusive thrombus formation in the IVC in response to FeCl₃-induced injury

Although both GPIIb and vWF are both required for stable, occlusive thrombus formation in the IVC in response to FeCl₃-induced injury, our experiments did not determine whether or not GPIIb-vWF binding was required for this response. To examine this question, chimeric GPIIb (2V)-Fc was used as it prevents platelet GPIIb from binding to vWF [34]. GPIIb (2V)-Fc is composed of the amino terminal 290 amino acids of GPIIb – including two gain-of-function valine mutations (G233V and M239V) in the regulatory loop – and an IgG1 Fc. As a consequence of these valine mutations, GPIIb (2V)-Fc has significantly enhanced affinity for vWF compared to wild type GPIIb [34]. The inclusion of IgG1 Fc increased the *in vivo* stability of the chimera. GPIIb (2V)-Fc was used at a level of 10 µg/g of body weight; this amount was found to be optimal through trial and error. Hanks Balanced Salt Solution [HBSS] lacking GPIIb (2V)-Fc was used as a control. The data in Figure 5 show that infusion of GPIIb (2V)-Fc into the tail veins of mice prevented occlusive thrombus formation in response to FeCl₃-induced injury of the IVC, whereas HBSS had no effect.

Discussion

The data presented here demonstrate that FeCl₃ treatment of the IVC can induce platelet/GPIIb-vWF-dependent, stable, occlusive thrombus formation in mice. The significance of these observations is that platelets can form stable, occlusive GPIIb-vWF interaction-dependent thrombi even in a large vein under conditions of venous shear rates and therefore supports the hypothesis that platelets can play an important role in DVT and VTE despite the effects of shear rate on GPIIb-vWF interaction [16–19, 21, 22]. These data extend and confirm the results of Brill et al. which demonstrated that a vWF-dependent, vWF/GPIIb interaction mediated venous thrombosis in response to flow (stenosis and stasis) disturbance in the IVC [24]. Thus, results obtained from two different IVC thrombosis model systems confirm that shear rate may not be an essential component platelet/vWF mediated thrombus formation in the IVC.

This study provides new information directly demonstrating the requirement for platelets to support FeCl₃-induced occlusive thrombus formation in the IVC. Previous studies of FeCl₃-induced thrombus formation in the IVC did not measure blood flow occlusion [31–33], nor did they evaluate the requirements for platelets and their receptors for thrombus formation. Our data demonstrate that platelets, the extracellular domain of GPIIb and vWF are required for occlusive thrombus formation in the IVC in response to damage induced by FeCl₃. These observations extend those made using a flow disturbance model that provided direct and indirect evidence for the respective roles of vWF and platelets in thrombus formation in the IVC [24].

Our data are consistent with those obtained studying FeCl₃-induced thrombus formation in carotid arteries and mesenteric arterioles, but conflict with data obtained using venules. Studies of FeCl₃-induced occlusive thrombus formation in murine carotid arteries [9, 11, 12] and mesenteric arterioles [10] demonstrated a requirement for GPIIb. The shear rate in the mesenteric arterioles (~0.1 mm in diameter) was approximately 1300 sec⁻¹ [13]. GPIIb was found not to be required for FeCl₃-induced thrombus formation in mesenteric venules [15]. The shear rate for the venules was about 120 sec⁻¹, and the diameter of the venules (0.280 mm) was about 2.8-fold greater than the diameter of the mesenteric arterioles. Thus, in contrast to all other occlusion studies, GPIIb was not absolutely required for occlusive

thrombus formation in response to FeCl₃-induced injury in the venule study (Figure 5C in [15]). Rather, the presence of GPIb enhanced the rate of thrombus formation, although thrombus formation still occurred in the absence of GPIb after a delay of about 6 minutes (19.5 minutes versus 25 minutes). Those results prompted one group to posit “that vWF likely uses other adhesive receptors besides GPIb in thrombus growth under venous shear conditions” [15]. Interestingly, that group’s more recent study using GPIb (2V)-Fc [24] support our results which directly demonstrate the requirement for GPIb – not another adhesive receptor – in FeCl₃-induced occlusive thrombus formation in the IVC.

Although experimental results do not provide an unequivocal explanation for the conflicting GPIb requirements for venules and this IVC, an analogous lack of agreement (concerning recombinant fibrinogen, not GPIb) between FeCl₃-induced occlusion in arterioles and a lack of occlusion in the carotid arteries was explained primarily on the basis of differing vascular dimensions [28]. A similar explanation may account for the requirement for GPIb to support occlusive thrombosis in the IVC versus the lack of that requirement in venules. The diameter of the IVC in our isoflurane-anesthetized mice (n=12) was about 0.71 mm whereas the diameter of the mesenteric venules was about 0.280 mm [15]. Assuming that the morphology of the IVC was circular under those conditions, the cross-sectional diameter of the IVC was about 6X greater (2.5×2.5 mm) than that of the venules, meaning that a thrombus with about a six-fold greater cross-section is required to occlude the IVC than that required for occluding mesenteric venules. Whereas α IIb β 3 may be able to mediate the growth and stability of the thrombus in the venules, GPIb may also be required for mediating the growth and stabilization of the thrombus in the IVC. However, this argument does not explain the basis of initial attachment of the platelets to the damaged endothelium, which may have been mediated by GPVI [9]. In agreement with the former explanation, not only did platelets lacking the extracellular domain of GPIb fail to incorporate into FeCl₃-induced growing thrombi in mesenteric arterioles of WT mice (even though those platelets expressed α IIb β 3), α IIb β 3^{-/-} platelets expressing GPIb were incorporated efficiently into the growing thrombi [10]. In that situation α IIb β 3 might not have been able to mediate thrombus growth due to the high shear rate in arterioles relative to that in venules. Finally, although not measured in this study, an estimate based on relevant literature [35, 36] supports a shear rate of approximately 50–100 sec⁻¹ for the IVC; hence, differences in shear rates between the IVC and the venules (120 sec⁻¹) probably do not contribute significantly to the lack of a GPIb requirement in the venules. Further work is required to resolve this interesting situation.

