Heparin Impairs Glycocalyx Barrier Properties and Attenuates Shear Dependent Vasodilation in Mice

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Abstract—The endothelial glycocalyx is a hydrated mesh of polysaccharides and adsorbed plasma proteins that forms the true interface between the flowing blood and the endothelium. We hypothesized in the present study that competitive binding of heparin to glycocalyx-associated proteins would affect glycocalyx barrier properties and mechanotransduction of shear stress to the endothelium. In anesthetized mice, the clearance of 70-kDa dextrans from the circulation was increased ($P<0.05$ versus saline) 1 hour after heparin (1.25 U) and glycocalyx degradation with hyaluronidase (35 U; amount cleared in 30 minutes after saline: $11\pm5\%$; after heparin: $45\pm8\%$; after hyaluronidase: $30\pm3\%$). Clearance of 40-kDa dextrans increased ($P<0.05$ versus saline) to a lesser extent after both treatments (saline: $46\pm3\%$; heparin: $60\pm5\%$; hyaluronidase: $60\pm2\%$). The dilator response of second-order arterioles in cremaster muscle during reactive hyperemia was reduced for $\equiv90$ minutes after heparin as reflected by a decrease ($P=0.008$) in $t_{50}$ of diameter recovery, and this effect was associated with a diminished NO bioavailability. Infusion of hyaluronidase resulted in reductions ($P<0.05$) in baseline and peak reactive hyperemic diameter, whereas, despite an increase in wall shear rate at the beginning of reactive hyperemia, $t_{50}$ of diameter recovery was not affected. In conclusion, our data in mice show that a heparin challenge is associated with increased vascular leakage of dextrans and impaired arteriolar vasodilation during reactive hyperemia. Our data suggest that protein–heparan sulfate interactions are important for a functional glycocalyx.

Key Words: glycocalyx ▪ mechanotransduction ▪ heparin ▪ hyaluronidase ▪ reactive hyperemia

Heparin is a major clinical anticoagulant and widely used in most cardiovascular procedures.1 Undesirable adverse effects of heparin therapy have, however, been reported, of which heparin-induced thrombocytopenia is the most important life- and limb-threatening event.2 In addition, there is evidence that heparin can induce antibody-independent platelet activation, and this effect has been associated with an impaired endothelial NO production3 and NO-dependent vascular reactivity.4 On the other hand, increases in endothelial NO production and associated arteriolar vasodilation by heparin have been reported as well.5,6 Heparin exerts its anticoagulation effect primarily by catalyzing the inhibition of thrombin by the serine protease inhibitor antithrombin III, apparently as a result of a conformational change within antithrombin III.3,7 In addition to antithrombin III, heparin can bind to and interact with a broad spectrum of other proteins by virtue of their heparin-binding domain (eg, proteases, growth factors, chemokines, and lipid-binding proteins).1,7,8 In this manner, heparin may interfere with various biological processes other than coagulation.

