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Resuscitating the Endothelial Glycocalyx in Trauma and Hemorrhagic Shock

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Abstract: The endothelium is lined by a protective mesh of proteins and carbohydrates called the endothelial glycocalyx (EG). This layer creates a negatively charged gel-like barrier between the vascular environment and the surface of the endothelial cell. When intact the EG serves multiple functions, including mechanotransduction, cell signaling, regulation of permeability and fluid exchange across the microvasculature, and management of cell-cell interactions. In trauma and/or hemorrhagic shock, the glycocalyx is broken down, resulting in the shedding of its individual components. The shedding of the EG is associated with increased systemic inflammation, microvascular permeability, and flow-induced vasodilation, leading to further physiologic derangements. Animal and human studies have shown that the greater the severity of the injury, the greater the degree of shedding, which is associated with poor patient outcomes. Additional studies have shown that prioritizing certain resuscitation fluids, such as plasma, cryoprecipitate, and whole blood over crystalloid shows improved outcomes in hemorrhaging patients, potentially through a decrease in EG shedding impacting downstream signaling. The purpose of the following paragraphs is to briefly describe the EG, review the impact of EG shedding and hemorrhagic shock, and begin entertaining the notion of directed resuscitation. Directed resuscitation emphasizes transitioning from macroscopic 1:1 resuscitation to efforts that focus on minimizing EG shedding and maximizing its reconstitution.

Keywords: endothelial glycocalyx, hemorrhagic shock, resuscitation

INTRODUCTION

From the time of Galen's ancient, influential theory on the circulation of blood to the contemporary delineation of the ultrastructure of the glycocalyx, our understanding of blood flow in terms of the anatomy of the endothelium has evolved and continues to change with advancements in technology.[1,2] Electron microscopy allowed researchers to recognize a certain heterogeneity in the endothelium as well as to deduce variability in function. The glycocalyx was then introduced as an extracellular polysaccharide coating on cells.[3] Some 50 years ago, with the invention of the electron microscope, Luft was able to visualize the glycocalyx.[4] However, the significance of this delicate structure remained largely unknown. With the refinement of staining techniques and improved technology, it is apparent that this carbohydrate mesh has importance extending beyond its barrier function. This 'sweet-husk' stands front and center in trauma-related hemorrhage and the ensuing endotheliopathy that occurs. It is a potential cause or indicator of the severity of pathology as well as a promising therapeutic target. The following paragraphs seek to give the reader a brief introduction regarding the structure and function of the endothelial glycocalyx (EG), review and summarize significant findings noted in hemorrhagic shock, and trauma, and suggest future directions for research and exploration.

STRUCTURE

The glycocalyx is a negatively charged, carbohydrate-rich layer composed of a core backbone of proteoglycans and glycoproteins attached to the endothelial cell.[4] This structure extends approximately 0.5–5 μm (sometimes even as much as 1 μm) into the vascular lumen. The thickness depends on the type of blood vessel.[5] There is some variability in the published thickness of this multilayered structure because current fixation techniques may damage it. Attached to the core proteoglycans and glycoproteins are glycosaminoglycans (GAGs) and oligosaccharide chains, respectively, to which plasma proteins, peptides, and other soluble proteoglycan components are bound.[5] Collectively, these components interact to create a continuous layer that provides structural support and actively regulates homeostatic endothelial cell functions.

The EG is rich in heparan sulfate (HS) proteoglycans, including syndecans (that contain a membrane-spanning domain that associates with the cellular cytoskeleton) and glypicans (that are anchored to the cell membrane by glycosylphosphatidylinositol anchors). Additional proteoglycan structures include mimecan, perlecan, and biglycan, which are secreted and become part of the larger glycocalyx structure.[4] The GAG chains vary in length and number. The more frequently seen GAG chain is HS, comprising 50%–90% of the GAG chains in the glycocalyx, and it has an important impact when shed.[3,4] HS chains are primarily formed in the Golgi apparatus and bind to the core proteoglycan structure. They consist of a chain of disaccharides that are altered by sulfation, acetylation, or deacetylation. Other GAG structures within the EG include keratin sulfate, chondroitin sulfate (CS), and hyaluronic acid (HA).

