Shedding of the Endothelial Glycocalyx in Patients Undergoing Major Vascular Surgery With Global and Regional Ischemia

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Background—The astonishing thickness of the endothelial glycocalyx, which rivals that of endothelial cells in the microvasculature, was disclosed in the last 15 years. As already demonstrated, this structure plays a key role in the regulation of inflammation and vascular permeability.

Methods and Results—Two components of the glycocalyx, syndecan-1 and heparan sulfate, were measured in arterial blood of 18 patients undergoing surgery of the ascending aorta with cardiopulmonary bypass (n = 12 with and n = 6 without deep hypothermic circulatory arrest) and of 14 patients undergoing surgery for infrarenal aortic aneurysm. Basal values of syndecan-1 (1.2 μg/dL) and heparan sulfate (590 μg/dL) of patients were similar to those of control subjects. Anesthesia and initiation of surgery caused no changes. Global ischemia with circulatory arrest (n = 12) was followed by transient 42- and 10-fold increases in syndecan-1 and heparan sulfate, respectively, during early reperfusion (0 to 15 minutes). After regional ischemia of heart and lungs (cardiopulmonary bypass; n = 6), syndecan-1 increased 65-fold, and heparan sulfate increased 19-fold. Infrarenal ischemia was followed by 15- and 3-fold increases, respectively (n = 14). The early posts ischemic rises were positively correlated (r = 0.76, P < 0.001). Plasma concentrations of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 did not change. Circulating polymorphonuclear granulocytes and the level of postischemic heparan sulfate corresponded negatively. Immunohistochemical imaging and immunoassay of isolated hearts (guinea pig) substantiated syndecan-1 and heparan sulfate as components of the endothelial glycocalyx released into the coronary venous effluent. Electron microscopy revealed shedding of the glycocalyx after ischemia/reperfusion.

Conclusions—This study provides the first evidence in humans for shedding of the endothelial glycocalyx during ischemia/reperfusion procedures. (Circulation. 2007;116:1896-1906.)

Key Words: aneurysm ■ endothelium ■ glycocalyx ■ inflammation ■ ischemia

A healthy endothelium is coated by a “thick” endothelial glycocalyx. Intravital microscopic studies have shown that an exclusion zone for circulating red cells is present adjacent to the endothelial surface in which plasma motion is significantly retarded. This zone, also termed the “plasma layer” or “endothelial surface layer,” has a thickness of ≈0.4 to 0.5 μm in microvessels (in some regions, it is even thicker than the endothelial cells themselves) and contains fluid and plasma proteins in dynamic equilibrium with the flowing plasma.1,2 In larger vessels, an endothelial surface layer of up to 3 μm is assumed.3 Various experimental models showed this large structure to be fundamentally involved in numerous physiological and pathophysiological actions in the circulation. Damage to the endothelial glycocalyx decreases vascular barrier function and increases platelet and leukocyte adhesion. Moreover, heparan sulfates linked to core molecules of the endothelial glycocalyx play a pivotal role in inflammation.4,5 Previous experimental models have demonstrated that ischemia/reperfusion damages the endothelial glycocalyx.5–8 In patients experiencing perioperative ischemia due to aortic clamping and in patients undergoing cardiopulmonary bypass (CPB), vascular leakage with edema formation is a common complication. This complication is due at least in part to an inflammatory response that can even induce postoperative multorgan failure.9,10 On the basis of recent findings, it appears that damage to the endothelial glycocalyx could be a trigger for postoperative inflammation, edema, and other postoperative complications in patients. However, until now, perioperative damage to the endothelial glycocalyx has not been described in humans. Previous
studies from our laboratory, conducted on the isolated guinea pig heart, suggested that shedding of the endothelial glycolyx is associated with greatly increasing levels of syndecan-1 and heparan sulfate in the circulation.\textsuperscript{11,12} We now present the first evidence for an acute destruction of the endothelial glycolyx in humans undergoing vascular surgery associated with ischemia/reperfusion injury. Shedding of the glycolyx was investigated in patients with aortic surgery and global ischemia (deep hypothermic circulatory arrest [DHCA]) or regional ischemia (with and without CPB) by determining levels of syndecan-1 and heparan sulfate in blood at various phases of the procedure.\textsuperscript{2,13} To substantiate these findings, we present the first evidence for an acute destruction of the endothelial origin of these substances, intravascular shedding of syndecan-1 and heparan sulfate concentrations were also measured in guinea pig hearts with and without global ischemia, combined with electron microscopic visualization of the endothelial glycolyx and immunohistochemical tissue staining of syndecan-1 and heparan sulfate.

