Coimmobilized Native Macromolecular Heparin Proteoglycans Strongly Inhibit Platelet-Collagen Interactions in Flowing Blood

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Abstract—We coimmobilized mast cell–derived heparin proteoglycans (HEP-PGs) of very high molecular weight (750 kDa) or unfractionated heparin (UFH) on coverslips together with collagen without altering the amount of immobilized collagen. Subsequently, platelet-collagen interactions were studied under both flowing and static conditions in D-phenylalanyl-D-prolyl-L-arginine chloromethyl ketone–anticoagulated blood and platelet-rich plasma (PRP), respectively. At a high shear rate (1600 1/s), the mean platelet deposition (PD) on collagen monomers was 7.5±6.1×10⁶/cm² (n=5). When the monomers were coimmobilized with UFH, PD was inhibited by 73% (2.0±1.2×10⁶/cm²), whereas HEP-PG completely blocked it (0.42±0.38×10⁶/cm²; P<0.05). Also, when collagen fibrils were used for coating, HEP-PG significantly inhibited PD. At a low shear rate (200 1/s) and under static conditions in PRP, the inhibitory effect of HEP-PG on PD was less marked. Inhibition of glycoprotein Ib/IIa did not affect PD on coimmobilized HEP-PG in contrast to coimmobilized UFH or collagen alone. As a sign of inactivation, platelets adhering to the HEP-PG surface released considerably less β-thromboglobulin than did those adhering to pure collagen. In summary, immobilized HEP-PG strongly inhibited PD on collagen by attenuating adhesion-induced platelet activation. The stronger effect on collagen monomers suggests the inhibition of glycoprotein Ia/IIa–mediated activation. (Arterioscler Thromb Vasc Biol. 2000;20:e113-e119.)

Key Words: collagen • heparin proteoglycans • mast cells • platelets

Acute thrombotic complications after vascular interventions remain a clinical problem despite better understanding of the response to injury and pathophysiology of atherothrombosis.¹ ² Recently, the selection of antithrombotic drugs has become more extensive, and several well-controlled studies have shown the efficacy of these systemic drug therapies.³ ⁴ Platelets interacting with exposed collagen in the vascular wall play a pivotal role in the regulation of hemostasis.⁵ If there is a defect in the platelet glycoprotein (GP) receptors or if the synthesis of collagen is impaired, bleeding occurs.⁶ However, after arterial injury, be it due to plaque rupture or to angioplasty, a far more prevalent and clinically demanding end state is unregulated platelet activation leading to thrombosis. Therefore, local control of the thrombotic response in vascular procedures would be highly beneficial not only acutely, but very likely also chronically, to limit plaque growth or restenosis subsequent to arterial injury. Thus far, however, local approaches to prevent arterial thrombosis have been rather limited.

Experimental studies have suggested that GP Ia/IIa (integrin α₂β₁) is the key receptor mediating platelet-collagen interactions.⁷ Clearly, then, our therapeutic attempts to prevent collagen-induced platelet activation should focus on this receptor. As yet, however, we have lacked therapeutic tools to interfere with this particular interaction in a clinical setting. Now it seems that mast cell–derived heparin could be such a tool. Mast cells, which are the only source of heparin in the body, are located throughout the connective and mucosal tissues. Heparin can be stored and secreted by these cells in the form of macromolecular proteoglycans (PGs).⁸ In the skin, where mast cells are especially numerous and appear around small vessels, there is a significant concentration of heparin with antithrombotic potential.⁹ Indeed, in the skin, platelet plug formation and subsequent thrombin generation were blunted on allergic activation of mast cells.¹⁰ Moreover, collagen-induced platelet activation can be inhibited by the very large (750-kDa) macromolecular heparin proteoglycans (HEP-PGs) derived from rat serosal mast cells.¹¹ This inhibition by soluble HEP-PGs involves their tight binding to von Willebrand factor (vWF) and relies on impaired GP Ia/IIa–mediated platelet activation.¹¹ ¹² Because mast cells are also present in the arterial intima and because they can release their contents into the surrounding collagen-containing matrix, our objective here was to assess whether immobilized mast cell–derived HEP-PGs can affect platelet-collagen interactions. We found that collagen-immobilized HEP-PGs strongly inhibited platelet activation and aggregation and that this inhibitory response was enhanced at a high shear rate. Thus, not only soluble, but also collagen-immobilized, HEP-PGs are strong physiological inhibitors of platelet-collagen interactions.
Methods