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Glycoproteins include endothelial cell adhesion molecules, such as selectins and integrins, which span and compose a significant portion of the glycocalyx.[4] These structures bind to a proteoglycan/glycoprotein core to create a continuous, negatively charged, mesh-like structure. The EG also contains soluble components more superficially located in the glycocalyx. These include plasma proteins and soluble proteoglycan components. These proteins provide a unique characteristic to the endothelium in its environment. They are dynamic and ever-changing and help maintain this layer's selectivity and negative charge.

Though this surface comes together to form a negatively charged aqueous gel layer, studies suggest that the GAG and bound proteins are well intermixed in the EG. However, the GAG structures are not bound to each other. This was demonstrated when CS and HA were digested but did not affect HS.[6] This delicate and often overlooked structure coats the endothelium and plays a vital role in cell-cell signaling, and signal transduction, and has a major role as a mechanosensor and modulator of the microvascular environs.

FUNCTION

The complex and dynamic structure of the endothelial glycocalyx layer (EGL) serves several functions that are important during hemorrhagic shock, including mechanotransduction and cell signaling, oxygen radical scavenging, and managing endothelial permeability. The absence of these functions may be responsible for further physiologic derangements.

Mechanotransduction

The structure and function of the EG are tightly intertwined. Mechanotransduction describes the sensing or perception of extracellular signals for transmission or signaling into the intracellular environment. This multilayered structure is dynamic and appears to adapt to surrounding environmental demands by functioning as a mechanosensor and transducer.[6] There are 3 major types of mechanical stress that the EG responds to: cardiac cycle-generated hemodynamic forces that are perpendicular to the endothelial surface, tangential forces (such as shear stress), and circumferential stretch.[6,7] Together this transmits a certain pattern of force. The ultrastructure of the EG functions as a sort of "Wind in the Trees" model, where the wind (or force) is sensed by the outer branches, composed of GAGs, and transmitted through the glycoprotein core (tree trunk) through the cell membrane.[7] This transmission is responsible for intracellular signaling. Results from animal studies show that the application of a steady amount of shear stress to the vessel wall results in increased nitric oxide (NO) production from baseline. With the enzymatic breakdown of the EG components, however, NO production was stunted, but shear stress-induced prostacyclin production remained unchanged.[7] NO is a soluble gas synthesized within the endothelium from the amino acid L-arginine.[8] It is responsible for smooth muscle relaxation in the vascular wall.[8] Production of NO helps in the regulation of basal vascular tone, and its production is increased with resulting vasodilation in response to shear stress. Destruction of any one of the components may not produce the same effect. As studies have shown, preservation of NO-mediated vasodilation with the digestion of CS but not with HS, HA, and sialic acid.[7] Other studies found endothelial nitric oxide synthase production decreases in reaction to shear stress when HS is digested from the EG; favoring the conclusion that HS may play a role in the mechanotransduction of shear stress.[9] Understanding the EG component breakdown in critically ill patients is important for the purpose of targeting resuscitation.

As exposure to shear stress changes, so does the conformation of the EG components and the cytoskeleton within the endothelium, resulting in potential changes to the permeability of the glycocalyx.[10] This mechano-remodeling of the cells may include elongation of the endothelial cells in the direction of the undisturbed flow. In cases of disturbed flow with significant oscillations, endothelial cells have been shown to assume a cobblestone appearance.[11] The purpose and importance of the glycocalyx lies in its ability to sense the intravascular environment and serve as a liaison, via signaling, to the endothelial cell, while protecting it from the dynamic stresses of the intravascular environment.

Evidence suggests that high shear stress or dysregulated flow affects glycocalyx thickness and its composition.[12] When Gouverneur et al[9] exposed human umbilical vein endothelial cells to nonpulsatile shear stress, there was an increase in glucosamine-containing GAGs, such as hyaluronan, incorporated in the glycocalyx. Other studies with much higher shear stress showed the EG incorporated with sulfated components. The carotid artery is an example of the variations seen based on flow-related shear stress. The carotid sinus has a significantly diminished EG thickness compared to the opposite wall. Bifurcations in the carotid artery show varying thicknesses of the EG. Studies in which 2 different shear stress intensities were delivered to the EG demonstrated greater incorporation of sulfated GAGs with greater stress.[12] The EG is important in the conversion of mechanical forces to signal transduction. Areas of high shear stress or disturbed flow promote changes in glycocalyx thickness and change the permeability of the underlying endothelium. The EG's reaction to shear stress patterns, and the implications when these same components are shed, are important to understand when targeting therapeutics toward the glycocalyx.