The results presented here also conflict with data obtained from FeCl₃-induced injury studies in arterioles [13]. Thrombus formation in response to FeCl₃-induced injury in mesenteric arterioles was found not to be vWF-dependent [13]. Although vWF deficiency delayed thrombus formation in response to FeCl₃-induced injury in mesenteric arterioles, occlusion occurred at the sites of FeCl₃-induced injury in 50% of the mesenteric arterioles tested. Fibronectin was thought to compensate for the absence of vWF [14]. By comparison, our results reported that vWF was required for FeCl₃-induced occlusive thrombus formation in the IVC (Figure 4).

The data presented here clearly demonstrate that GPIb and vWF are necessary for the formation of stable, occlusive thrombi in the IVC in response to FeCl₃-induced injury. Our earlier characterization of FeCl₃-induced injury of the carotid artery demonstrated that GPIb-vWF binding was required for occlusive thrombus formation [11]; this interaction is thought to underlie most, if not all arterial thrombus formation. The data presented in Figure 5 obtained using GPIb (2V)-Fc demonstrate that GPIb-vWF binding is required for FeCl₃-induced stable thrombus formation in the IVC.

Our conclusion that GPIb-vWF interaction-dependent stable thrombus formation occurs under conditions of low shear extend the results of Brill et al. [24]. In that study, ligation of the IVC apparently causing cessation (stasis) of blood flow also resulted in thrombus formation being prevented by vWF deficiency and GPIb (2V)-F_C, respectively thereby demonstrating that GPIb-vWF binding-dependent thrombus formation can occur under conditions of blood flow stasis, possibly in the absence of damage to the endothelium. Our observations combined with those of Brill et al. [24] demonstrate that the concept that high shear rate is required to support thrombus formation dependent on GPIb-vWF interactions does not appear to be supported by a variety of *in vivo* observations. Presumably, vWF exposed on the luminal aspect of veins is configured such that stable GPIb binding occurs even under conditions of low shear rate [37]. Further work is required to resolve this issue.

In summary and conclusion, the data presented here demonstrate that FeCl₃-induced thrombus formation occurs in the IVC of the mouse in a platelet, GPIb-vWF-interaction dependent manner despite the large diameter and venous shear rates of the IVC. These results support the hypothesis that platelets can play an important role in DVT and VTE despite the low shear rates characteristic of veins. This latter point is supported by the data of Brill et al. [24].

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Figure 1. FeCl₃-induced thrombus formation in the IVC is platelet-dependent

(A) The tracings in this figure represent blood flow obtained from the mice treated with anti-mouse GPIIb mab (top panel) and the control rat IgG (bottom panel) after treatment with 20% FeCl₃ for 10 minutes. Blood flow was monitored for 45 minutes. The arrow represents the time of removal of the FeCl₃ containing strip of Whatman filter paper. (B) The graphs represent the box plots for the anti-mouse GPIIb mabs and the control rat IgG. The mean time for the occlusion for control mice was 25 minutes.

Figure 2. GPIb is required for FeCl₃-induced thrombus formation in the IVC

(A) The figures represent blood flow data obtained from GPIb^{-/-} mice treated with 20% FeCl₃ for 10 minutes. The arrow indicates the time of removal of the FeCl₃ strip. Blood flow was monitored for 45 minutes. (B) The graphs represent the box plots for the GPIb^{-/-} and WT mice. As shown in the box plot, the mean time of occlusion for the WT mice was 27 minutes.

Figure 3. The extracellular domain of GPIb is required for FeCl₃-induced thrombus formation in the IVC

(A) The figure represents tracings of the blood flow of IL-4R/GPIb^{-tg} and WT mice. All the mice were treated with 20% FeCl₃ for 10 minutes after exposing the IVC. The arrow indicates the time of the removal of the FeCl₃ strip. Blood flow was monitored for 45 minutes. (B) Box plots for the GPIb^{-/-} and WT mice. The mean time of occlusion for WT mice was 27 minutes.

Figure 4. VWF is required for GPIIb-IIIa-dependent thrombus formation in the IVC

(A) The figure represents the blood flow data for the VWF $-/-$ and WT mice in response to 20 % FeCl_3 treatment to the IVC for 10 minutes. Blood flow was monitored for 45 minutes. The arrow indicates the time for the removal of the filter paper saturated with 20 % FeCl_3 .

(B) Box plot for VWF $-/-$ and WT mice. The average time for occlusion for the WT mice was 20.8 minutes.

Figure 5. VWF/GPIb interaction is required for occlusive thrombus formation in the IVC in response to FeCl₃-induced injury

(A) The figure represents the blood flow data for control mice injected with Hanks Balanced Salt Solution (upper panel) and experimental mice injected with GPG-290 chimeric protein (lower panel) after treatment with 20 % FeCl₃ for 10 minutes. The arrow indicates the time of removal of FeCl₃ strip. Blood flow was monitored for 45 minutes. (B) Box plot for the GPG-290 chimeric protein and Hanks Balanced Salt Solution (control) injected mice. The average time of occlusion for the control mice was 17 minutes.