Isolation of HEP-PGs From Rat Peritoneal Mast Cells

The lavage method used for isolating mast cells from rat peritoneal cavities has been described elsewhere. After isolation, the mast cells were treated with compound 48/80 (Sigma Chemical Co) to induce their degranulation. The degranulated mast cells and their exocytosed granules, ie, granule remnants, were then sedimented by 2 consequent centrifugations to obtain granule-free supernatant. The concentration of the very large, soluble HEP-PGs (750 kDa; range, 500 to 1000 kDa) in the supernatant was determined by the Alcian blue method (Fluka, Buchs) or by Blyscan glycosaminoglycan (GAG) assay (Biocolor Ltd), after which the supernatant was divided into aliquots and frozen at −20°C until use.

Blood Collection, Preparation of Platelet-Rich Plasma, and Labeling of Platelets With [3H]Serotonin

The study was approved by an institutional review board. After they had given their informed consent, blood was collected from healthy volunteers (n = 49), who reported not having taken any medication during the previous 14 days. The volunteers avoided coffee, tea, and dietary fats in the morning before blood sampling but were not required to fast completely. The superficial cubital vein was cannulated (Venflon 2, 17-gauge; BOC Ohmeda AB), and 9 volumes of free-flowing blood was collected into 1 volume of 300 mmol/L D-phenylalanil-L-prolyl-L-arginine chloromethyl ketone (PPACK; Calbiochem-Novabiochem Co). Platelet-rich plasma (PRP) was prepared by centrifugation (180g, 12 minutes, 22°C). The final platelet count in PRP was adjusted to 300,000/L by the addition of platelet-poor plasma (300,000/L). The platelets in PRP were labeled with [3H]serotonin as previously described and validated. After being labeled, PRP was added to the remaining blood components to reconstitute the blood used in perfusions. To stabilize the blood, perfusion experiments were not started until 30 minutes had elapsed after reconstitution.

Preparation of Collagen-Coated (±Unfractionated Heparin or HEP-PGs) Coverslips for Studies of Platelet Interaction in PRP and for Perfusion Studies

The coverslips used for platelet interaction studies in PRP were coated with collagen type I extracted with acetic acid from bovine Achilles tendon and treated with pepsin to yield monomers. In these experiments altogether, 6 different coating solutions were used. The solutions were prepared by mixing 2 different concentrations of collagen (coll, 10 or 50 μg/mL) with either unfractionated heparin (10 μg/mL UFH; Laid克 or with 10 mg/mL HEP-PGs. The coating suspensions (200 μL) were then added to round Thermax (Nunc) coverslips and incubated for 60 minutes at 37°C in a humid atmosphere. The coating solutions were removed, 200 μL of 2% human serum albumin (HSA) was added as a blocking solution, and the incubation was continued for another 60 minutes at 37°C. Each time, the coating process was performed similarly by the same trained investigator within a time frame of 2 hours before platelet interaction studies in PRP or before perfusion experiments. To ensure that the coating was homogeneous, some of the coverslips were stained with Coomassie brilliant blue R250 and viewed by light microscopy. Two different type I collagen preparations were employed for perfusion studies. Collagen monomers (final concentration, 50 μg/mL) were used as a coating substrate for most of the whole-blood perfusion studies. For comparison, we also studied the effects of collagen fibrils on platelet interactions (collagen reagent Horm, Nycomed Arzneimittel). The amount of collagen adhering to the coverslips was quantified by a specific dye-binding assay (Sircoll, Biocolor Ltd). Because it was observed that higher amounts of fibrils than monomers adhered to the surface during the coating procedure, collagen fibrils at a concentration of 16 μg/mL were used to make the coating comparable to that with 50 μg/mL of monomers. In the perfusion studies, we also compared the effects of collagen-immobilized HEP-PGs (coll–HEP-PG, 50 μg/mL of collagen monomers or 16 μg/mL of collagen fibrils with 10 μg/mL of HEP-PGs) with those of UFH. Here, however, we used a 1000-fold concentration of UFH (10 mg/mL) together with the collagens, as reported above. The Thermax coverslips (22×60 mm) were divided into 4 identical pieces to fit the perfusion chamber. Then, 400 μL of coating suspension was incubated over the coverslips, and the coating was finished with 2% HSA, as described above.