Permeability and the Microcirculation

Regulating permeability is noted to play a major part in the glycocalyx's ability to maintain a homeostatic environment for the endothelium. To gain a better understanding of the importance of the EG in the permeability of the endothelium, it is important to discuss Starling's original model of fluid movement within a capillary. Starling's principle showed that fluid movement in and out of the capillary is influenced by a balance of hydrostatic and osmotic forces within and outside the vascular lumen.[13,14] Nearly one hundred years later, discrepancies noted between the original principle and new quantitative findings were satisfied after the EG was considered.[14] The EG appears to contribute to the colloid pressure gradient between the vascular space and the interstitial space, signifying the important effect its integrity has on permeability and fluid movement. When a loss of GAGs is mediated by enzymatic treatment, significant subendothelial fluid retention is noted in rats.

Oxygen Radical Scavenging

The proteoglycan structure plays a role in scavenging oxygen radicals by harboring various enzymes that break down reactive oxygen species (ROS).[12] Superoxide dismutase dismutates oxygen radicals into oxygen and hydrogen peroxide. This enzyme is bound to proteoglycans within the glycocalyx and aids in decreasing radical-mediated damage to the endothelium. When the glycocalyx is damaged or shedding occurs, superoxide dismutase is shed as well and leaves a peri-endothelial environment favoring the persistence of oxygen radicals.[12]

SHEDDING AND RESUSCITATION

The EG diligently maintains a homeostatic sub-environment so that mechanotransduction, signaling, impermeability, and a negatively charged environment are created. Shedding of the EG components, secondary to a traumatic event, changes the balance from an anti-inflammatory, negatively charged

antioxidant state, to a pro-inflammatory one, attracting inflammatory cytokine binding, coagulation on the endothelial surface, and increased permeability. These changes have negative downstream effects that increase ROS generation, worsen coagulopathy, and increase vascular permeability. The resulting disturbances are related to increased morbidity and mortality. The degree of shedding has shown to be dependent upon the degree of insult.[14]

Several studies have addressed a heightened sympathoadrenal response following glycocalyx dysfunction. Xu et al[15] reported on the association between sympathoadrenal activation and endothelial damage. Following hemorrhage, sympathetic denervation suppressed serum catecholamines, soluble thrombomodulin, and syndecan-1 (Sdc-1) levels. Sympathectomy was endothelial protective and anti-inflammatory and produced anti-fibrinolytic effects in this model of acute traumatic coagulopathy.[15] Hofmann et al.[16] found that the extent of hemorrhagic shock severity, as assessed by a base deficit and/or lactate, determined the amount of glycocalyx shedding and subsequent sympathoadrenal activation. Severe hemorrhagic shock was noted to result in endothelial injury even before resuscitation.[16] Its shedding is also noted to be a mediator and impact coagulopathy.[17,18]In a trauma patient, a host of factors collectively occur to create a hostile environment for the EG. The initial insult promotes primary injury to the EG—systemic cytokine activation and a large sympathetic response results in an increase in ROS from ischemic/reperfusion injury.[18] The occurrence of these events promotes the shedding of the EG components, a thinner and more attenuated structure, and a changed microenvironment. Leukocyte adherence to an attenuated EG becomes easier.[19] With injury and hemorrhagic shock, a combination of shear stress, ischemia-reperfusion (I/R) injury, a sympathoadrenal activation, with increased generation of pro-oxidants create a microenvironment conducive to shedding.[20,21] Coagulopathy, third spacing, and microvascular dysfunction are associated with EG shedding.[15,20–24] The severity of pathology observed from trauma-related endotheliopathy is associated with the severity of glycocalyx shedding.[25]

