The role of endothelial glycocalyx in sepsis

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ABSTRACT

The surface of endothelial cells is filled with various membrane-bound molecules that form the glycocalyx. The endothelial glycocalyx is a surface layer mainly consisted of glycosaminoglycans that include heparan sulfate, chondroitin sulfate, and hyaluronic acid and its core proteins. Previous studies suggest that endothelial surface glycocalyx shedding could play a role in endothelial dysfunction and inflammation. This article will review the endothelial glycocalyx and its role in sepsis.

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INTRODUCTION

Endothelial glycocalyx produced by endothelial cells occupies a very important position on the endothelial surface of blood vessels that interact with plasma proteins and lipids. Endothelial glycocalyx is not only present on the surface of endothelial cells, but also in the space between endothelial cells and on the side of endothelial cells that are close to the basement membrane. The space between endothelial cells is called "endothelial clefts" (ETC) where glycocyte plays an important role in the regulation of vascular permeability.[1–3]

Endothelial glycocalyx is composed of glycoproteins or proteoglycans as their backbone. Proteoglycans and glycoproteins attached to the endothelial by various types of bonds. On the luminal side, they bind to carbohydrates called glycosaminoglycans (GAG), which are negatively charged. The combination of GAG along with glycoproteins/proteoglycans forms a complex sequence.[2]

The thickness of glycocalyx reaches 400-500 nm, which exceeds the length of endothelial cell receptors involved in leukocyte attachment to selectins and integrins. Studies of the integrin β2 receptor lengths are 20 nm and 30 nm for selectin E and P. Endothelial glycocalyx thickness decreases according to the diameter of the blood vessels and reaches a thickness of 0.2 μm on the microvascular. In addition to electron microscopy, the endothelial glycocalcular thickness can also be measured using *orthogonal polarization spectral imaging*. By using indocyanine green, the endothelial glycocalyx volume in humans is estimated to be 700 ml, with an outer surface estimated at 350 m^2.[4]

Dynamic balance occurs between dissolved components of glycocalyx and blood flow, which affect the composition and thickness of the glycocalyx. The interaction between endothelial glycocalyx and substances dissolved in plasma will determine the biosynthesis and release of the glycocalyx components.

PROTEOGLYCANS

Proteoglycans are proteins that make up endothelial glycocalyx which is the backbone of endothelial glycocalyx and bind one or more GAGs. Glycosaminoglycans that are bound to proteoglycans vary and can change depending on the stimulus given by substances contained in the plasma.[2,5,6]

Proteoglycans are composed by core proteins which consist of syndecan, which attach to the endothelial through the membrane-spanning, while glypican attaches to the surface of the vascular endothelial through the GPI anchor. Perlecan, versican, biglycan, decorin, and mimecan are proteoglycans which after being formed and undergo modification in the GAG, the blood vessel lumen will be secreted. Each proteoglycan molecule can bind GAG that is different depending on the stimulus received by cells.[2,5,6]

Syndecan is the most glycoprotein composed of endothelial glycocalyx with molecular weights ranging between 19-35 kDa. Research on the umbilical vein endothelial found that syndecan-1 was expressed the most, followed by syndecan-2, syndecan-3, syndecan-4, and glypican-1.[7]

Glycosaminoglycans are bound to the sides of the core protein consisting of heparan sulfate (HS), chondroitin sulfate, dermatan sulfate, hyaluronan, and keratan sulfate. Heparan sulfate is the highest GAG in endothelial glycocalyx, ranges from 50 to 90%, and has a ratio of 4:1 to chondroitin sulfate, the second largest GAG.[8]

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Glycosaminoglycans, such as heparan sulfate and chondroitin sulfate contain different sulfating sequences, sulfuric diversity and to specific sulfates that cause glycocalyx to bind various molecules. Some of the molecules capable of being bound by glycocytes are antithrombin III, heparin cofactor II, tissue factor pathway inhibitors, thrombin, protein C inhibitors. Another molecule that is able to be bound is superoxide dismutase (SOD), L and P selectin, TGF β, lipoprotein lipase, hepatic lipase, fibronectin, and collagen type I, III, and V.[9]

ENDOTHELIAL GLYCOCALYX FUNCTION

Maintaining permeability

The concept of endothelial glycocalyx as a regulator of permeability was first put forward by Danielli in 1940, who stated that there are non-cellular layers on the endothelial surface called the endocapillary layer. This layer turns out to have a very important role to maintain and regulate blood vessel permeability.[1]

