Syndecan-1 restitution by plasma after hemorrhagic shock

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During the past 7 years, there have been a number of retrospective studies demonstrating that the early and empiric use of fresh frozen plasma in patients in hemorrhagic shock and receiving a massive transfusion is beneficial.[1–3] More recently, a prospective observational multicenter massive transfusion study (PROMMTT) confirmed that increased ratios of plasma to red blood cells and platelets to red blood cells decreased early mortality from hemorrhage.[4] A prospective randomized optimum platelet and plasma ratios (PROP:P:R) study was recently completed and currently under analysis. Results of these studies have dramatically changed the manner in which bleeding trauma patients are resuscitated,[5] although the mechanism of protection remains unclear. We hypothesized that central to plasma's protection is the endothelium. The important role of the endothelium to the pathophysiology of hemorrhagic shock has been coined the endotheliopathy of trauma.[6] Injury to the endothelium from trauma and hemorrhage results in alterations in coagulation, inflammation, vasoregulation, and organ-specific barrier integrity. This review will focus on the endothelium as a therapeutic target to mechanistically explain the protection provided by plasma to the endothelium.

GLYCOCALYX

Overview

The glycocalyx is a network of soluble plasma components that project from the cell surface of both epithelial and endothelial cells and is believed to play a key role in stabilization of membrane integrity. The glycocalyx is composed of both proteoglycans and glycoproteins. The proteoglycans are composed of a protein core to which attach a variety of glycosaminoglycans, primarily heparan sulfate. The major cell surface proteoglycan is syndecan, the focus of the current review. Glycoproteins are important to coagulation and include antithrombin III, heparin cofactor II, and thrombomodulin.[7] Other glycoproteins include cell adhesion molecules such as selectins and intracellular adhesion molecules. Shedding of the endothelial glycocalyx exposes adhesion receptors to circulating neutrophils, thus enhancing endothelial-neutrophil adhesion.[8]

Glycocalyx in Different Diseases

In models of cardiac ischemia, shedding of the glycocalyx was associated with vascular hyperpermeability, an effect mitigated by antithrombin, highlighting the interplay of the glycocalyx with coagulation.[9] Alterations in the endothelial glycocalyx have also been reported to be responsible for vascular leakage and leukocyte adhesion after cardiac arrest.[10] Finally, shedding of the syndecan-1 backbone and heparin sulfate moieties occurs in patients undergoing abdominal aortic aneurysm repair.[11] A dysfunctional glycocalyx has also been implicated in sepsis, diabetes, and atherosclerosis, as well as renal failure and hypervolemia (related to atrial natriuretic peptide).[12–15]

Role of the Glycocalyx After Hemorrhagic Shock

Alterations in the endothelial glycocalyx have only recently been recognized to occur after hemorrhagic shock and to be modulated by resuscitation. We showed in a rat model of pressure-controlled resuscitation that the endothelial glycocalyx, imaged using electron microscopy in the small bowel mesentery, was virtually ablated 2 hours after hemorrhagic shock.[16] Figure 1 illustrates the virtual absence of the endothelial glycocalyx after hemorrhagic shock compared with shams. Glycocalyx thickness after resuscitation by lactated Ringer's solution was similar to shock alone, whereas plasma significantly restored thickness. In a similar study by Torres et al.,[17] the cremaster muscle was imaged by intravital microscopy in a volume-controlled resuscitation model of hemorrhagic shock. Glycocalyx thickness after lactated Ringer's solution was 50% lower than in shams or rats resuscitated with fresh frozen plasma.

SYNDECAN-1

Syndecans are a family of heparin sulfate proteoglycans expressed on both epithelial and endothelial cells. They are transmembrane proteins with an extracellular domain that may be shed in response to a variety of stimuli. There are four members of the syndecan family, but syndecan-1 has been the focus of most laboratory and clinical studies.