Plasma Sdc-1 and other proteoglycans and GAGs are known to be indirect measures of EG shedding, which have been shown to correlate to the shedding activity at the level of the endothelium after injury and/or hemorrhage.[26] Though there are several isoforms in the syndecan and glypican families, the most cited shed marker in trauma and hemorrhagic shock is Sdc-1, an HS proteoglycan.[27] HS is the dominant GAG present on the endothelial surface. Finally, HA, a GAG not bound to a core protein is frequently shed as well.[28] Differing downstream effects have been cited for these shed components. Higher levels of HA, for example, have been associated with abnormalities in the coagulation profile of trauma patients.[29] With increased shear stress as seen in trauma and hemorrhage, increased incorporation of HA has been noted as well.[9] HS plays a large role in NO production when exposed to shear stress. When digested by heparinase III there was a significant decline in NO production by the endothelium.[27] During times of injury/shock, reduction of HS in the EG matrix may negatively impact the individual's ability to respond to shear stress. There is also a suggestion of auto-heparinization secondary to increased circulating HS shed from the EG.[30] Sdc-1 shedding appears to be primarily related to the severity of the microvascular injury and is selectively shed by heparinase.[26,31]However, much more exploration remains to delineate the exact impact of each shed component during injury.

THE ROLE IN HEMORRHAGIC SHOCK

Hemorrhagic shock induces EG shedding as demonstrated in the postcapillary venules of skeletal muscle and mesentery of rats. Following hemorrhage, Torres Filho et al[27] found that greater decreases in glycocalyx thickness significantly correlated with larger reductions in blood flow and a positive correlation with vascular permeability in the microcirculation.[26,32] Interestingly, Abdullah et al[17] found that glycocalyx shedding is not evenly distributed among all vascular beds in the setting of hemorrhagic shock. Glycocalyx shedding was most prominent in the endothelium of the pulmonary and intestinal vasculature when compared to cerebral vessels, the coronary system, skeletal muscle capillaries, portal veins, and sinusoids. The areas with the greatest shedding (pulmonary and intestinal vasculature) demonstrated the greatest level of ROS generation within the endothelium.[17] The authors explain that different patterns of shedding is likely secondary to each tissue bed experiencing varying levels of hypoxia, oxidative stress, and I/R injury from resuscitation.[17,20] Phenotypic differences of the endothelial cells and endothelial nitric oxide synthase activity in the individual tissue bed may play a role in differential shedding as well.[17,21] In addition, the inclusion of polytrauma with hemorrhagic shock may have dissimilar changes as opposed to hemorrhagic shock alone, in vascular barrier permeability and patterns of shedding.[33] In a study by Guerci et al[34], rats experiencing hemorrhagic shock without trauma had glycocalyx shedding without barrier disruption.[33] In another study, estrogen, not testosterone, modulated the disruption of the EG.[34] Another consideration to help explain differential shedding in tissue beds is the presence of signaling mechanisms, unique to each tissue bed, dictating varying sheddase activity. Dialysis patients have been found to have circulating hyaluronan and Sdc-1 with higher hyaluronidase activity.[21] In I/R injury to the heart, mast cell degranulation is associated with heparanase expression.[21] In terms of tissue characteristics Suzuki et al[36] investigated the EG in various tissue beds and types of vessels.[35] She found that each tissue had unique EG characteristics suggesting that thickness, coverage, and composition were specific to the function of each tissue bed.[35] Much remains to be explored as to why certain tissue beds have a differing EG, how each bed is impacted by I/R, the true influence of sexual dimorphisms, and the phenotypic properties of the endothelial cells. Hemorrhage and injury may need to be viewed separately, as their individual impact on shedding as well as vascular barrier disruption may be different.[33] EG thickness and Sdc-1 levels are often used as biomarkers of glycocalyx integrity. There is a negative correlation between glycocalyx thickness and plasma levels of both Sdc-1 and HS, both components of the EG.[26] A systematic review by Hahn et al[37] critically appraised the glycocalyx literature and found that trauma regularly reported a 3–4 fold increase in degradation products, but a mixed picture in surgical patients alone was much more dependent on the intraoperative course and type of surgery.[36]

In a study by Hofmann et al[16], severe hemorrhagic shock was shown to induce endothelial injury at the time of the event, before resuscitation.[16] Despite various studies showing an association between EG dysfunction and increased vascular permeability, Guerci et al[38] found that during degradation of the EG, vascular barrier permeability remained intact following plasma fluid resuscitation in a model of nontraumatic hemorrhagic shock in rats.[37–39]

