J Anesth (2015) 29:269–278
DOI 10.1007/s00540-014-1887-6

REVIEW ARTICLE

Implications of recent accumulating knowledge about endothelial glycocalyx on anesthetic management

Ghada M. N. Bashandy

Received: 2 May 2014 / Accepted: 30 June 2014 / Published online: 1 August 2014

Abstract The endothelial glycocalyx is a labile, fine structure coating the luminal membrane of intact healthy vascular endothelium. For many decades, no physiologic importance was linked to this structure. It is crucial for vascular barrier function. There has been an immense interest in recent years for studying this important structure, and research is needed to disclose more information about it. Perioperative damage of the glycocalyx has been demonstrated, and is linked with morbidity and even mortality in surgical patients. Research on the glycocalyx should change many of the current perioperative management guidelines, and focusing on its protection is plausible. The present article reviews what we already know about the glycocalyx and how this knowledge has changed anesthesiologist perspectives.

Keywords Endothelial glycocalyx · Third space loss · Sepsis · Perioperative fluid management

Background

The endothelial glycocalyx (EG) is a fine structure coating the luminal membrane of healthy vascular endothelium that is crucial for vascular barrier function. It is a dynamic layer that extends luminally from several tens of nanometers up to three micrometers [1]. In some regions, the glycocalyx is even thicker than the endothelial cells themselves [2, 3]. It is a labile structure and may be rapidly affected by metabolic and inflammatory events [4]. For many decades, no physiologic importance was linked to this structure because it is destroyed upon conventional tissue fixation and is optically transparent in most light microscopic examinations. At best, it was detected only as an 'exclusion' zone for erythrocytes in blood perfused vessels [5]. Perioperative damage of the glycocalyx has been demonstrated [6]. While some transfusion strategies proved to cause shedding of the EG, sevoflurane showed to be protective [7]. Currently, no drugs are known to increase synthesis or directly prevent degradation of the glycocalyx. Therefore, perioperative prevention of EG damage and incorporation of potentially protective agents should be considered [8].

History

Seventy-three years ago, Danielli was the first to postulate that the polysaccharides layer coating the endothelial surface, together with trans-membrane proteins, serves as a permeability barrier [9]. Subsequently, by histochemical analysis, Bennett termed this barrier the "glycocalyx," derived from the Latin for "sweet husk," due to its predominant polysaccharide constituents [10]. A few years later, the EG was first visualized by conventional electron microscopy using ruthenium red. An anatomical width of only some tens of nanometers was suggested by the first electron microscopic visualizations, which relied on traditional fixation techniques [11]. As a result of more recent intense research, more details about the glycocalyx are being discovered.

Glycocalyx structure and visualization techniques

The endothelial surface layer (ESL) is formed by a glycocalyx basal skeleton interacting intensely and

G. M. N. Bashandy (✉)
Department of Anesthesiology and Pain Management, National Cancer Institute, Cairo University, 1 Fom Alkhalij, Kasr Alainy St., Cairo 11211, Egypt
e-mail: ghada_pashandy@yahoo.com