Syndecan-1 Ectodomain Shedding

Ectodomain shedding is an important posttranslational mechanism that modulates diverse pathophysiologic processes that are not well understood.[18] In rodent models of sepsis, syndecan-1 shedding protects against gram-positive toxic shock by inhibiting dysfunctional inflammation.[19] Shedding facilitates resolution of inflammation by binding to chemokines to aid in the removal of proinflammatory mediators.[20] More recently, Johansson et al.[21] demonstrated an association between the sympathoadrenal activation, fibrinolysis, and syndecan-1 shedding in a small clinical study in septic patients, suggesting that the catecholamine surge of sepsis may lead to endothelial damage.

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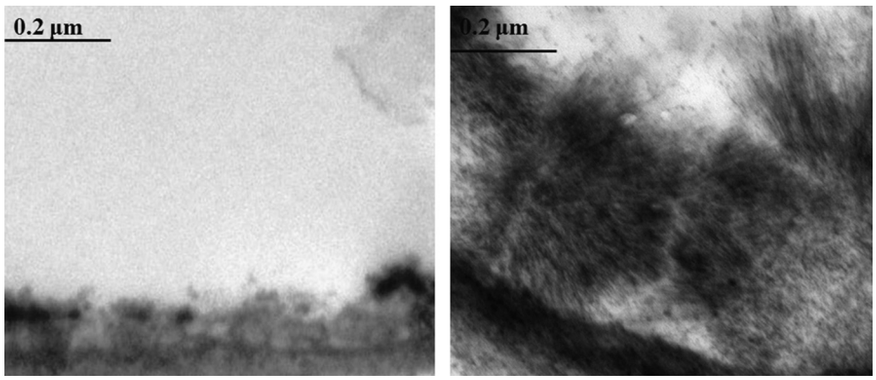

Figure 1. Virtual absence of the endothelial glycocalyx following hemorrhagic shock. Rats were subjected to 90 minutes of hemorrhagic shock then 2 hours of reperfusion. The small bowel mesentery was harvested and perfused with uranyl acetate and lead citrate to stain the glycocalyx as we have described.[16] Representative images of postcapillary venules are shown. The left image is following hemorrhagic shock alone and illustrates the virtual absence of the gycocalyx compared with the sham in the right image. Magnification is 20,000×.

Oxidative stress–induced shedding caused neutrophil chemotaxis and aberrant wound healing in a model of pulmonary fibrosis.[22,23] Hemorrhagic shock–induced shedding seems to be injurious, resulting in the exposure of the injured endothelium to proinflammatory mediators and in alterations to the structural integrity of the endothelium with resultant hyperpermeability.

We have shown in a small pilot study that syndecan-1 is shed at the time of injury in severely injured patients in hemorrhagic shock.[24] Our data also suggested that patients with higher postresuscitation syndecan-1 levels had a higher mortality (survivors, 144 ± 141 ng/mL vs. nonsurvivors, 289 ± 226 ng/mL; $p = 0.15$). Shed syndecan-1 negatively correlated with three proinflammatory cytokines, interferon-γ, fractaline, and interleukin-1β, whereas interleukin-10 was positively correlated. These four cytokines interestingly all play a role in endothelial integrity. More recently, Johansson et al.[25] demonstrated increased mortality in patients with high admission shed syndecan-1 levels and that shedding correlated with inflammation and coagulopathy. To begin to understand the mechanisms underlying the pathologic role of shedding in injured patients in shock and, importantly, the mechanisms responsible for plasma's protective role, we used an in vitro model of shock, hypoxia-reoxygenation, to examine endothelial integrity.[24] Hyperpermeability induced by shock was mitigated by fresh frozen plasma (FFP) but not lactated Ringer's solution. In addition, adherens junction protein vascular endothelial cadherin, which is responsible of endothelial integrity, was similarly disrupted by shock and lessened by FFP but not lactated Ringer's solution. Using a rat pressure-controlled model of hemorrhagic shock and the lung as an end-organ damaged after injury, we next demonstrated that lung histopathology was present after shock, not improved by lactated Ringer's solution resuscitation, but significantly lessened by plasma.[16] Pulmonary syndecan-1 mRNA and cell surface syndecan-1 immunostaining were similarly increased by plasma but not lactated Ringer's solution. These findings were expanded in a coagulopathic mouse model of volume-controlled resuscitation. Hemorrhagic shock led to systemic shedding of syndecan-1 and lessened pulmonary syndecan-1 immunostaining—changes that correlated with lung hyperpermeability and inflammation.[26] FFP compared with lactated Ringer's solution abrogated these pathologic changes. Changes in permeability and inflammation by FFP were mimicked by spray-dried plasma.[27]