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Methods

The study was approved by the ethics committee at our institution (trial registration No. 339/02), with all patients giving their written informed consent. A total of 32 patients with aortic surgery were studied; among these, 12 (9 men and 3 women) underwent surgery of the ascending aorta with CPB and DHCA (DHCA group), 6 (5 men and 1 woman) underwent only CPB during repair of an aortic aneurysm and/or the aortic valve (CPB group), and 14 (all men) had surgery for an infrarenal aortic aneurysm without CPB (AAA group). Patients were excluded who had impaired renal function (serum creatinine >2.0 mg/dL), congestive heart failure (New York Heart Association class IV), or brain infarction during the last 6 months, as well as those who were classified as American Society of Anesthesiologists physical status IV. Patients were premedicated with an oral benzodiazepine. General anesthesia was induced with intravenous etomidate, fentanyl, or sufentanil and atracurium and was maintained with isoflurane 0.7 to 1.5 vol%. Mechanical ventilation was performed to maintain PaO$_2$ at 33.3 to 40.0 kPa and PaCO$_2$ at 5.3 kPa. Intraoperative monitoring included end-tidal PCO$_2$, ECG, pulse oximetry, esophageal and rectal temperatures, arterial blood gases, direct arterial blood pressure, central venous pressure, and, in most patients, pulmonary artery pressure. Figure 1 illustrates the experimental protocols.

Patients With Surgery of the Ascending Aorta With DHCA

After systemic heparinization (heparin 400 IU/kg), femoral artery cannulation, median sternotomy, and bicaval cannulation, CPB and core cooling were started. The surgical procedure included repair of the ascending aorta extending into the aortic arch and, in 3 patients, additional aortic valve replacement. The circulation was arrested after core temperature had reached 18°C. When repair of the aorta had been completed, gradual rewarming of the patients was begun by means of CPB to a core temperature of 36.5°C. Arterial blood had been completed, gradual rewarming of the patients was begun by means of CPB to a core temperature of 36.5°C. Arterial blood was first withdrawn to measure baseline values under stable anesthetic conditions before surgical incision (T0), 2 minutes after beginning CPB (T1), 2 minutes before aortic declamping (T2), and 2 hours after surgery (T3; Figure 1). Twenty-four hours after surgery, blood samples were taken again from all patients in whom the arterial line was still in place (n = 11; T4).

Patients With Surgery of the Ascending Aorta Without DHCA

After systemic heparinization (heparin 400 IU/kg), femoral artery cannulation, median sternotomy, and bicaval cannulation, CPB was started. The surgical procedure included repair of the ascending aorta and, in 3 patients, additional aortic valve replacement. In this group, the ischemic regions are chiefly the lungs and the heart during the ongoing CPB. During the operative period, the patient’s core temperature was kept constant at 32°C by CPB. Arterial blood samples were taken to measure syndecan-1 and heparan sulfate under stable anesthetic conditions before surgical incision (T0), 2 minutes after beginning CPB (T1), 2 minutes before aortic declamping (T2), 2 minutes before completion of CPB, ie, with reperfusion of heart and lungs already initiated (T3), and 60 minutes after CPB (T4; Figure 1).

Patients With Repair of Infrarenal Aortic Aneurysm

Intraoperative exclusion criteria were suprarenal clamping and aortic aneurysm that extended into the iliac arteries. After arrival in the operating theater, thoracic epidural catheters were placed in all patients. During the operative period, the patient’s core temperature was kept constant by use of fluid warmers and warming blankets. Arterial blood was first withdrawn to measure baseline values under stable anesthetic conditions before surgical incision (T0). The next blood samples were taken 2 minutes after clamping of the aorta (T1), 15 minutes after declamping (T2), and 2 hours after surgery (T3; Figure 1). Patients were excluded who had impaired renal function (serum creatinine >2.0 mg/dL), congestive heart failure (New York Heart Association class IV), or brain infarction during the last 6 months, as well as those who were classified as American Society of Anesthesiologists physical status IV. Patients were premedicated with an oral benzodiazepine. General anesthesia was induced with intravenous etomidate, fentanyl, or sufentanil and atracurium and was maintained with isoflurane 0.7 to 1.5 vol%. Mechanical ventilation was performed to maintain PaO$_2$ at 33.3 to 40.0 kPa and PaCO$_2$ at 5.3 kPa. Intraoperative monitoring included end-tidal PCO$_2$, ECG, pulse oximetry, esophageal and rectal temperatures, arterial blood gases, direct arterial blood pressure, central venous pressure, and, in most patients, pulmonary artery pressure. Figure 1 illustrates the experimental protocols.