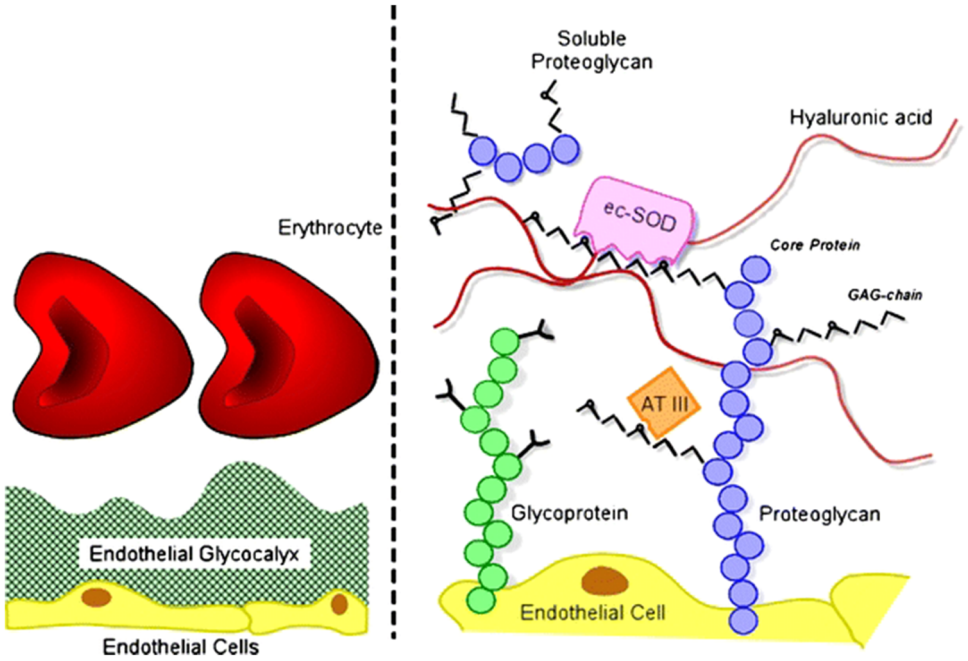

Fig. 1 Schematic representation of the main components of the EG. *Left* The EG can be observed in vivo as a red blood cell exclusion zone. *Ec-SOD* extracellular superoxide dismutase, *AT III:* antithrombin III [12]

dynamically with plasma constituents in vivo. This represents the real physiological layer at the interface between flowing blood and the vessel wall [2, 12, 13].

Glycocalyx is composed of: (see Fig. 1)

- Proteoglycans: these are the most important "backbone" molecules of the glycocalyx. They consist of a core protein of syndecans (four types) and glypicans (six types), to which one or more glycosaminoglycan (GAG) chains are linked [12, 14]. GAG chains are linear polymers of disaccharides with variable lengths. There are five types of GAG chains: heparan sulfate, chondroitin sulfate, dermatan sulfate, keratan sulfate, and hyaluronan [15–20] Heparan sulfate proteoglycans represent roughly 50–90 % [21]. There are also soluble proteoglycans, which either reside in the glycocalyx or diffuse into the blood stream [22].
- Glycoproteins: like the proteoglycans, they are also regarded as "backbone" molecules. They are characterized by relatively small (2–15 sugar residues) and branched carbohydrate side chains [12]. Endothelial cell adhesion molecules are well-defined glycoproteins that play a major role in cell recruitment from the bloodstream and in cell signaling. The three families of cell adhesion molecules present in the endothelial glycocalyx are the selectin family, the integrin family, and the immunoglobulin superfamily [23] Glycoproteins with coagulation, fibrinolysis, and hemostasis functions are also harbored in the EG [26].
- Soluble components: various types of proteins, e.g., albumin and orosomucoid, and soluble proteoglycans are embedded within and layered on top of the mesh of proteoglycans and glycoproteins. These components are derived from either the endothelium or from the bloodstream [27].

The glycocalyx dimension

The thicknesses of the EG reported by various authors are widely heterogenous due to differences in the applied techniques, sample preparation procedures, species and organs used, and cultivation conditions, as well as the use of in vivo, ex vivo, and in vitro models. The thickness of the EG in the first images in 1966 measured approximately 20 nm in capillaries [11]. By 1979, a theoretical estimate of glycocalyx dimensions was up to 1 μm thick [28]. By 2003, a new staining protocol with Alcian blue 8GX in rat myocardial capillaries revealed that endothelial cells are covered by a 200–500 nm thick glycocalyx [29]. In 2004, an improved electron microscope staining protocol using fluorocarbon-based oxygen carrying fixatives revealed the glycocalyx to be as thick as 60–200 nm in glomerular capillaries, and 50–100 nm in intestinal fenestrated capillaries [30]. It was reported that glycocalyx thickness is 40 nm under shear stress conditions [31]. By 2006, an estimate of the thickness of the ESL was almost 2 μm by calculation, after knowing the amount of sequestrated plasma within the EG and a total endothelial surface area of 350 m^2 [2].