Heparan sulfates are the biological counterparts of heparin in blood vessels.8–10 After injection, heparin has been shown to interact rapidly and specifically with the endothelium11 and to induce the release of heparan sulfate–bound proteins from the endothelium into the bloodstream, such as lipoprotein lipase, superoxide dismutase (SOD), and xanthine oxidase.12–15 At the luminal side of the endothelium, heparan sulfate proteoglycans are common constituents of the endothelial glycocalyx.16,17 The glycocalyx forms a highly hydrated mesh of polysaccharide structures and adsorbed plasma proteins, which excludes flowing blood and large macromolecules.17,18 Experimental studies in which the glycocalyx was treated with glycosaminoglycan-degrading enzymes have shown that this layer contributes to the barrier properties of the vascular wall19–21 and that it participates in the mechanosensing and transduction of shear forces to the endothelium.18 Florian et al22 demonstrated recently that NO production in response to fluid shear stress was impaired in cultured endothelial cells after enzymatic removal of heparan sulfates from the cells by heparinase III, demonstrating that a heparan sulfate component participates in shear-mediated NO production. Heparan sulfates were also shown to have the ability to modulate vasodilator effects of endothelial receptor-dependent, NO-mediated, agonists.23 On the other hand,
plasma proteins have been shown to modify the glycocalyx and to preserve vascular permeability.\textsuperscript{24,25} In the current study in mice, we hypothesized that a heparin challenge, presumably by displacing proteins bound to heparan sulfate proteoglycans, would affect the functional properties of the glycocalyx and that this consequently would impact on vascular leakage and shear dependent vasodilation. As a reflection of the glycocalyx barrier properties, we measured the clearance of fluorescently labeled 70-kDa and 40-kDa dextrans from the systemic circulation.\textsuperscript{26} This measurement is based on intravital microscopic observations in cremaster tissue, which show that, under baseline conditions, fluorescein isothiocyanate–labeled 70-kDa dextrans (Dex-70) are partly excluded from the vascular wall by the glycocalyx in capillaries, whereas Texas red–labeled 40-kDa dextrans (Dex-40) rapidly access the glycocalyx domain.\textsuperscript{18,27} Shear dependent vasodilation was assessed in 2A arterioles of the cremaster muscle from the diameter and red blood cell velocity response during a reactive hyperemia (RH).\textsuperscript{28} We found in a previous study that this maneuver elicits robust NO-mediated vasodilation in these vessels, with the duration of dilation being related to the wall shear rate (WSR) increase on release of the occlusion.\textsuperscript{28} To test for a change in NO bioavailability after heparin administration, NO production was blocked with an L-arginine analogue, and involvement of an enhanced NO degradation by superoxide anions was checked by adding free radical scavengers to the muscle. For reference, additional experiments were performed in which hyaluronidase was infused. Hyaluronidase degrades hyaluronic acid glycosaminoglycans from the glycocalyx and was shown previously to impair glycocalyx exclusion of Dex-70 in cremaster capillaries\textsuperscript{28} and to diminish flow-dependent NO production in isolated canine femoral arteries.\textsuperscript{30}

Methods

**General Surgery and Anesthesia**

All of the procedures and protocols were approved by the animal care and use committee of the Academic Medical Center in Amsterdam. Studies were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Experiments were performed on C57/B6 mice (20 to 25 g; n=33; Charles River Europe) that received standard chow and water ad libitum. At the beginning of an experiment, mice were anesthetized with an IP injection of ketamine hydrochloride (125 mg/kg) and medetomidine (0.2 mg/kg) and tracheotomized to ensure airway patency. Depth of anesthesia was maintained according to stability of blood pressure, respiration rate, and lack of toe pinch reflex by supplemental administration (every hour) of anesthetic (ketamine: 1.25 U; Leo Pharma), or hyaluronidase (35 U, Type IV-S; Sigma-Aldrich), and 60 minutes later fluorescent dextrans were administered. The choice for this time point was based on the observation of Henry and Duling\textsuperscript{29} that glycocalyx exclusion in cremaster capillaries was maximally impaired 1 hour after hyaluronidase administration.

**Arteriolar Vasodilator Response During Reactive Hyperemia**

The mouse was placed in a supine position on a custom-built animal platform, and the right cremaster muscle was prepared\textsuperscript{28} (for details, see the online data supplement available at http://hyper.ahajournals.org). The preparation was equilibrated for 30 minutes, during which time the arteriolar network was scanned and a schematic diagram drawn to identify sites for data collection. In each experiment, 30-second occlusions of a first-order (1A) or second-order (2A) arteriole in the proximal and central region of the muscle were performed and responses during reactive hyperemia measured in the 2A arteriole distal from the occlusion.\textsuperscript{28} One observation site was examined per experiment. Occlusions were performed every 10 to 15 minutes; this period of time between repetitive occlusions was sufficient for resting tone to recover while obtaining reproducible responses. Arteriolar responses were measured during a control period of 60 minutes. Hereafter, animals were given a bolus of saline, heparin, or hyaluronidase (as described earlier) after which measurements were resumed for another 90 minutes (saline and heparin) and 120 minutes (hyaluronidase). At 90 minutes postheparin, NO synthase was blocked in 1 group of animals by adding N-nitro-L-arginine (L-NNA; 10\textsuperscript{-4} mol/L, Sigma-Aldrich) to the physiological salt solution (PSS) superfusing the muscle,\textsuperscript{28} whereas in a second group, SOD (50 U/mL; Sigma-Aldrich) and catalase (CAT; 50 U/mL; Sigma-Aldrich) were added to the physiological salt solution.\textsuperscript{31} Arteriolar responses were re-evaluated \textlessspace\textgreater 30 minutes after initial addition of the blocker or free radical scavenger. At the end of an experiment, sodium nitroprusside (10\textsuperscript{-5} mol/L; Sigma-Aldrich) was added to the physiological salt solution and maximum diameters determined.