Concentrations in Blood Plasma

Syndecan-1 concentrations were determined directly in plasma as previously reported by use of an ELISA (Diaclone Research, Besancon, France).\textsuperscript{11} This kit uses a solid-phase monoclonal B-B4 antibody against an extracellular domain of syndecan-1. Heparan sulfate was measured after pretreatment of plasma with actinase E (Sigma, St. Louis, Mo) by use of a special ELISA kit (Seikagaku Corp, Tokyo, Japan). Because no normal values for this variable are described in the literature, we also measured heparan sulfate in the blood of 10 healthy volunteers (5 men and 5 women).

Concentrations of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) were determined directly in plasma by ELISA (Biosource International Inc, Camarillo, Calif). These kits use horseradish peroxidase–conjugated monoclonal antibodies against human ICAM-1 and VCAM-1.

Animal Experiments

The animal investigation conformed with the National Research Council’s Guide for the Care and Use of Laboratory Animals. Permission to conduct the experiments was granted by the ethics committee installed by the government of Upper Bavaria (file No. 209.1/211-2531.3-2/99).

Guinea pig hearts were isolated and perfused in a Langendorff mode as described previously.\textsuperscript{11,14} In brief, animals (males, weight 200 to 250 g) were stunned by neck dislocation with a specially designed instrument, and immediately after the thorax was opened, the hearts were arrested with cold isotonic saline. The aorta was cannulated quickly and retrogradely perfused at constant aortic pressure (80 cm H$_2$O) and 37°C with a modified Krebs-Henseleit buffer gassed with 94.5% O$_2$ and 5.5% CO$_2$, pH 7.40±0.05. Hearts were removed from the thorax, perfused for 15 minutes, and then subjected to fixation (see below) either immediately or after 20 minutes of warm global ischemia plus 1 minute of reperfusion.

Electron Microscopy

Electron microscopy (Philips CM, Aachen, Germany) was performed with lanthanum nitrate fixative as previously published.\textsuperscript{11,14–16} Lanthanum is a trivalent cation and binds to negatively charged glycoprotein moieties of the glycolyx, stabilizing the
structure during fixation. In brief, the coronary vessels were flushed free of blood in situ and then perfused with a fixation solution (2% glutaraldehyde, 2% sucrose, 0.1 mol/L sodium cacodylate phosphate, 2% lanthanum nitrate). Afterward, diced pieces of tissue were soaked for 12 hours at 20°C in a solution of 2% H₂O₂, 2% sucrose, 0.1 mol/L sodium cacodylate phosphate, and 2% lanthanum nitrate and then washed (18 mL of 0.1N NaOH, 27 mL of H₂O, and 9 mL of sucrose 12%).

**Light Microscopy and Immunohistochemistry**

Four hearts were perfusion-fixed after minimal perfusion (<1 minute) by the addition of formaldehyde to the flowing Krebs-Henseleit buffer to a resulting concentration of 1%. After 4 minutes, the hearts were removed from the apparatus and stored in 4% formaldehyde solution for 24 hours. Paraffin sections (5 μm) were immunohistochemically stained with monoclonal antibody against heparan sulfate (Seikagaku Corp) or syndecan-1 (Biosource). The primary antibodies, applied to generate an avidin-biotin horseradish peroxidase complex and with the Vectastain kit (Vector, Burlingame, Calif), were diluted and handled as follows: anti-heparan sulfate 1:100, tissue preincubation with 0.2% trypsin at 37°C; anti-syndecan-1 1:50, tissue pretreatment by microwave irradiation. Controls, in which the primary antibody was replaced with buffer, were treated identically. Diaminobenzidine or aminoethylcarbazole was used as chromogen.

**Measurement of Syndecan-1 and Heparan Sulfate in Guinea Pig Hearts**

Syndecan-1 and heparan sulfate concentrations were determined in the coronary effluent of hearts without (n=10) and with (n=8) 20
minutes of warm (37°C), global, stopped-flow ischemia (ELISA kits as above). All samples were preconcentrated over 10-kDa cutoff membrane filters (Millipore, Eschborn, Germany).