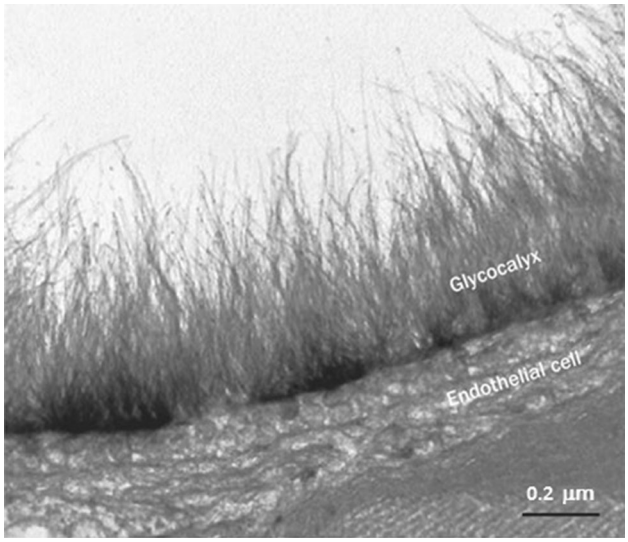

Fig. 2 Electron microscopy image of the EG

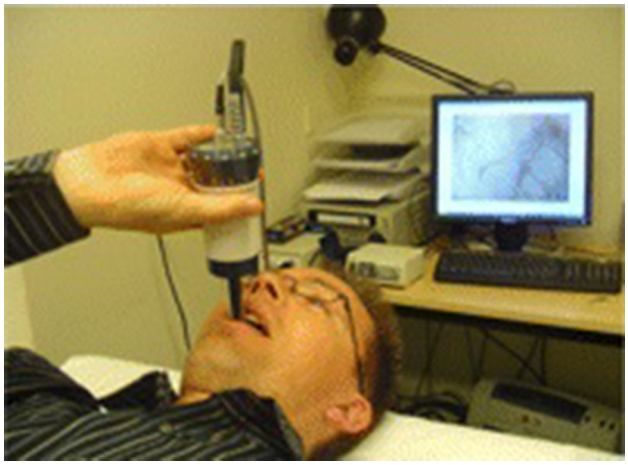

Fig. 3 Determining the change in erythrocyte width in a sublingual capillary, as a measure of the EG, by imaging the sublingual microcirculation using orthogonal polarization spectroscopy (OPS). Images of the microvasculature are projected directly onto a screen

Visualization techniques

In vivo visualization of the glycocalyx in humans is extremely difficult, mainly because of its fragility. The state of the glycocalyx and the ESL can be indirectly investigated by measuring plasma levels of their constituent parts, e.g., heparan sulfate, syndecan-1, or hyaluronan [32]. In order to establish the exact role of the glycocalyx, direct visualization techniques should be available. Ways in which the EG has been visualized up to now can be summarized as follows:

Electron microscopy (TEM)

Transmission electron microscopy (TEM) can provide information on the charge, composition, and structure of the glycocalyx. However, TEM cannot be used in vivo and the EG is often aberrant and is lost during standard tissue fixation techniques (Fig. 2).

Intravital microscopy

Study of the ESL requires use of intravital microscopy to visualize the endothelial glycocalyx in vivo. The glycocalyx can be measured indirectly as a gap between the flowing red blood cells and the endothelium. In addition, when the plasma was labeled by a fluorescent dextran, the glycocalyx appeared as a plasma exclusion zone [36]. Recent promising techniques are orthogonal polarization spectral imaging (OPS, measured in the sublingual area) (Fig. 3) and side-stream dark field imaging (SDF, measured on the nail fold) [39, 40].