Immobilization of vWF, Collagen, UFH, and HEP-PGs on Microtiter Wells for PRP Studies Under Static Conditions

For some platelet interaction experiments in PRP, plastic microtiter wells (Labsystems) were coated with vWF, 10 μg/mL (Calbiochem), collagen (10 μg/mL), UFH (10 μg/mL), or HEP-PGs (10 μg/mL) or with combinations of these substrates. Here, too, the wells were blocked with 2% HSA, and the principle of the coating procedure was the same as presented above.

Quantification of Collagen and GAGs Deposited on Coverslips After Coating

The effect of coinmobilization of UFH and HEP-PGs on the amount of collagen was also quantified by Sircoll assay. Analogously, adherence of the GAGs to the coll–UFH and coll–HEP-PG coverslips was analyzed by Blyscan assay, which is a quantitative method based on the specific binding of the cationic dye 1,9-dimethylmethylenyl to PGs and sulfated GAGs. Because the substrates to be quantified were immobilized on the plastic coverslips at relatively low amounts, we modified the original instructions accordingly. In brief, the color reagents were added to 3 coverslips, and the detached coatings were pooled before dissociation of the color with the specific dissociation reagent provided. Finally, the absorbance of the bound dye was determined by using a spectrophotometer, and the amount of immobilized substrate per coverslip was approximated.

Platelet-Collagen and Platelet-vWF Interactions in PRP

The platelet-collagen and platelet-vWF interaction studies were performed in PRP under static conditions as previously described. In brief, the coated Thermax coverslips were placed on the bottom of precoated (2% HSA) 24-well plates (Nunc). One milliliter of [3H]serotonin (10 nmol/L)–labeled PRP (platelet count adjusted to 300×10^4/mL) was then added to the wells to be incubated for 30 minutes at 37°C under slow rotation (100 rpm). To reduce the amount of substrates needed for the coatings, some experiments were performed with a slight modification by using precoated, detachable microtiter wells. After incubation, the coverslips or the wells were washed with PBS to remove unattached platelets, and the deposited ['H]scintillation activity was measured. In some experiments, 10 μg/mL of c7E3, a GP IIb/IIIa inhibitor (abciximab; ReoPro, Centocor), was added to block thrombus formation. Furthermore, some experiments were performed with unlabeled PRP, and the binding of PRP-derived vWF to collagen was detected immunologically. After incubation under rotation, the coverslips were washed meticulously with 0.1% Tween-PBS (3×5 minutes) and placed on new 24-well plates. They were then incubated with peroxidase-conjugated rabbit anti-human vWF (diluted 1:4000; Dako) for 2 hours at 22°C. After additional washing of the coverslips with 1% bovine serum albumin in PBS, the color reaction was developed with the use of TMB microwell peroxidase substrate system (Kirkegaard & Perry Laboratories Inc), and the absorbance was measured at 450 nm with a Labsystems multiscan MCC.

β-Thromboglobulin Assay

Platelet-collagen interaction experiments were performed in PRP as described above. After 30-minute incubation at 37°C, 450 μL of PRP was added to 50 μL of anticoagulant mixture containing (final concentrations) citric acid (3.5 mmol/L), trisodium citrate (7.5 mmol/L), dextrose (13.6 mmol/L), EDTA (0.6 mmol/L), adenosine (0.6 mol/L), hirudin (2.5 U/mL), and UHF (2.5 U/mL). The anticoagulated PRP was then centrifuged (1500g) for 5 minutes at 4°C, and the middle third portion of the supernatant was separated.
and frozen at −40°C. The β-thromboglobulin contents of these samples were determined by ELISA (Asserachrom β-TG, Diagnostica Stago; normal range in plasma, 10 to 40 IU/mL) within 2 weeks of sample collection.