**Statistical Analysis**

Normally distributed data (tested by Kolmogorov-Smirnov tests) are presented as mean±SEM, and nonnormally distributed data are presented as median and quartile difference (Q3–Q1). Results from statistical tests were regarded as significant if \( P<0.05 \). For repeated assessments of normally distributed data, comparisons were made with repeated-measures ANOVA. Post hoc comparisons with \( \text{T0} \) used a Bonferroni correction; local probability values <0.01 were regarded as significant, thus maintaining a global \( \alpha \)-level of 0.05. For nonnormally distributed data, comparisons were made with ANOVA on ranks for multiple comparisons and Wilcoxon tests as appropriate. Correlation between variables was evaluated with Spearman rank correlation coefficients. The probability values were 2-sided and were computed from Student \( t \) distribution with \( n−2 \) degrees of freedom, \( n \) being the size of the sample (Systat Software, Inc, San Jose, Calif).

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

**Results**

**Patients and Procedural Characteristics**

The operation protocols are outlined in Figure 1. Median age of the patients was 67.5 years (range 37 to 86 years), height was 173 cm (range 160 to 188 cm), and weight was 80 kg (range 59 to 100 kg). Median duration of operation in the DHCA group was 273 minutes (range 235 to 685 minutes), whereas it was 159 minutes (range 92 to 331 minutes) in the CPB group and 115 minutes (range 55 to 155 minutes) in the AAA group. In patients with surgery of the ascending aorta with circulatory arrest (median duration of 25 minutes, range 8 to 65 minutes), aortic clamping time was 100 minutes (range 55 to 227 minutes); it was a median of 91 minutes (range 60 to 154 minutes) in patients without circulatory arrest. Clamping time for infrarenal aortic surgery was 40 minutes (range 24 to 65 minutes). Eight patients who underwent surgery of the ascending aorta received erythrocyte concentrates (1 to 2 U) during the investigational period, but no patient who underwent surgery of the abdominal aorta did.

**Syndecan-1 and Heparan Sulfate Plasma Concentrations Increase Multifold After Ischemia**

In the literature, normal values for plasma syndecan-1 are a median of 1.7 \( \mu \text{g/dL} \) (range 0 to 30.8 \( \mu \text{g/dL} \)). The value for heparan sulfate determined by us in the blood of 10 healthy volunteers was a median of 559 \( \mu \text{g/dL} \) (quartile 1 to quartile 3, 482 to 794 \( \mu \text{g/dL} \)). Basal values of syndecan-1 and heparan sulfate in patients in the present study were a median of 1.2 (0.72 to 1.42) and 590 (453 to 841) \( \mu \text{g/dL} \), respectively. Figure 2 illustrates the multifold increases in syndecan-1 and heparan sulfate concentrations in blood plasma of patients undergoing aortic surgery. Global ischemia in patients with circulatory arrest (DHCA group) led to a median 42- and 10-fold increase in syndecan-1 and heparan sulfate concentrations, respectively, as detected directly after aortic declamping (T3). It should be noted that the heart and lungs were already being reperfused 2 minutes before the end of CPB (T3). All data are corrected for hemodilution (see figure legend). Interestingly, some increment was also present at T2, the time at which DHCA had come to an end. As soon as 1 hour after the end of CPB, both syndecan-1 and heparan sulfate concentrations had practically normalized (Figure 2A).

Figure 2 also illustrates the increases in syndecan-1 and heparan sulfate concentrations for patients undergoing surgery of the ascending aorta who had need of CPB but not of circulatory arrest (CPB group). Two minutes before termination of CPB, 65- and 19-fold increases had occurred in the median levels of syndecan-1 and heparan sulfate, respectively. Again, both syndecan-1 and heparan sulfate concentrations had returned to basal levels by the end of surgery (Figure 2B). In patients with repair of abdominal aortic aneurysm who had no CPB but had infrarenal total ischemia (AAA group), a 15-fold increase in the median syndecan-1 concentration had occurred 15 minutes after declamping. In these patients, heparan sulfate increased slowly after surgery (2-fold). The protocol for the AAA group also included measurements 24 hours after surgery. At this time, a selective 2.5-fold rise in heparan sulfate levels had also occurred; syndecan-1 shedding was unchanged versus the preoperative level (Figure 2C).