Internal vessel diameter, measured from the internal edges of the vessel lumen, was determined off-line during video playback from the monitor using video calipers calibrated against a stage micrometer (resolution: \textlessspace\textless 2 \textmu m). Centerline red blood cell velocity was monitored on-line with an optical Doppler velocimeter (Microcirculation Research Institute, Texas A&M University); effective slit width ranged from 12 to 18 \textmu m. Data were acquired at 100 Hz using a PowerLab 4/SP system (ADInstruments) coupled to a Pentium-based personal computer.

**Data Analysis**

**Dextran Clearance**

Dextran concentrations were normalized to the amount injected, and for each individual experiment, concentration–time curves of both tracers were fitted with a monoequponential function.\textsuperscript{26,32} Initial distribution volume of each dextran was determined from the extrapolated dilution at the start of tracer injection, and clearance was defined as the percentage decrease in tracer concentration at the final sample point (t=30 minutes) compared with the extrapolated concentration at the start of tracer injection (t=0 minutes). Effects of...
Heparin or hyaluronidase on dextran clearance were compared with control (saline) mice using t tests. Results were considered statistically significant with \( P \leq 0.05 \). Summary data are reported as mean±SEM with “n” referring to the number of animals studied.

**Arteriolar Dilation**

For each individual occlusion, diameter and red blood cell velocity were averaged for ~30 seconds before the occlusion (baseline), for the last 5 seconds of the occlusion period (end occlusion), and for every 5 seconds after release of the occlusion (RH). Diameters (\( D \)) were normalized from their baseline (\( D_0 \)) to their maximum value (\( D_{max} \)) during reactive hyperemia, that is, \( D_{norm} = (D - D_0)/(D_{max} - D_0) \). As an index of the diameter recovery rate, the time to 50% recovery of \( D_{norm} \) after release of the occlusion, \( t_{50} \), was used. Blood flow and WSR were calculated as equal to \( \left[ \pi (D/2)^2 V_r \right] \) and \( 8V_r/D \) with mean red blood cell velocity (\( V_r \)) calculated from red blood cell velocity using a correction factor of 1.3. WSR at the beginning of RH was estimated from \( V_r \) during the first 0.5 seconds after release of the occlusion and vessel diameter at end occlusion.

Repeated observations in an individual vessel during the 60-minute control period were averaged. Measurements after administration of heparin and hyaluronidase were split into sequential 30-minute periods after injection, and effects were determined using 1-way repeated-measures ANOVA. In case of significant main effects, posthoc comparisons were performed using Tukey tests. Repeated observations in an individual vessel during superfusion with 1-NNa or SOD and CAT were averaged and their effects assessed by paired t tests. Results were considered statistically significant with \( P \leq 0.05 \). Summary data are reported as mean±SEM, with “n” referring to the number of vessels studied.

**Results**

Mean arterial pressure and heart rate were 60 to 70 mm Hg and 300 to 400 bpm during experiments and did not change during respective treatments.