Research by Rehm and colleagues on the guinea pig coronary arteries proves that endothelial glycocalyx has a role in maintaining and limiting the transfer of fluid from intravascular to interstitial.[10] Research on the mesenteric arteries of mice also proved that the glycocalyx layer plays a role in determining vascular permeability.[2,11]

The endothelial glycocalyx structure that contains many of the GAG chains that are overlapped, results in glycocalyx having a negative charge. This negative charge on the surface causes the glycocalyx to be able to regulate vascular permeability by limiting the entry of certain molecules based on the electric charge of the molecule. Glycocyte will reject negatively charged cells/molecules such as erythrocytes, leukocytes, and platelets.[2,5,6] Ueda proved that neutralizing the charge from endothelial glycocalyx will cause an increase in vessel permeability, resulting in increased albumin uptake by endothelial cell cultures.[2,12]

Disruption of endothelial glycocalyx causes a loss of glycocalyx function to maintain vascular permeability, which in turn may lead to edema.[6] This can be proven from studies in mice, where the destruction of mice myocardial endothelial glycocalyx causing myocardial edema.[13]

Preventing leukocytes and thrombocyte attachments with endothelial

Endothelial glycocalyx also functions as a barrier to platelet and leukocyte adhesion in the vascular wall. There are two mechanisms that prevent adhesion of blood cells to the endothelial membrane. The first mechanism is the negative charge of endothelial glycocalyx which rejects the blood cell to attach to the glycocalyx surface. The second mechanism is the thickness from glycocalyx endothelial.

Endothelial glycocalyx thickness exceeds the dimensions of cellular adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and L-selectins, thus preventing blood cell interactions with this molecule. Under normal conditions, leukocytes are protected from contact with adhesion molecules because of the presence of endothelial glycocalyx. Exposure to pro-inflammatory mediators such as IL-1, IL-2, IL-6, TNF-α, and other molecules released during acute inflammation such as bradykinin, thrombin, VEGF, and histamine.[14] Inflammation causes damage and detachment of endothelial glycocalyx, which in turn causes leukocytes to be easily exposed to adhesion molecules, resulting in the attachment of leukocytes to blood vessel membranes.[6] The adhesion molecules then stimulate leukocyte rotation, adherence, and migration causing inflammation of the endothelial lining and tissues.

Damage to the endothelial subsequently causes more severe glycocalyx degradation and shedding followed by a progressive increase in paracellular permeability. During this shedding process, adhesion molecules are released and can be found in the blood circulation. Thus, in patients with sepsis, circulating levels of VCAM-1 and ICAM-1 are parallel to IL-6.[14]

The above theory was proven by research in the cremaster venules of mice that had been damaged by heparitinase, which causes an increased leukocyte attached to the endothelial. The higher the level of heparitinase given, the higher the leukocyte attached to the endothelial. The administration of ox-LDL (oxidized low-density lipoprotein) or TNF-α also causes endothelial glycocalcular smoothness which results in increased rolling and endothelial leukocyte adhesion.[2] Other studies showed an increase in leukocyte attachment to the cremaster venule endothelial after administration of ox-LDL.[15] Leukocytes, platelet, and endothelial interactions are also increased after the destruction of endothelial glycocalyx by ox-LDL.[16]

Mechanotransduction

The glycocalyx structure delivers the mechanical and biochemical forces that pass through the glycocalyx into biochemical signals. Fluid shear stress (FSS) due to increased flow/blood flow will cause

increased production of local nitric oxide (NO), which causes vasodilation and increased vascular permeability. Vasodilation after FSS is a mechanism for protecting blood vessels against stress. Damage to endothelial glycocalyx disturbs the mechanism of vasodilation induced by NO. Vink et al. proved that the damage of endothelial glycocalyx after exposure to oxidized lipoproteins causes a loss of endothelial ability to produce NO, so vasodilatation does not occur after the flow went through these veins.[17]

The main evidence supporting the role of large endothelial glycocalyx in mechanotransduction comes from experiments using selective enzymes to degrade specific components of endothelial glycocalyx, followed by a reassessment of the glycocalyx function after being given FSS.[18–20]

Research to prove the association between FSS, endothelial glycocalyx and cytoskeleton rearrangement due to FSS found that endothelial glycocalyx is an FSS transduction tool, and there is an FSS threshold that triggers the cytoskeleton rearrangement. Observations on intact endothelial glycocalyx have severe disturbances in thick peripheral actin bands (dense peripheral actin band, DPAB), stress fiber formation, and vinculin migration to the cell edge after FSS exposure. Although this has been seen in many previous studies, this rearrangement disappears completely when the integrity of endothelial glycocalyx is impaired.