Confocal microscopy and other methods

Lectin labeling of the glycocalyx of cultured human umbilical vein endothelial cells and subsequent confocal laser scanning microscopy (CLSM) imaging revealed a surface layer as thick as 2.5 ± 0.5 µm [41]. Another promising technique to directly visualize the glycocalyx in larger vessels, both ex vivo and in vivo, is two-photon laser scanning microscopy (TPLSM). The combination of enhanced penetration depth, good resolution, optical sectioning, and low phototoxicity makes TPLSM a suitable technique to visualize the delicate endothelial glycocalyx in intact larger vessels. With TPLSM, the glycocalyx thickness was found to be 4.5 ± 1.0 µm in intact mouse carotid arteries [42].

Glycocalyx physiological functions

A new circulatory compartment

A new, big, and probably very important compartment of circulation has been revealed after the discovery of the really large dimension of the EG/ESL, it being non-circulating plasma trapped within the EG (1.7 ± 0.2 L) [34]. That compartment was demonstrated when none of the expected rises in plasma protein levels were detected in patients undergoing surgery of the ascending aorta who received a median of 5 Unites (1 L) of fresh-frozen plasma. It was suggested that was due to a strong incorporation within the glycocalyx [43].

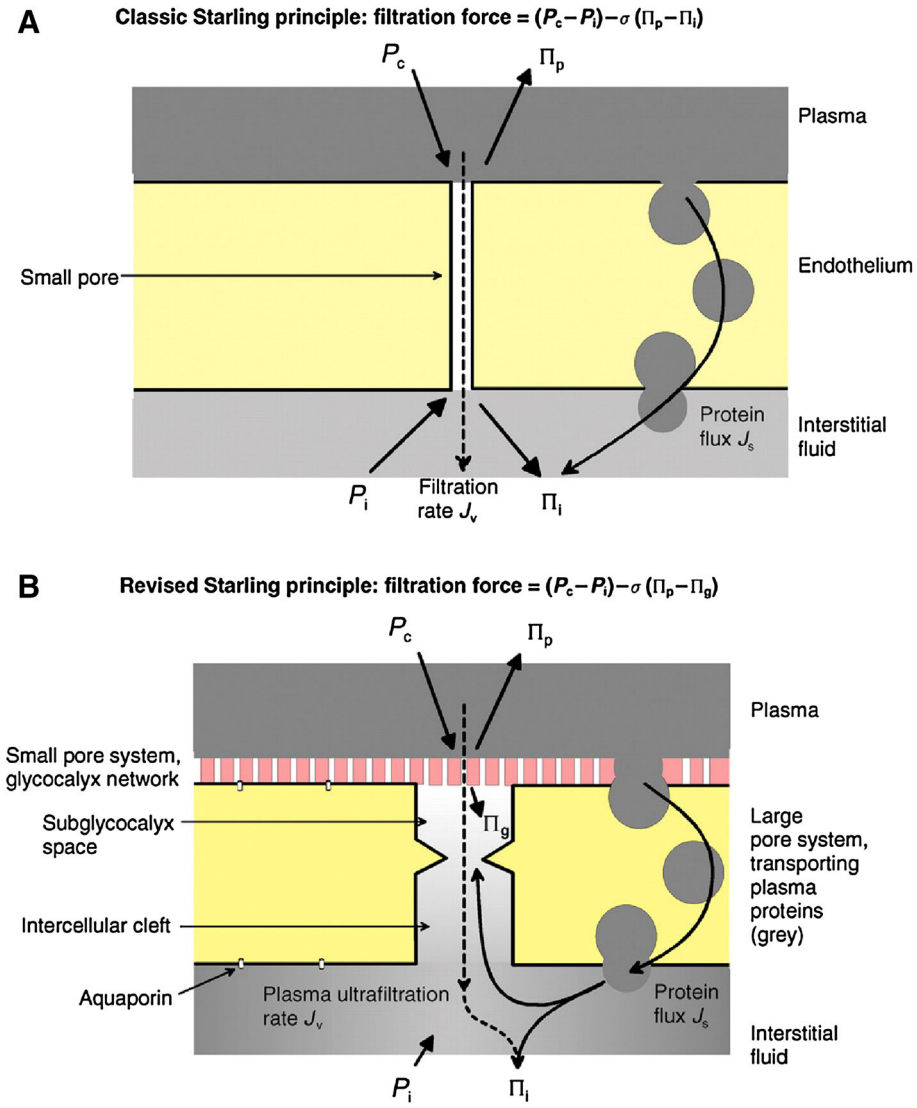

Fig. 4 Comparison of traditional Starling forces and glycocalyx model of the endothelial semipermeable membrane and the forces acting on it. *Grey shade* denotes concentration of plasma protein [49]. License Number: 3406970330618

Vascular barrier function

One hundred and twenty years ago, Starling hypothesized the forces governing vascular permeability [44]. Until recently, limitation of fluid filtration was thought to depend on a balance between hydrostatic and colloid osmotic pressure (COP) gradients across the vessel wall. At the arteriolar end, fluid is filtered to the interstitial space under a dominant hydrostatic pressure gradient of capillaries, while at the venular end, it was believed that fluid was absorbed back under a dominant COP gradient. Recent research found that both capillary filtration and subsequent reabsorption are less than originally thought by Starling, and that the only way for the return of fluid to the circulation is via the lymphatics. A lymph flow paradox appeared when the net capillary filtration rate calculated from tissue-averaged Starling forces was much greater than the observed