Platelet-Collagen Interactions Studied Under Whole-Blood Perfusion

Details of the whole-blood perfusion experiments are described elsewhere. The blood to which [3H]serotonin-labeled platelets were added was divided into aliquots (30 mL) and prewarmed for 5 minutes at 37°C before the perfusion. A coated coverslip was placed in a Badimon perfusion chamber with well-defined rheological characteristics. The prewarmed blood was then recirculated for 5 minutes through the chamber in which the coated coverslip was exposed to flowing blood. The flow rate was either 30 mL/min (high shear) or 10 mL/min (low shear), corresponding to wall shear rates of 1600 and 200 1/s, respectively. Immediately after the perfusions, the unattached platelets were flushed away by perfusing the coverslip with PBS for 40 seconds by using the same flowing conditions, after which the deposited H scintillation activity was determined. The blood samples for platelet count, total blood H scintillation activity, and plasma H scintillation activity (serotonin release) were collected just before and after the perfusion as previously described.

Scanning Electron Microscopy

The platelet deposition on coll, coll–UFH, and coll–HEP-PG was also studied by scanning electron microscopy (SEM), and in these experiments, whole blood without platelet labeling was used. After the perfusions, the coverslips were prepared for SEM by immediate fixation in 2.5% glutaraldehyde–PBS for 2 hours at 22°C. The fixed samples were carefully rinsed in PBS to remove excess fixative and kept in PBS until dehydration treatment with a series of increasing concentrations of ethanol. The dried SEM samples were sputter-coated with platinum and examined on a JEOL SEM 820 at the Electron Microscopy Unit, Institute of Biotechnology, University of Helsinki, Helsinki, Finland.

Statistical Analysis

The results are shown as mean±SD unless otherwise indicated. The statistical significance of the differences observed in platelet deposition on the differently coated surfaces was evaluated by a nonparametric Wilcoxon signed rank test. Statistical significance was set at P<0.05.

Results

Quantification of Collagen Monomers and GAGs Deposited on Coverslips After Coating

To quantify the adherence of collagen, UFH, and HEP-PGs, the contents of collagen and GAGs on the coated coverslips were studied by quantitative dye-binding assays and viewed by light microscopy after Coomassie blue staining. The observed mean content of collagen was 3 µg/cm², and there were only minor differences between the coll, the coll–UFH, and the coll–HEP-PG coverslips (Table). The collagen coating was homogenous in the presence of UFH and HEP-PGs when viewed after Coomassie blue staining. The mean content of GAGs on the coll–HEP-PG–coated coverslips was 130 ng/cm², and the amount of GAGs was only slightly higher on the coll–UFH coverslips despite the 1000-fold higher GAG concentration in the coll–UFH coating solution.

Interaction of PRP With Collagen Versus Collagen-Immovilized UFH or HEP-PGs

To evaluate the ability of collagen-immobilized UFH and collagen-immobilized HEP-PGs to inhibit platelet-collagen interactions, we incubated PRP (rotation 100 rpm, 30 minutes, 37°C) on coverslips coated with collagen monomers and quantified the attached platelets. When either a smaller (10 µg/mL) or larger (50 µg/mL) amount of collagen was used, both additional UFH and HEP-PGs decreased platelet deposition. The effect of HEP-PGs was consistently better than that of UFH (Figure I). The addition of c7E3 to PRP before incubation decreased platelet deposition on the coll and coll–UFH coverslips. In contrast, platelet deposition on the coll–HEP-PG coverslips was not affected, suggesting that HEP-PG blunted platelet aggregation induced by collagen (Figure II).