A study by Ostrowski et al[31] found increased levels of plasma Sdc-1 in trauma patients who had evidence of acute endogenous heparinization on their thromboelastogram purported an association between the two. Patients with increased endogenous heparinization had more severe endothelial damage, enhanced inflammation, and hyperfibrinolysis.[30]

In a randomized control trial of 75 patients, Johansson et al[23] found that despite similar injury severity scores (ISS), patients with elevated Sdc-1 levels demonstrated 3-fold higher mortality levels. Elevated levels of Sdc-1 expression were significantly associated with lower O_2 saturation at the scene of the accident, increased lactate and glucose levels, increased markers of inflammation (IL-6, IL-10), greater markers of tissue and endothelial

damage (histone-complexed DNA fragments, high-mobility group box 1, and thrombomodulin), and greater markers of fibrinolysis (D-dimer, tissue-type plasminogen activator, and urokinase-type plasminogen activator). After adjusting for age and ISS, Sdc-1 levels were shown to be independently predictive of 30-day mortality. Collectively, this study postulated that downstream effects of the injury rather than the injury itself resulted in glycocalyx degradation that resulted in hyperfibrinolysis and increased mortality due to endothelial dysfunction, loss of vascular integrity, and downstream organ damage.[22,40]

A study by Rahbar et al[41] found that decreased plasma colloid osmotic pressure correlated with increased injury severity and Sdc-1 shedding.[40] In a prospective observational study, increased HA and adrenaline, in addition to Sdc-1, were found in patients with low plasma colloid osmotic pressure compared with a control group of patients with normal plasma colloid osmotic pressure. Despite this association, there was no detectable difference in other components of the EG, namely CS and heparin sulfate, when comparing patients with normal and low colloid osmotic pressure. This suggests the idea that HA may have a role to play in the maintenance of the EGL and vascular integrity during hemorrhagic shock.[41]

Sphingosine-1-phosphate, a regulator of vascular permeability, is decreased in polytrauma patients compared with healthy controls, but not significantly so between polytrauma patients with or without hemorrhagic shock. Sdc-1 was strongly increased in patients with polytrauma and hemorrhagic shock over their time course of observation even when normalized to plasma protein. Higher Sdc-1 levels on day 1 were associated with higher maximal Sequential Organ Failure Assessment scores.[41] Higher Sdc-1 levels also correlated significantly with activated partial thromboplastin time levels. This study concluded that hemorrhagic shock is a driver for glycocalyx dysfunction early in polytrauma patients.

In a 410-patient study by Gonzalez Rodriguez et al[42], patients with Sdc-1 levels of ≥60 ng/mL had the highest rates of blood transfusions and 30-day in-hospital mortality. Sdc-1 levels of the nonsurvival cohort were significantly higher than those of the survival cohort (nonsurvival cohort median of 40.4 ng/mL; survivor cohort median of 24.7 ng/mL). This study concluded that Sdc-1 serves not only as a biomarker of EG breakdown but rather as an index of endotheliopathy of trauma.[25]

THE ROLE IN TRAUMA

Trauma is accompanied by a significant inflammatory process that promotes the activation of damage-associated molecular patterns, increased cytokine activation and sympathoadrenal response, and leukocyte adherence, reactivity, and downstream signaling.[18] A more severe injury is associated with a more severe inflammatory process.[18,42] The degree of inflammation is associated with an increased neutrophil activation, resulting in an increase in oxidative stress to the EG, leading to breakdown.[18] However, shedding is not straightforward as in recent multiomic analyses of trauma patients cluster patterns of shed markers and increases in inflammatory cytokines differ by patient and injury type.[43,44]