No correlation was present between the increase in syndecan-1 and heparan sulfate plasma concentrations and the transfusion of red blood cells (not shown). In addition, the increase in these substances did not correlate with the transfusion of any other blood product, because these were only given after T3 in patients with repair of the ascending aorta. When compared for all patients, syndecan-1 and heparan sulfate levels immediately after reperfusion were positively correlated (\( r=0.76, P<0.001 \); Figure 3). An even better correlation was obtained when the individual changes versus basal levels were compared (\( r=0.85, P<0.001 \)). Surprisingly, the increases in syndecan-1 and heparan sulfate during early reperfusion were rather poorly correlated with the preceding duration of ischemia (\( r=0.68 \) and \( r=0.58 \), respectively, each \( P<0.001 \); Figure 4). This counterintuitive finding reveals that there must be other determinants of glycocalyx shedding triggered by the ischemic challenge that exhibit great interindividual variability. Interestingly, the percentage of polymorphonuclear granulocytes in the blood of patients in the immediate reperfusion phase (T2 to T3 of Figure 1) tended to correlate negatively with the level of heparan sulfate (\( y=-99x+1.1789; r=0.51, P=0.006 \)). No significant correlation was present between polymorphonuclear granulocytes and syndecan-1 (\( r=0.19, P=0.5 \)), which suggests enhanced adhesion of polymorphonuclear granulocytes with increasing shedding of the negative glycocalyx charges.

**Plasma Concentrations of ICAM-1 and VCAM-1 Did Not Change During Ischemia/Reperfusion**

In contrast to the multifold increases in circulating glycosaminoglycans (Figure 2), no increases in ICAM-1 and VCAM-1 occurred in patients in the present study. Conclusively, heparan sulfate and syndecan-1 appeared to be more sensitive markers of the early distress of endothelial cells (Figures 5A and B).
Syndecan-1 and Heparan Sulfate Are Components of the Endothelial Glycocalyx

Electron microscopic photographs illustrating the endothelial glycocalyx of the coronary vessels of the guinea pig heart are depicted in Figure 6A. An endothelial glycocalyx with a thickness of \( \approx 0.2 \) to \( 0.3 \) \( \mu \text{m} \) is readily apparent. Figure 6B shows that after 20 minutes of global warm ischemia, the endothelial glycocalyx could no longer be visualized. Shedding of the endothelial glycocalyx was clearly connected with an increase of syndecan-1 and heparan sulfate content in the

Figure 2. Individual and median fold change of normalized syndecan-1 (CD 138) and heparan sulfate concentrations (left and right, respectively) at different operative stages. To account for hemodilution, individual syndecan-1 and heparan sulfate concentrations were related to the individual total protein concentration. Top, DHCA group. Patients undergoing surgery of the ascending aorta with circulatory arrest (global ischemia at T2). Middle, CPB group. Patients undergoing surgery of the ascending aorta without circulatory arrest but with CPB (regional ischemia at T2). Bottom, AAA group. Patients undergoing surgery of the infrarenal aorta (regional ischemia at T1). *Significantly different from T0, \( P<0.01 \); #significantly different from T0, \( P<0.001 \).
coronary effluent and therefore in the circulation. Light microscopy after immunohistochemical staining of nonischemic hearts evidenced syndecan-1 and heparan sulfate as components of the endothelial glycocalyx (Figure 7).

**Discussion**

The principal finding of this study is that components of the glycocalyx, syndecan-1 and heparan sulfate, are released from the tissue and can be detected in the circulating blood of patients with perioperative global or regional ischemia. To the best of our knowledge, this is the first quantitative description of this effect in humans. In the past, shedding of the endothelial glycocalyx due to ischemia was demonstrated in different animal models after very long periods of experimental ischemia. In patients in the present study, the low basal values and multifold increases of syndecan-1 and heparan sulfate concentrations indicate that syndecan-1 and heparan sulfate may serve as sensitive markers for shedding of the endothelial glycocalyx. Such an origin is suggested by the immunohistochemical localization of both syndecan-1 and heparan sulfate in the endothelial lining, including that of coronary vessels in the heart of the guinea pig. Using the isolated heart model, we demonstrated that shedding of the endothelial glycocalyx can be caused by ischemia and is associated with a multifold increase in circulating syndecan-1 and heparan sulfate (Figure 6). Because no change took place in levels of ICAM-1 and VCAM-1, integral membrane proteins, in patients in the present study, circulating components of the endothelial glycocalyx appear to be more sensitive markers of early endothelial cell distress.