β-Thromboglobulin

To study α-granule release during the platelet-collagen interaction, we determined the content of β-thromboglobulin from PRP, which was incubated on coverslips coated as described above. The highest β-thromboglobulin levels (809±148 IU/mL, mean±SD) were found in PRP that had been in contact with coverslips coated with collagen (50 µg/mL) alone, and they differed markedly from those (480±157 IU/mL; P<0.05) obtained with albumin-coated coverslips. Addition of UFH or HEP-PGs to the coating mixture lowered the β-thromboglobulin levels (699±139 and 576±130 IU/mL, respectively; P<0.05) in PRP. The difference between UFH and VEH did not differ statistically.

![Figure I. Platelet deposition (mean±SD) in platelet-collagen interaction studies in which PRP (300×10^3/mL) was rotated (100 rpm) in contact with the precoated coverslips for 30 minutes at 37°C. The following coating suspensions were used: 2% HSA; VEH, collagen monomers (coll) 10 µg/mL and vehicle (PBS); UFH, coll 10 µg/mL and UFH 10 µg/mL; HEP-PGs, coll 10 µg/mL and macromolecular HEP-PGs 10 µg/mL; UFH, coll 50 µg/mL and vehicle, UFH, coll 50 µg/mL and UFH 10 µg/mL; HEP-PG, coll 50 µg/mL and HEP-PGs 10 µg/mL. The data were collected from 5 subsequent experiments made in duplicate. *P<0.05 compared with the collagen standard; †P<0.05 UFH vs HEP-PGs. Platelet deposition on coll 50 µg/mL (VEH) was higher than on HSA (A) (P<0.05), but coll 10 µg/mL (VEH) and HSA did not differ statistically.](image-url)
and HEP-PGs was also significant ($P<0.05$), compatible with stronger HEP-PG–induced platelet inactivation.

Detection of Platelet-Derived vWF

To assess the amount of PRP-derived vWF deposited on the surfaces, some experiments were performed with unlabeled PRP, and the surface-bound vWF was immunologically detected. The mean absorbance representing bound PRP-derived vWF on the collagen-coated coverslips was $1.21 \pm 0.18$ (n=3, mean $\pm$ SD). In the presence of a high concentration (10 mg/mL) of UFH, the surface-associated vWF was markedly decreased (absorbance $0.65 \pm 0.04$), whereas HEP-PGs (10 mg/mL) nearly abolished the platelet-derived deposition of vWF (absorbance $0.27 \pm 0.09$). The latter absorbance values did not differ from those obtained with vWF alone (5 $\mu$g/mL of purified vWF in 2% BSA, absorbance 0.27) or with the platelet-poor plasma standard (absorbance 0.20). Because HEP-PGs reduced the amount of platelet deposition to the level detected on albumin (Figure I) and similarly the vWF binding to the level of plasma concentration of vWF, this observation is consistent with the inhibition of platelet activation and $\alpha$-granule release.

Interaction Between Platelets and Immobilized vWF

Incubation of PRP on vWF-coated coverslips at moderate rotation showed that vWF-immobilized HEP-PGs decreased platelet deposition (Figure III). Immobilization of collagen together with vWF increased the basal deposition of platelets, as expected, whereas addition of either HEP-PGs or UFH to the vWF-collagen coating mixture decreased platelet deposition, the effect of HEP-PGs being slightly weaker than that of UFH.

Platelet Deposition on Collagen in Flowing Whole Blood

In perfusion studies, we wanted to examine whether the results obtained in the platelet interaction studies would also apply under flowing conditions. First, we coated the coverslips with collagen monomers and used a low shear rate (200
1/s) for the blood perfusions. At the low shear rate, the HEP-PGs (10 µg/mL) immobilized together with collagen considerably decreased platelet deposition (2.5±0.8 versus 1.5±0.2×10⁶/cm²; *P*<0.05), whereas UFH (10 mg/mL) failed to have an effect of similar magnitude (1.8±1.4×10⁶/cm²; NS) (Figure IVA). When collagen-induced platelet deposition was studied at a high shear rate (1600 1/s), the mean platelet deposition on collagen increased 3-fold and was 7.5±6.1×10⁶/cm² (Figure IVB). At the higher shear rate, both UFH and HEP-PGs had a strong inhibitory effect on platelet deposition (2.0±1.2 and 0.42±0.38×10⁶/cm², respectively; *P*<0.05), and HEP-PGs actually fully abolished platelet deposition. We also made several attempts to apply HEP-PGs to the coverslips after collagen coating, but these experiments failed to show any significant effect of HEP-PGs on platelet deposition, although the HEP-PGs were found to bind to collagen (data not shown).