**Dextran Clearance**

Figure 1 shows average concentration–time curves of Dex-70 (top panel) and Dex-40 (bottom panel) 1 hour after infusion of a bolus of saline (\( n = 6 \)), heparin (\( n = 4 \)), or hyaluronidase (\( n = 3 \)). Dex-70 distribution volume, inferred from the extrapolated plasma concentration at the start of tracer infusion (\( t = 0 \) minutes), was 0.93 ± 0.06 mL in the control mice and did not differ in the mice that received heparin (0.98 ± 0.05 mL) or hyaluronidase (1.00 ± 0.01 mL). As shown before, Dex-70 was slowly cleared from the circulation in control mice, such that only 11.2 ± 5% of the injected amount was lost from the circulation at the end of the 30-minute sampling period (Figure 1, top). Clearance of Dex-70 was 3- to 4-fold increased after the administration of heparin and hyaluronidase (clearance at \( t = 30 \) minutes: heparin: 45 ± 8%; hyaluronidase: 30±5%; both \( P \leq 0.05 \) compared with saline).

Distribution volume of Dex-40 was larger than that for Dex-70 and did not differ between treatments (saline: 0.98 ± 0.04 mL; heparin: 1.10 ± 0.06 mL; hyaluronidase: 1.04 ± 0.01 mL). Under control conditions, Dex-40 was rapidly lost from the circulation, as reflected by a clearance of 46 ± 3% after 30 minutes (Figure 1, bottom). Dex-40 clearance was modestly increased (\( P < 0.05 \) versus saline) in the mice that received heparin (60 ± 5%) and hyaluronidase (60 ± 2%). The ratio of Dex-70 to Dex-40 clearance was 0.23 ± 0.10 after saline and was increased to 0.73 ± 0.07 with heparin (\( P < 0.05 \) versus saline). With hyaluronidase, the ratio of Dex-70 and Dex-40 clearance tended to increase as well.

**Arteriolar Dilation**

Figure 2 shows average arteriolar diameter responses during the RH maneuver. Figure 2A depicts diameter responses in controls and for every half hour after the heparin challenge (\( n = 10 \)), with the top panel illustrating the absolute diameter response and the bottom panel showing normalized diameter (see data analysis in Methods section). On proximal occlusion, vessels distal from the occlusion started to dilate within 2 to 3 seconds and reached steady-state dilation after ~20 seconds. When occlusion was released (\( t = 0 \)), arteriolar diameters increased further, reaching a peak within 5 to 10 seconds, after which they returned to their preclosure values. The duration of the dilatory response varied among vessels of different animals, yet was reproducible in an individual vessel, and diameters consistently returned to their baseline on repeated occlusions during an experiment. Infusion of 0.05 mL of saline in control mice (\( n = 4 \)) did not change baseline diameters or reactive hyperemic responses for ~90 minutes after infusion (data not shown).

Heparin impaired the dilatory response by diminishing the duration of RH as reflected by a decrease (\( P = 0.008 \)) in \( t_{50} \) of recovery (Figure 2A, bottom). The impaired dilation was evident within the first half hour after infusion already and remained for 90 minutes postinfusion. Baseline, end occlusion, and peak RH diameter did not change after the heparin
Baseline red blood cell velocity was 7.1 ± 0.8 mm/s in control and did not change after heparin (0 to 30 minutes: 6.5 ± 0.7 mm/s; 30 to 60 minutes: 6.6 ± 0.8 mm/s; 60 to 90 minutes: 6.9 ± 0.7 mm/s). Baseline blood flow was 2.3 ± 0.5 nL/s in control and not changed after heparin (2.5 ± 0.6 nL/s at 90 minutes). Also, peak RH blood flow was not affected by the heparin challenge (control: 5.0 ± 1.0 nL/s; 0 to 30 minutes: 5.9 ± 1.4 nL/s; 30 to 60 minutes: 5.0 ± 1.2 nL/s; 60 to 90 minutes: 6.3 ± 1.5 nL/s).