Traumatic brain injury (TBI) is a significant target of investigation of EG shedding. The EG plays an important role in the blood-brain barrier.[35] Its major functions include regulating permeability and acting as a mediator of inflammation and anticoagulation on its surface.[45,46] When the EG is impaired, brain microcirculation is affected and an increase in permeability leads to neuroedema and secondary injury.[45,47] Literature suggests that the severity of isolated TBI is associated with a rapid increase in degradation products which is associated with poor outcomes.[46] Gonzalez Rodriguez et al[49] found that isolated TBI patients had significantly less Sdc-1 shedding than non-TBI polytrauma and TBI with polytrauma patients.[48] In a different study by Genét et al[50] traumatic injuries not including the brain were associated with a greater ensuing endotheliopathy based on shed EG biomarkers.[49] There is evidence that shows released damage-associated molecular patterns from damaged astrocytes may increase EG shedding after TBI, potentially contributing to secondary brain injury.[47]

Individual organ system contribution to EG shedding was investigated by Suzuki et al[43] They found that of the 6 defined body regions via the abbreviated injury scale scores, chest, and abdominal/pelvic regions were associated with higher Sdc-1 levels.[42]

RESUSCITATION FLUIDS AND IMPACT ON GLYCOCALYX

The type of resuscitation fluid has shown to play a role in the integrity of the glycocalyx after hemorrhagic shock. In several studies, crystalloids, more than colloids are associated with increased EG shedding.[26,50] Torres Filho et al[27], demonstrated that the type of resuscitation fluid impacted the thickness of the glycocalyx after hemorrhage. However, Ergin et al[51] demonstrated that permeability did not change despite increased shedding, while other investigators showed different results. Several papers have delineated the effects of different types of resuscitation on EG integrity, with fresh frozen plasma (FFP) providing the most favorable outcomes.[26,38,39,51–54]

In a murine model of hemorrhagic shock, glycocalyx degradation was partially restored by plasma but not by lactated ringers (LR). Rats subjected to hemorrhagic shock with plasma resuscitation demonstrated early signs of restoration after 2 hours of resuscitation measured by glycocalyx thickness on electron microscopy. Sdc-1 mRNA isolated from the lung increased after plasma resuscitation suggestive of glycocalyx restoration. The protective effects of resuscitation through plasma may be due in part by restoration of the EG after hemorrhagic shock.[51]

A study by Peng et al[40] found similar results and reported that FFP resuscitation compared to LR resuscitation suppressed injurious effects following hemorrhagic shock. Lung permeability was significantly increased after hemorrhagic shock measured by extravasation of an intravenously delivered fluorescently conjugated dextran. FFP resuscitation significantly decreased lung permeability compared to LR resuscitation. Additionally, Sdc-1 shedding was reduced by FFP compared to LR. Systemic Sdc-1 shedding correlated with decreased pulmonary Sdc-1 and increased pulmonary vascular permeability. Glycocalyx restoration may play a role in tissue perfusion after shock.[39]

Torres et al[53] also reported improved outcomes with FFP, which demonstrated increased microvascular perfusion associated with a restored EG. FFP resuscitation restored Sdc-1 levels to control levels and restored EG thickness of a cremaster muscle after just an hour of resuscitation comparable to baseline in a hemorrhagic shock model.[53] Torres Filho et al[27] compared 7 different resuscitation strategies on glycocalyx restoration and found fresh whole blood and FFP to be superior to packed red blood cells (pRBC), 5% albumin, LR, normal saline, and 3% hypertonic sodium chloride. Albumin only partially restored glycocalyx thickness whereas FFP provided near full restoration. Nelson et al[54] note that favorable outcomes with FFP resuscitation following hemorrhage can be explained by improved plasma volume expansion compared to crystalloids.[54,55]

Hofmann et al[55] demonstrated that resuscitation with FFP restored HS to baseline values but not Sdc-1. The addition of coagulation factor concentrates had no effect on Sdc-1 shedding. Compared with crystalloids, colloid resuscitation resulted in higher mean arterial pressure, improved base deficit and lactate as well as decreased concentrations of epinephrine and HS.[55]

Wu et al[39] purported that other members of the syndecan family may be contributing to the effects FFP has on the EG.