Protection of endothelial cell

Glycocalyx also acts to protect endothelial cells from various damage due to various mediators such as oxidative stress. Under normal circumstances, glycocalyx store various antioxidants such as SOD (superoxide dismutase) which is bound to heparan sulfate. SOD serves to reduce oxidative stress by eliminating/extinguishing the effects of oxygen radicals and maintaining bioavailability of NO so it can prevent endothelial dysfunction.[6]

Reducing inflammatory effects

Endothelial glycocalyx is thought to play an important role in protecting endothelial cells from damage caused by various mediators of oxidative stress. Under physiological conditions, endothelial glycocalyx contributes to the vasculoprotective effect because its capacity binds to superoxide dismutase (SOD) enzymes. Extracellular superoxide dismutase is bound to heparan sulfate proteoglycans, which plays a role in reducing oxidative stress by extinguishing oxygen radicals and maintaining NO bioavailability.[6]

Dilatation of blood vessels due to FSS followed by the release of NO is also a protection system of blood vessels because dilation of blood vessels will reduce stress on the endothelium.[21] Research on the human umbilical vein shows that elevated FSS will increase the production of hyaluronic acid in glycocalyx which also functions to protect blood vessels.[1] Endothelial glycocalyx can also bind to cytokines, which have a major influence on composition and synthesis or even modulate inflammatory responses by increasing the bond between cytokines and receptors on the cell surface.[6,22,23]

GLYCOCALYX ENDOTHELIUM IN SEPSIS

Vascular endothelium is first affected in the systemic or local inflammatory processes, either due to infection, diabetes mellitus, surgery, ischemic reperfusion injury, and atherosclerosis.[3,24,25] As explained earlier, optimal endothelial glycocalyx function is highly dependent on intact structures. Structural damage can be minor to severe damage. Loss of glycocalyx constituents is called *shedding*.[3,25]

Shedding of endothelial glycocalyx is found in arterioles, capillaries, and venules. It is a result of the release of various inflammatory mediators in sepsis, such as cytokines and chemoattractant, shown in various inflammatory model experiments.[3,25,26] At the beginning of the process of decreasing the endothelial glycocalyx layer, the supply of nutrients will improve, but microvascular changes due to the release of glycocalyx cause reduced blood vessel protection which adversely affects the function of the blood vessels.[27] Endothelial glycocalyx degradation increase leukocyte access to attach to endothelial adhesion molecules.[24]

The release of inflammatory mediators in sepsis will directly damage the structure of endothelial glycocalyx which certainly interferes with endothelial function. Inflammatory reactions also activate a subset of leukocytes such as polymorphonuclear (PMN), macrophages, and mast cells, to release various enzymes that contribute to endothelial glycocalyx degradation.[27]

TNF-α released during sepsis causes damage to the glycocalyx through proteases, which cause shedding of GAG, and in more severe circumstances, causes the release of core proteins (syndecan).[23,24,28] TNF-α causes acute shedding of coronary arteries and followed by the occurrence of myocardial edema which indicated an increase in vascular permeability. TNF-α will activate mast cells to release cytokines, proteases, and also heparanase. Heparanase causes heparan sulfate degradation. TNF-α also stimulates nitric oxide synthase

to produce NO which causes extensive vasodilation, hypotension, and hyporeactivity to adrenergic agents.[24] n the study using the sepsis model, high TNF-α levels were associated with reduced syndecan expression and changes in hyaluronan and sialic acid.[14]

Research conducted on cremaster's microvessel of rats shows that reactive oxygen species (ROS) is also an agent that causes endothelial glycocalyx damage. Ischemic injury, reperfusion, and activated neutrophils cause increased production of ROS followed by increased penetration of dextran 70-kDa, but this condition will improve after superoxide dismutase (SOD) is given. This evidence shows that ischemic injury and reperfusion cause the release of ROS, which can be proven by a reduced adverse effect of ROS after SOD administration. This study also found that xanthine-oxidoreductase (XOR) can increase the production of ROS, which can be inhibited by allopurinol so that the effects of reperfusion ischemic injury will decrease after administration of allopurinol.[29] At the time of ischemic injury and reperfusion, XOR will be released in very large amounts into the circulation, due to cell damage. Circulating XOR undergoes proteolysis with the help of enzyme serine protease to XO, which is then immobilized/binds to the glycocalyx endothelium.