**Dimension and Physiological Role of the Endothelial Glycocalyx**

The endothelial glycocalyx was discovered nearly 40 years ago in conjunction with the first electron microscopic investigations. Owing to insufficient staining techniques at that time, this structure was primarily regarded as a layer of membrane-bound proteoglycans and glycoproteins having a thickness of only a few tens of nanometers. Accordingly, no important role was attributed to it. In reality, however, an intact and healthy vascular endothelium is coated by a thick glycocalyx of 0.3 to 0.5 μm, further augmented by intercalated and adsorbed plasma components. This insight is the result of more recent direct and indirect estimations, performed in microvasculature of different animal models. Because of its fragility, only very special and careful staining techniques conserve the true “body” of the endothelial glycocalyx. Consequently, studies evaluating the role of the endothelial glycocalyx in human diseases have been extremely rare. Very recently, however, Nieuwdorp and co-workers demonstrated that hyperglycemia can lead to damage to the endothelial glycocalyx/endothelial surface layer by measuring plasma hyaluronan and assessing the total volume of the endothelial surface layer via tracer dilution techniques. The authors estimated in normal subjects a very large total volume of noncirculating plasma trapped within the endothelial glycocalyx, namely, 1.7 ± 0.2 L. By a comparable tracer dilution technique (indocyanine green instead of dextran 40 for measuring total intravascular plasma volume), our group had previously established a total volume of the endothelial surface layer of ≈ 720 mL in healthy gynecological patients after induction of anesthesia. By taking this amount and a total endothelial surface area of 350 m², Pries and Kuebler estimated the average thickness of the endothelial surface layer to be ≈ 2 μm. The discovery of the really large dimension of the endothelial glycocalyx/endothelial surface layer reveals a new, big, and probably very important compartment of the circulation.

In healthy vessels, the endothelial glycocalyx constitutes the primary structure that maintains the colloid osmotic competence of the vascular barrier. Almost 110 years ago, Starling’s hypothesis about the forces governing vascular permeability was born without any knowledge of the existence of an endothelial glycocalyx. Starling described vas-
cular fluid flux as a function of the differences between intravascular and interstitial hydrostatic pressures and between intravascular and interstitial colloid osmotic forces. Indeed, until recently, it was a commonly accepted belief that the high intravascular colloid osmotic force, as opposed to a very low interstitial protein content, limits fluid filtration and prevents edema formation. Within the past few years, however, interstitial colloid osmotic force was demonstrated to be much higher than Starling assumed. Van den Berg and co-workers demonstrated that damage to the huge glycocalyx structure leads to tissue edema. According to the “double barrier concept,” vascular barrier function is provided by 2 competent components, the endothelial glycocalyx and the endothelial cell bodies, in a heterogeneous, probably even dynamic system, which regulates vascular permeability. Furthermore, involvement of the glycocalyx in regulating adhesion of leukocytes and blood platelets to the vessel wall is more than likely. Moreover, this structure is probably the main sensor for shear stress–induced vasodilation and nitric oxide release. In the present study, patients undergoing surgery of the ascending aorta received a median of 5 U (1 L) of fresh-frozen plasma in the postoperative phase. Although this should have elicited measurable increases in plasma protein levels, no such rises were detected (results not shown). This suggests a state of marked extravasation of colloid but can also indicate a stronger incorporation within the glycocalyx.

Interindividual Variability and Kinetics in Human Glycocalyx Shedding

The high interindividual difference in increases of syndecan-1 and heparan sulfate in patients in the present study indicates that pathogenesis of glycocalyx disruption correlates with more than just ischemic intensity. Mathematical analysis corroborates this conclusion: Although the peak level of heparan sulfate during early reperfusion and the individual duration of ischemia correlated only with an r of 0.58, the correlation with syndecan-1 was 0.68 (Figure 4). We presume that the great variability may originate from a different individual thickness of the glycocalyx, different basal turnover values, and the speed and severity of deterioration after ischemia/reperfusion. Several experimental conditions are known to be connected to the destruction of the endothelial glycocalyx. In addition to enzymatic degradation (eg, with heparinase, neuraminidase, or pronase), destruction of the endothelial glycocalyx can be induced by tumor necrosis factor-α, oxidized lipoproteins, and, of special relevance during ischemia, reactive oxygen species. Heparanases, produced by platelets or inflammatory cells, can also degrade heparan sulfate chains. All these potential mechanisms may act in human beings with a considerable interindividual variability. In addition, constituent parts of the glycocalyx appear to be excreted rapidly via the kidney, and therefore, we cannot be sure of always having sampled the blood at peak shedding time. Disruption of the endothelial glycocalyx due to atrial natriuretic peptide was demonstrated in guinea pig hearts both by measuring the release of syndecan-1 from coronary vessels and by direct visualization with electron microscopy. Accordingly, atrial natriuretic peptide is probably responsible for the marked extravasation of colloids seen in patients with volume loading. This was also found to have a great