When pulmonary Sdc-1 expression was silenced in mice, FFP lost its protective effects of the glycocalyx compared to LR resuscitation. Permeability was increased as well, suggesting a role for pulmonary Sdc-1 in modulating pulmonary protection by FFP after hemorrhagic shock.[39]

The Prehospital Air Medical Plasma (PAMPer) trial, a study that looked at the efficacy of prehospital plasma on trauma patients compared to standard resuscitation found it safe to transfuse thawed plasma in the prehospital setting.[56,57] When performing hierarchical clustering analysis on admission samples to determine patterns of elevation, 2 clusters emerged (A and B) with differing injury severity, type, and patterns of inflammatory and endothelial marker elevations.[45] Cluster A patients had a higher ISS, more blunt trauma, and TBI, whereas Cluster B patients had more penetrating trauma.[45] Cluster A patients had an improved 30-day survival with prehospital plasma whereas this was not seen in Cluster B patients.[45] This further supports FFP's use as a modulator of traumatic endotheliopathy secondary to EG shedding. In a study by Wu et al[44], a multi-omic analysis was performed on injured patients to explore the effect of injury on each -omic layer. The authors classified injury based on various endotypes with or without a concomitant TBI. They found that prehospital FFP had a limited impact on lowering the shed EG markers in TBI and nonbrain injury patients.[44] Though FFP did not appear to show a beneficial effect in terms of EG modulation, the importance of this study is to show that there was a certain subtype that appeared to benefit. Different injury patterns and individual phenotypic responses to injury seem to impact FFP responders versus nonresponders.[44]

A separate study found that fresh whole blood or pRBC/LR resuscitation was superior in restoring glycocalyx thickness, permeability barrier, and blood flow following hemorrhage compared with pRBC/LR or LR alone. Given that plasma has been shown to induce glycocalyx restoration, the authors hypothesized that residual plasma in pRBC may be responsible for the restoration of the glycocalyx and its permeability.[58,59]

Aksu et al[59] compared balanced crystalloid resuscitation versus unbalanced crystalloid resuscitation and found that neither restored the glycocalyx (measured through hyaluronan levels) in a rat model of hemorrhagic shock. Normal saline has been shown to have deleterious effects on the endothelium. Torres et al[60] found that normal saline prolonged endothelial injury compared to resuscitation with LR, albumin, or FFP.

Patients in hemorrhagic shock demonstrate elevated plasma Sdc-1 levels upon arrival to the emergency department, which decreased significantly after resuscitation, but still well above controls. Postresuscitation Sdc-1 levels were elevated, but not statistically significant, in patients who did not survive (survival mean 144 ± 141 ng/mL; non-survival 189 ± 226 ng/mL). IL-10 was also shown to positively correlate with Sdc-1 levels in shock patients.[61]

ADJUNCTS OF RESUSCITATION

There is a fair amount of literature describing adjuvant therapy following hemorrhage and resuscitation in murine models. Torres Filho et al[62] tested the effects of different adjuncts (ALM: adenosine + lidocaine + magnesium; BHB/M: beta-hydroxybutyrate plus melatonin; P-188: poloxamer 188) on EG integrity. Following hemorrhage, resuscitation with each adjunct significantly restored EG thickness compared to hemorrhage models. However, resuscitation with these adjuncts did not significantly restore plasma Sdc-1 levels following hemorrhagic shock.

Hydroxyethyl starch has recently been described as beneficial for the EG. Uzawa et al[63] found a protective effect of hydroxyethyl starch (HES130) in suppressing vascular permeability during resuscitation, as well as improved pH and lactate levels compared to a cohort of resuscitation with either normal saline or normal saline with albumin.[63] Zhao et al[64] found that HES recovered glycocalyx thickness as well as other components of the EG via downregulation of the expression of heparinase, hyaluronidase, and neuraminidase. Administration of these EG degrading enzymes (heparinase, hyaluronidase, and neuraminidase) suppressed the beneficial effects of HES on permeability.[64,65]

Other studies have shown hydrogen gas inhalation to be protective of the glycocalyx. Hydrogen gas inhalation following hemorrhagic shock with LR resuscitation suppresses Sdc-1 expression. Degradation of the glycocalyx is mediated by ROS and pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α. Plasma Sdc-1 levels and TNF-α levels negatively correlate with mean arterial pressure measurements. Remarkably, TNF-α and Sdc-1 plasma levels positively correlated with each other. In the presence of an anti-TNF-α monoclonal antibody, there was an observed reduction of plasma TNF-α and Sdc-1 shedding as well as improved hemodynamic stability following hemorrhagic shock. There were no additional therapeutic effects when the anti-TNF-α monoclonal antibody was administered in the presence of hydrogen gas inhalation after hemorrhagic shock with resuscitation, which ultimately demonstrates that hydrogen gas inhalation exerts its effects through suppression of TNF-α mediated EG shedding.[65] Sato et al[66] found similar therapeutic effects of hydrogen gas inhalation in terms of a resultant thicker glycocalyx as well as longer survival time after hemorrhagic shock resuscitated with normal saline.