Some studies have successfully evaluated syndecan-1 and GAG in septic patients.[30,31] Circulation levels of syndecan-1 are associated with endothelial damage and endothelial glycocalyx degradation. If there is degradation of endothelial glycocalyx, there will be shedding of fragments such as syndecan-1 into the blood circulation. A cross-sectional study reported a significantly higher syndecan-1 level in the septic shock group compared to controls (246 ng/mL (140-496) vs. 23 ng/mL (26-31), p <0.001). This study also found high GAG levels in septic shock patients compared to controls, and GAG levels were significantly higher in patients who died. In the same study, the syndecan-1 level also increased and turned out to be positively correlated with the Sequential Organ Failure Assessment score (SOFA) scores.[30]

The study of 150 subjects suffering from severe sepsis, septic shock, and post-digestive surgery with no systemic inflammatory response syndrome (SIRS) compared to healthy subjects showed a significant increase in syndecan-1 and heparan sulfate levels in the ill-subjects compared to the healthy subjects. The highest increase in syndecan-1 levels was obtained in the sepsis and postoperative groups. The high levels of syndecan-1 in sepsis patients turned out to be positively correlated with IL-6 levels.[32] Sallisami et al. reported a significant increase in the level of syndecan-1 in the septic shock group compared to the control group (p <0.01). The study also reported a positive correlation between VICAM-1 and the first-day SOFA scores.[33]

Other study reported that the syndecan-1 levels were positively correlated with the severity of sepsis based on the score of Acute Physiology and Chronic Health Evaluation (APACHE) II (r = 0.425, p = 0.001), SOFA score (r = 0.476, p = 0.003), and Multiple Organ Dysfunction Syndrome (MODS) (r = 0.529, p = 0.001). The study also found an increased level of heparin sulfate was correlated to the severity of sepsis.[31]

THE SHEDDING OF ENDOTHELIAL GLYCOCALYX

Various factors may cause the shedding of endothelial glycocalyces, such as ischemic injury, reperfusion injury, and inflammation due to sepsis. It turned out that several factors can also cause shearing of endothelial glycocalyx. Shedding causes the loss of some or complete function of glycocalyx, causing various endothelial and systemic problems. Atrial natriuretic peptide (ANP) is one of the causes of shedding. The ANP is known to increase vascular permeability. Bruegger et al. injected ANP into the lumen of the guinea pig coronary arteries, causing an increase in extravasation of hydroxyethyl starch (HES) 6% by 29%.[34]

A study conducted by Chappell et al. showed that in elective surgical patients with good cardiopulmonary conditions, after the administration of 6% HES fluid loading was 20 ml/kg body weight, there was a significant increase in ANP, followed by an increase in shedding from syndecan-1, hyaluronan in the blood, but not followed by an increase in heparin sulfate. Improved syndecan-1 is also detected in urine. This study also proved the release of ANP that occurs after fluid loading causes shedding of endothelial glycocalyx. This study strengthens the role of ANP in glycocalyx shedding.[35]

In patients who underwent Caesarean section with spinal anesthesia who received 750 ml bolus of crystalloid fluid, there was a significant increase from syndecan-1 and heparan sulfate but no significant increase in ANP levels. This is different from other studies, and it was probably due to the average bolus volume of 9 ml/kg of body weight, which is thought to not causing any significant stretches of the atrial wall to release ANP.[36]

Acute hyperglycemia has been shown to cause shedding of endothelial glycocalyx especially hyaluronan, followed by increased vascular permeability and plasma permeability. Hyperglycemia is a potent pro-oxidant and is a pro-inflammatory stimulus that is associated with shedding glycocalyx

through the radical oxygen pathway. Shedding glycocalyx is followed by increased procoagulant activity, as evidenced by increased coagulation of factor VIIa activity and stimulation of tissue factor. Endothelial glycocalyx is also the site of attachment of antithrombin III, so there is no doubt that the glycocalyx is followed by an increased coagulation activity.[37]

CONFLICT OF INTERESTS

The authors report no conflict of interests in the writing of this review.

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