Subsequent superfusion of the muscle with L-NNA (performed in n=5 of 10 experiments) resulted in a decrease in arteriolar diameter at baseline, at end occlusion, and at peak RH (Figure 2B, top), whereas recovery of diameter was not further reduced by L-NNA after heparin (Figure 2B, bottom). Baseline red blood cell velocity tended to decrease after L-NNA (control: 6.4 ± 0.5 mm/s; 90 minutes postheparin: 6.4 ± 1.2 mm/s; L-NNA: 4.1 ± 0.8 mm/s; P<0.1 compared with postheparin), and there was a significant decrease in baseline blood flow during NO blockade (control: 2.7 ± 0.8 nL/s; 90 minutes postheparin: 2.3 ± 0.8 nL/s; L-NNA: 1.0 ± 0.3 nL/s; P<0.05 compared with postheparin). Addition of SOD and CAT in the other 5 animals did not restore the effect of heparin on duration of reactive hyperemia (t50: control: 51.0 ± 14.4 s; 90 minutes postheparin: 29.0 ± 7.7 s; SOD + CAT: 30.0 ± 7.1 s).

Infusion of hyaluronidase resulted in a baseline vasoconstriction (P=0.016) and reduced diameters at end occlusion (P<0.001) and at peak reactive hyperemia (P<0.001; Figure 3, top). This effect occurred gradually in time and was most profound at 120 minutes after the injection. Duration of vasodilation did not significantly change after hyaluronidase (Figure 3, bottom). Despite the vasoconstriction, baseline red blood cell velocity (control: 5.8 ± 0.9 mm/s; obtained in n=4 of 6 animals) did not change after hyaluronidase (0 to 30 minutes: 6.4 ± 0.8 mm/s; 30 to 60 minutes: 5.8 ± 0.4 mm/s; 60 to 90 minutes: 6.2 ± 0.7 mm/s; 90 to 120 minutes: 5.9 ± 0.4 mm/s). Baseline blood flow was 1.8 ± 0.3 nL/s in control and not significantly affected after hyaluronidase (1.4 ± 0.3 nL/s at 120 minutes). Also, peak RH blood flow did not change after hyaluronidase (control: 5.2 ± 0.7 nL/s; 0 to 30 minutes: 4.9 ± 0.5

Figure 2. Heparin impairs NO-dependent arteriolar vasodilation during reactive hyperemia. A, Diameter response of n=10 arterioles during control and for 90 minutes after heparin challenge. Top, absolute diameter; bottom, normalized diameter (see Methods section). Error bars are representative; those not shown are omitted for clarity. Maximum (sodium nitroprusside) diameters: 36.0 ± 2.4 μm. Dashed lines indicate occlusion period; actual duration of occlusion (30 seconds) is longer than depicted. Time = 0 seconds indicates release of occlusion. Vertical dotted lines indicate t50 of diameter recovery. *P<0.05, main effect of heparin on t50 of recovery. B, Subsequent diameter response during NO blockade in n=5 vessels (top, absolute diameter; bottom, normalized diameter). *P<0.05 vs baseline, end occlusion, and peak RH diameter postheparin. #P<0.05, t50 of recovery postheparin vs control.

Figure 3. Hyaluronidase induces baseline arteriolar vasoconstriction and reduces peak reactive hyperemia. Diameter response of n=6 arterioles during control and for 120 minutes after hyaluronidase infusion. Top, absolute diameter; bottom, normalized diameters. Error bars are representative; those not shown are omitted for clarity. Maximum diameters: 35.7 ± 1.1 μm. *P<0.05, main effect of hyaluronidase on baseline, end occlusion, and peak RH diameter.
In a previous study, we demonstrated that the $t_{50}$ of recovery in 2A arterioles was related to the WSR at the beginning of RH. Therefore, we considered our current observations also in light of (changes in) WSR at the beginning of RH. Baseline WSR was 2344±363 s⁻¹ before heparin and did not change after its infusion. WSR at the beginning of RH was 2347±257 s⁻¹ and also was not changed after the heparin administration. The reduction in $t_{50}$ of recovery after heparin, therefore, occurred in the face of an unchanged WSR. Before hyaluronidase infusion, baseline WSR was 1627±270 s⁻¹ and also did not change after infusion of the enzyme. WSR at the beginning of RH was 2195±231 s⁻¹ before injection and, in contrast to heparin, increased after hyaluronidase ($P=0.011$; main effect). Despite this increase in WSR at the beginning of RH, $t_{50}$ of recovery was not changed after hyaluronidase treatment (Figure 3).