In a study by Alves et al[67], sphingosine-1-phosphate resulted in improved outcomes on the glycocalyx following hemorrhagic shock and resuscitation by way of acting through mitochondrial complex III. Mitochondrial complex III inhibition decreased HS expression, a component of the EG. Treatment with sphingosine-1-phosphate after mitochondrial complex III inhibition preserved HS expression maintaining glycocalyx integrity.[67]

Other novel adjuncts, such as Tubastatin A (a histone deacetylase 6 inhibitor), malate, and the use of hemoglobin-based oxygen carrier, have also been shown to be promising therapeutics in maintaining glycocalyx integrity following hemorrhagic shock.[68–70]

AGING AND THE ENDOTHELIAL GLYCOCALYX

Given that a greater proportion of injured adults are younger, with less comorbidities, the assumption is that the EG before the injury is intact and healthy, with shedding severity primarily associated with injury severity. However, this may not be the case in all trauma patients. A growing population of injured adults are older and are accompanied by greater comorbidities and associated vasculopathies, such as diabetes and cardiovascular disease.[71,72] Morbidity and mortality are worse in this older cohort compared to their younger counterparts.[73] A deeper dive shows animal, in-vitro, and human studies in which aging, is associated with greater endothelial dysfunction, a thinner EG, as well as aberrant shedding.[74–78] In patients with renal disease and uremia, EG is also much thinner.[22] Combined with an altered innate immune function in injured older adults from cumulative disease and aging, EG response to injury appears to be different than in younger trauma patients.[79] Thus, age-related changes may make this population more vulnerable to poor outcomes from injury and optimal resuscitation in this group may be different than their younger counterparts and needs to be studied further.

CONCLUSION AND FUTURE DIRECTIONS

The clinical impact of the EG is substantial. The above studies show a similar pattern. Worsening injury or hemorrhage is associated with higher Sdc-1 and HS levels, decreased glycocalyx thickness, correlating to increased microvascular permeability, and overall worsening morbidity and mortality. In animal

studies, this relationship is easier to depict as the plasma levels of shed components can be directly correlated to microscopic findings of the EG. In human subjects, plasma levels of EG components serve as markers of increased shedding.

How will this information guide resuscitation in trauma and hemorrhagic shock? Before we ask this question other questions must be asked first; What is a balanced resuscitation? Does it strictly involve the transfusion of a certain ratio of products? Or is balanced resuscitation defined by the degree of EG shedding during resuscitation? And should our resuscitation be targeted towards minimizing shedding, and perhaps minimizing the morbidity of the initial insult as well as our resuscitation? Our definition of balanced resuscitation will likely change as our understanding of the microvascular changes of trauma and resuscitation evolve.

Based on the above studies, the future of resuscitation in hemorrhagic shock may be directed towards minimizing EG shedding and maximizing timely reconstitution, thereby making resuscitation more targeted and minimizing the negative downstream morbidity. This future entails a more directed resuscitation. The incorporation of adjuncts such as tranexamic acid, and vasopressors, with prothrombin complex concentrate is already occurring. But how these intermix to minimize shedding and maximize timely repopulation of EG is where the investigation must continue. If minimizing shedding and maximizing timely rebuilding is the goal, then an individual's EG health on presentation must be considered as resuscitation may differ.[75] Balanced transfusion for an injured older adult or a patient with particular multi-omic injury patterns may involve unique and personalized adjunctive treatments such as tranexamic acid and high-dose intravenous ascorbic acid with arginine vasopressin to modulate EG shedding, minimize volume overload, and allow for each transfused product to have a greater effect, as opposed to beginning with aggressive 1:1 product infusion. With the intensity of ongoing research, it appears that a directed resuscitation to restore the glycocalyx is where the future lies.

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