The use of native-type collagen fibrils (16 µg/mL) as the coating substrate (instead of collagen monomers) led to an expected increase in the deposition of platelets (Figure IVC). When immobilized collagen fibrils were exposed to flowing blood at the high shear rate, the coinmobilized HEP-PGs markedly inhibited platelet deposition (62% decrease, *P*<0.05), whereas UFH had a smaller but consistent lowering effect (12% decrease; *P*<0.05) (Figure IVC).

The actual presentation of platelets on the coverslips was examined by SEM. The surface coverage and size of aggregates appeared larger in the collagen monomer–coated coverslips than in the coll-UFH or the coll–HEP-PG coverslips, thus according with the quantitative data presented above (Figures VA through VF). Moreover, the coll-UFH coverslips presented some large aggregates, but most of the adhered platelets were located at the margins of the perfusion channel, where shear rates are known to be lower than in the middle of the channel (Figures VC through VD). The coll–HEP-PG coverslips showed only occasional aggregates, which likewise were mainly located in the marginal zone of the channel, and only few platelets were seen in the center of the channel (Figures VE and VF). Similarly, when collagen fibrils were exposed to flowing blood, the surface coverage and size of aggregates decreased on the coll-UFH and especially on the coll–HEP-PG coverslips (Figures VIA through VIF). However, on the surfaces coated with collagen fibrils and with UFH or HEP-PG, the platelet aggregates were more evenly distributed, and there were no large bare areas as typically seen in the center of the perfusion channels on surfaces coated with collagen monomers and HEP-PGs.

**Discussion**

We have shown here that macromolecular HEP-PGs, when immobilized together with type I collagen, can inhibit platelet-collagen interactions. To mimic the conditions during mast cell activation in the human vasculature when mast cells release their contents, including HEP-PGs, we decided to expose blood to immobilized collagen in the presence or absence of coinmobilized HEP-PGs.

In these perfusion experiments, the interaction between platelets and collagen monomers was severely compromised

![Figure V](https://via.placeholder.com/150)

**Figure V.** Visualization of platelet deposition by SEM after high shear-rate whole-blood perfusions over collagen monomers. All perfusions were performed with unlabeled platelets. A, Collagen alone (50 µg/mL) ×60. B, Collagen alone ×200. C, Collagen and UFH (10 mg/mL) ×60. D, Collagen and UFH ×200. E, Collagen and HEP-PGs (10 µg/mL) ×60. F, Collagen and HEP-PGs ×200. Note the significant reduction in both adhesion and size of aggregates in the middle part of the flow channel on the UFH-collagen surface (C and D) and especially on the HEP-PG-collagen (E and F) surface. Platelets have accumulated at the edges of the channel area in both cases.
by HEP-PGs under high shear-rate conditions, but the interaction was also inhibited, albeit to a lesser degree, at a low shear rate. Furthermore, HEP-PGs immobilized together with collagen fibrils inhibited platelet deposition, supporting our previous observations from studies with soluble HEP-PGs in blood interacting with collagen fibrils, optimally treated for fibril formation. UFH had an inhibitory effect on platelet deposition at a high shear rate, although it was much weaker than that of HEP-PGs and was absent at a lower shear rate. In the platelet interaction studies in PRP, both UFH and HEP-PGs markedly reduced platelet deposition, and again the effect of HEP-PGs predominated over that of UFH. These findings are compatible with the notion that HEP-PGs inhibit GP Ia/IIa–mediated platelet activation, which is crucial in mediating thrombus growth on collagen monomers at a high shear rate but not at a low shear rate. UFH was also seen on collagen fibrils, though less clearly than on monomers, suggesting additional platelet-activating domains on the fibrils. HEP-PGs also effectively inhibited release of β-thromboglobulin and blunted vWF exposure on the monomer-adherent platelets, in parallel with decreased activation. Finally, in contrast to that on collagen alone and to that on coimmobilized UFH, platelet deposition on HEP-PG-coated collagen surfaces could not be inhibited by c7E3.