### Discussion

There is controversial experimental evidence with regard to an effect of heparin on NO-dependent vascular reactivity in the literature. In the present study, we hypothesized that competitive binding of heparin to glycocalyx-associated proteins would affect the mechanotransduction of shear stress to the endothelium. We found that a clinically relevant dose of heparin ($≈50$ U/kg of body weight) was associated with an increased clearance of large dextrans from the systemic circulation and a reduction in the duration of arteriolar vasodilation during reactive hyperemia in the mouse cremaster muscle. This latter effect involved a decrease in NO bioavailability as evidenced by the lack of effect of sequential administration of L-arginine analogue. NO-dependent baseline vasomotor tone was not affected after heparin. Our data show that heparin administration impairs vascular barrier properties and attenuates NO-mediated vasodilation during dynamic changes in blood flow and suggest that interactions between heparan sulfate glycosaminoglycans and heparin-binding proteins might be important for barrier and mechanotransduction properties of the glycocalyx.

### Barrier Properties of the Glycocalyx

The glycocalyx participates in the barrier properties of the vascular wall by forming the first protective “sieve” for plasma molecules. Based on intravital microscopy studies in capillaries of the cremaster muscle, we used fluorescent dextrans to evaluate the glycocalyx barrier properties in the systemic circulation. Obviously, our systemic dextran clearance measurements do not distinguish between changes in permeability of the glycocalyx per se, and other mechanisms determining vascular permeability, nor do they provide information about the location(s) in the circulation from where the dextrans are lost. With respect to the kidneys, Jeansson and Haraldsson found that treatment of the glomerular glycocalyx with enzymes was associated with a selective increase in clearance of albumin compared with neutral Ficoll, suggesting that an impairment in glomerular barrier properties might contribute to the profound clearance of Dex-70 after heparin and hyaluronidase in our experiments.

The initial distribution volume of the Dex-70 did not change after treatment of the glycocalyx and was under control conditions only slightly smaller than that of Dex-40, indicating that glycocalyx exclusion of the larger dextrans was less than anticipated from intravital microscopy observations of a 0.4- to 0.5-μm exclusion zone for Dex-70. Nevertheless, an increase in Dex-70 clearance appears to reflect perturbation of the charge-dependent barrier properties of the glycocalyx. In support of this, clearance of Dex-70 in mice was found to increase during hyperglycemic conditions, in concurrence with a profound loss of glycocalyx volume in the systemic circulation under these conditions in humans.

### Glycocalyx and Shear Dependent Vasodilation

We established in our previous study that the 2A arterioles constitute a functional locus of shear dependent NO-mediated vasodilation during RH in the mouse cremaster muscle. Differences in RH dynamics during baseline (Figures 2 and 3, control) encompass the normal variation that is found among arterioles during this maneuver. This intervessel variation likely reflects differences in NO contribution to the response, possibly resulting from differences in prevailing shear rates at the beginning of RH. Nevertheless, RH responses within a vessel are very reproducible, and paired observations of pretreatment and posttreatment revealed distinct effects of heparin and hyaluronidase on arteriolar vasodilation in the present study.

Hyaluronidase resulted in a reduction in baseline and peak diameter but did not significantly change the $t_{50}$ of arteriolar diameter recovery. This apparent lack of effect on RH duration might have been masked by the significant increase in WSR at the beginning of RH. The vasoconstriction is suggested to reflect a decrease in NO bioavailability, in line with the observation of a greatly reduced flow-dependent production of NO in isolated canine femoral arteries after hyaluronidase treatment. Indeed, the decrease in baseline diameter was comparable with that during L-NNA superfusion in a previous study. The slow effect of hyaluronidase on baseline vasoconstriction seems to reflect the time needed for the enzyme to penetrate into the glycocalyx and degrade hyaluronan structures and complies with the observation of gradual impairment of glycocalyx exclusion in cremaster capillaries after a single systemic bolus of hyaluronidase. In the face of the 10% to 15% decrease in baseline diameter, the ≈20% decrease in baseline blood flow 2 hours after hyaluronidase was less than anticipated and indicates that total resistance of the microvascular bed may have decreased. Because the vessels proximal from the site of observation have very low vasomotor tone in these experiments, the resistance decrease must be confined to the distal microvessels and possibly reflects an increase in perfused microvascular volume resulting from glycocalyx degradation.