Available evidence suggests that the injurious changes to the endothelium after hemorrhagic shock are caused by loss of the syndecan-1 backbone and encompassing glycocalyx.[16,24–26] In uninjured vessels, an intact glycocalyx harbors adhesion molecules within its protective structure. With pathologic stimuli such as shock, exposure of the now injured endothelium to pathologic neutrophils can occur. Chappell et al.[28,29] have shown in models of ischemia/reperfusion that protection of the glycocalyx reduced both leukocyte and platelet adhesion and subsequent hyperpermeability and inflammation. Additional investigations using syndecan-1 loss in vitro or in vivo have confirmed a proinflammatory phenotype in response to shear stress.[30] However, studies specifically examining sequelae of endothelial loss of syndecan-1 using syndecan-1–null mice may be hampered. Savery et al.[31] have shown that the thickness of the endothelial glycocalyx in null mice was only slightly less than that of wild-type mice, suggesting that either synedcan-1 was not essential or these null mice may adapt to loss of syndecan-1 by increasing the expression of other proteoglycans. Endothelial cells also contain both syndecan-2 and syndecan-4,[31] and our preliminary data suggest that syndecan-1–null mice have increased levels of syndecan-2 (unpublished data). Studies to evaluate the role of syndecan-1 using syndecan-1–null mice in models of hemorrhagic shock are underway.

Potential Mechanisms by Which Plasma Reconstitutes Syndecan-1

Syndecan-1 shedding is controlled by outside-in signaling that initiates the proteolytic cleavage of the syndecan-1 ectodomain.[18] Little is known about the intracellular processes that lead to shedding. Hayashida et al.[32] have shown that shedding agonists stimulate dissociation of GTPase Rab5 from the cytoplasmic domain of syndecan-1, which then exposes syndecan-1 to sheddases. The metalloproteinases of the A Disintegrin And Metalloproteinase (ADAM) family are the largest group of sheddases. It is possible that hemorrhagic shock is a pathologic stimuli that activates matrix metalloproteinases or ADAMs

and that plasma reduces their activation. In addition to direct effects on matrix metalloproteinases and ADAMs, plasma could also reduce the activities of these enzymes by increasing the expression of endogenous inhibitors such as the tissue inhibitor of metalloproteinase (TIMP) family of glycoproteins.[33] In particular, TIMP3 has been shown to be a critical regulator of ADAM17 activity, with the balance of TIMP3 and ADAM17 being important for the regulation of tumor necrosis factor-α–induced inflammation.[34] Tumor necrosis factor-α is increased early after trauma, [35] and plasma is known to contain TIMP3 (unpublished data). The potential role of plasma's inhibition of sheddases and activation of inhibitors of sheddases is currently being investigated.

In summary, hemorrhagic shock causes shedding of the syndecan-1 ectodomain, which is associated with organ damage and worsened outcomes. Recent evidence suggests that resuscitation with plasma reduces shedding and reconstitutes the endothelial glycoclayx. Regulation of syndecan-1 shedding postinjury and/or reconstitution of syndecan-1 and the glycocalyx could potentially serve as viable therapeutic targets for novel drug discovery. As plasma is composed of thousands of circulating proteins, investigation of the specific component(s) of plasma responsible for its protective effects may warrant further investigation.

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DISCLOSURE

The authors declare no conflicts of interest.

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