In most experiments, we chose to use collagen monomers, which were obtained by extracting collagen with acetic acid from bovine Achilles tendons and subsequently subjecting the extracts to extensive pepsin treatment. This treatment proteolyzes the telopeptide ends of the collagen fibers, resulting in monomeric strands without the characteristic quaternary structure of collagen. Due to collagenolytic activity in eroded atherosclerotic plaques, collagen fibrils of the vessel wall may partly present themselves as monomers. Thus, platelet interactions with both fibrils and monomers seem to be pathophysiologically relevant. The stronger inhibition of platelet interaction with collagen monomers than with fibrils by HEP-PGs could be due to the efficacy of HEP-PGs in inhibiting GP Ia/IIa–mediated activation and subsequent recruitment of platelets to the monomers. Savage et al have recently shown that, at a high shear rate, blockage of GP Ia/IIa by a monoclonal antibody completely abolishes stable platelet attachment and thrombus formation on soluble collagen monomers.

We observed that collagen-immobilized HEP-PGs, likely owing to their macromolecular size and high negative-charge density, exceeded UFH in their inhibitory effect on platelet deposition. We also applied HEP-PGs to the coverslips after they had been coated with collagen. Interestingly, however, HEP-PGs did not significantly affect platelet deposition when applied to such preimmobilized collagen. Previously, we have shown that during short incubation periods, HEP-PGs do not bind to collagen, which is relevant for the strong inhibitory effect typical for soluble HEP-PGs. In the present study, however, incubation of HEP-PGs with immobilized collagen for 1 hour resulted in HEP-PG binding to the preimmobilized collagen. The lack of inhibitory effect of HEP-PGs in this system can be explained if HEP-PGs...
effectively bridged collagen and plasma vWF. As evidenced in the static experiments, HEP-PGs inhibited vWF-mediated platelet function less effectively than did UFH. HEP-PGs could also impair fibril formation, but this is an unlikely explanation because in our previous study, soluble HEP-PGs were highly effective when collagen was allowed to form fibrils before blood perfusion.

Regarding the potential roles of mast cells in the arterial intima, experiments in cell culture have suggested that degranulation of mast cells is potentially a prothromogenic event. Thus, stimulated mast cells promote the formation of foam cells when cocultured with macrophages or with smooth muscle cells. The potential roles of mast cells in the fibrous caps of advanced atherosclerotic plaques include mast cell protease-dependent activation of matrix-degrading metalloproteinases. If operative in vivo, this effect would tend to weaken the fibrous cap of the atherosclerotic plaque and so predispose to plaque rupture and initiation of thrombosis. On the other hand, the significant inhibitory capacity of the mast cell–derived HEP-PGs in platelet reactivity toward collagen would tend to limit thrombus growth. Although novel vascular therapies such as percutaneous transluminal coronary angioplasty have revolutionized the management of arterial disease, acute thrombotic complications, restenosis in particular, still limit the efficacy of these treatment options. Clearly, the local control of collagen-induced platelet activation during and after vascular interventions would be crucial to avoid early occlusions and perhaps also to improve long-term patency. This study shows that collagen-immobilized HEP-PGs strongly inhibit platelet deposition to collagen and subsequent release reaction under conditions comparable to those present in atherosclerotic coronary arteries. These observations suggest that degranulating mast cells at the site of vascular injury, by secreting HEP-PGs, actually tend to prevent thrombus growth and has also been noted as a regulatory element in cardiac (sub)endothelium after arterial thrombosis. However, further studies are needed to evaluate whether locally applied HEP-PGs or similar compounds can also affect platelet–vessel wall interactions in vivo.

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References

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