Figure 4. Correlation of peak, normalized plasma levels of syndecan-1 (A) and heparan sulfate (B) for individual patients in each group, with the respective duration of tissue ischemia.
interindividual variation. In the present study, we found a negative correlation between circulating polymorphonuclear granulocytes and the level of postischemic heparan sulfate. Normally, in most regions of microvasculature, circulating blood cells have direct contact with the endothelial glycocalyx but not with endothelial cells themselves. Therefore, diminution of the endothelial glycocalyx has an effect on the movement of erythrocytes through capillaries and results in an increase in vascular leukocyte adhesion and platelet aggregation. However, disrupted glycocalyx components can directly activate leukocytes, which then have the power to cut heparan sulfates from syndecans. Also, activation and aggregation of platelets causes release of a potent heparanase. This behavior has all the qualities of a vicious cycle, which makes it difficult to assign causalities, and can have a high interindividual variability per se.

In patients in the present study, the plasma concentration of these molecules increased multifold and decreased to normal very quickly, except for the heparan sulfate concentration in patients with repair of infrarenal aneurysm, which displayed a protracted, continuous rise. Presumably, the transient increases of syndecan-1 and heparan sulfate can be explained by proteolytic degradation of the glycocalyx with subsequent rapid clearance, especially via the kidneys. In the patients with repair of infrarenal aortic aneurysm, a general activation of leukocytes and platelets with an associated release of heparanases may have been responsible for a slower but ongoing systemic degradation of the side chains. Because of the lengthy observation in this group (24 hours versus 1 hour; Figure 1), lymphatic absorption and recirculation of glycocalyx of abluminal cellular origin may be occurring. Perhaps this is not a peculiarity of this particular type of operation, an aspect we intend to investigate in future studies. Likewise, it should be rewarding to analyze the kinetics of the initial transient with greater resolution in future work, as well as to analyze the glycocalyx compounds in urine to evaluate the rate of biosynthesis of glycosaminoglycans and regeneration of the glycocalyx.
Critical Evaluation of the Results

One fundamental question is: Were the entire multifold increases in syndecan-1 and heparan sulfate in patients in the present study only caused by damage to the endothelial glycocalyx? Endothelial cells also have a glycocalyx at their abluminal side, and many other cells build up a glycocalyx. However, the principal proteins that bind heparan sulfate to form proteoglycans are the transmembrane syndecans, and the major syndecan of both epithelial and vascular endothelial cells is syndecan-1.39 As clearly demonstrated by the present animal experiments (Figures 6 and 7), the main location of syndecan-1 and heparan sulfate appears to be the luminal side of the endothelium, and the multifold increases in the circulation coincide with visible destruction of the endothelial glycocalyx during ischemia/reperfusion in this model. In no way are we suggesting that the glyceal components in the plasma of the investigated patients originated from the coronary system alone. The heart merely served as a versatile experimental model. Another open question is the cause of the high interindividual differences in glycocalyx shedding in patients in the present study. This, however, can only be addressed when the mechanisms that lead to shedding

Figure 6. Top. Electron microscopic views of microvessels of guinea pig hearts stained to reveal the glycocalyx: A, after 15 minutes of blood-free perfusion; B, after an additional 20 minutes of warm (37°C), global, stopped-flow ischemia. Bottom, Syndecan-1 and heparan sulfate release in coronary venous effluent (n=10 and n=8, respectively) after 5 minutes of perfusion (A) or reperfusion (B). Values are mean±SEM per gram of heart weight. *P<0.01, intergroup difference vs control group without ischemia.
of the endothelial glycocalyx in humans are better understood.

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Disclosures

None.

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