### Heparin-Induced Glycocalyx Perturbation

Heparin reduced the duration of RH response within the first half hour after administration, and this impairment lasted for...
release of the occlusion.28 The decrease in NO contribution to NO-mediated vasodilation after heparin (Figure 2B, top). We found in a previous study that hypercholesterolemia was associated with a substantial reduction in NO contribution to baseline vasomotor tone and to the duration of reactive hyperemia dilation but that NO-mediated peak RH diameter was not affected.28 These and the current data, therefore, exemplify that “endothelial dysfunction” might be differently manifested among the various components of RH. Although we did not test whether agonist-induced NO-mediated dilation was affected after heparin, the impaired duration of NO-mediated vasodilation that we observed likely reflects a defect in the mechanotransduction response of the arteriole to the WSR increase on release of the occlusion.28 The decrease in NO contribution could not be accounted for by an enhanced degradation of NO by oxygen-free radicals, because addition of SOD and CAT did not restore the duration of RH. Furthermore, the lack of effect of free radical scavengers seems to indicate that the effect of heparin was not simply because of heparin-induced release of SOD from the glycocalyx. Indeed, based on findings in the pig showing that the release of extracellular SOD to plasma requires a ~10-fold higher dose of heparin than that needed to release other heparin sulfate binding factors,40 only a small amount of extracellular SOD is estimated to be released with the dose of 50 U/kg of body weight in the present study.

Further studies are needed to determine the precise mechanisms by which heparin affects vascular barrier properties and arteriolar vasodilation during RH. A heparin challenge is clinically used to release endothelial cell-bound proteins into the bloodstream. On the other hand, evidence has been provided that heparin can be taken up by the endothelial surface of blood vessels,11 and in isolated coronary arterioles it was shown that administration of heparin directly could stimulate vasodilation.6 In vivo, however, the association of heparin with the endothelial surface might be hindered by the glycocalyx itself. The dimensions of the glycocalyx are, under normal conditions, highly regulated resulting from a balance between the biosynthesis of polysaccharide structures and association with blood-borne substances on the one hand and shedding or release of components on the other hand.16,41 In case of degradation of the glycocalyx, such as may occur in an in vitro setting, heparin can, however, attach to the vessel wall and in this way restore glycocalyx function.42

Perspectives

Heparin is a sulfated polysaccharide that is used as anticoagulant in most cardiovascular procedures. Anticoagulation occurs when antithrombin III binds to a specific pentasaccharide sequence in heparin, whereas additional sequences enable heparin to interact with a wide variety of other proteins. In the present study, we hypothesized that competitive binding of heparin to glycocalyx-associated proteins would affect glycocalyx function and demonstrated that a heparin challenge in mice impaired both vascular barrier properties and NO-mediated arteriolar dilation during reactive hyperemia. Although the presence of plasma proteins within the glycocalyx has been shown to be important for reducing vascular permeability, and heparan sulfate proteoglycans have been described as mechanosensors in the NO-mediated response of cultured endothelial cells to fluid shear stress, our data suggest that interactions of proteins with the heparin-binding domain of the heparan sulfates might be important for a functional glycocalyx barrier and mechanotransduction of shear stress from blood to endothelium during dynamic changes in blood flow. In addition, the presence of hyaluronan within the glycocalyx seems to contribute to mechanotransduction of shear stress from blood to endothelium during baseline conditions of blood flow. Overall, our findings indicate that a heparin challenge has adverse effects on vascular homeostasis and suggest that a perturbation in glycocalyx might be involved.

Sources of Funding

This study was supported by the Netherlands Organisation for Scientific Research (No. 902-16-192) and the Netherlands Heart Foundation (No. 2005ST073 and No. 2003B181).

Disclosures

None.

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*Hypertension*. 2007;50:261-267; originally published online April 23, 2007;
doi: 10.1161/HYPERTENSIONAHA.107.089250

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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