tissue lymph production [45, 46]. Moreover, interstitial colloid osmotic force was demonstrated to be much higher than Starling assumed [47]. Experimentally, it was found that when albumin concentration in and out of the vessel wall was equal, the COP gradient from the lumen to the interstitium was 70 % of that when there was no surrounding albumin [48]. Here came the COP paradox, where properties other than the effect on COP gradient contribute to the capillary 'sealing' effect of albumin or plasma substitutes, explaining why capillary filtration is so much less than Starling predicted. The glycocalyx model assumed that in healthy vessels, the EG is the main structure that provides a colloid osmotic gradient and thus prevents tissue edema [46, 47]. That gradient is formed by the EG's high COP due to retained plasma proteins and the small space beneath, 'while still at the luminal side of the anatomical vessel wall,' which is practically protein free [49] (Fig. 4). Now, the double barrier concept, where vascular barrier is maintained by two components, the

endothelial glycocalyx and the endothelial cell bodies, is the accepted model of the vascular wall barrier [32].

Perioperative protection of the endothelial glycocalyx will prevent perioperative interstitial edema, and consequently will prevent perioperative morbidity due to tissue edema [52].

The ESL has a protective function against the shear stress of blood flow. Increased shear increases nitric oxide (NO) production by the ESL, which in turn dilates vessels and reduces stress [53].

Electrostatic properties of highly sulfated GAG chains play a role in the regulation of vascular permeability and fluid balance. Negative charges contribute to repulsion of red blood cells from the endothelium [45]. In addition to cells being repelled by negative charges, the body of the EG serves as a barrier against the inadvertent adhesion of platelets and leukocytes to the vascular wall, as intact EG exceeds the dimensions of cellular adhesion molecules (ICAM 1, VCAM 1, and P- and L-selectins). Thus, shedding of EG appears to be required for platelet and leukocyte adherence to the vessel wall [54].

The glycocalyx also has a role in protecting endothelial cells against damage by various mediators of oxidative stress, by binding to enzymes that scavenge oxygen radicals [55].

The EG also has an important role in the regulation of coagulation. Mediators, such as antithrombin III, heparin cofactor II, thrombomodulin, and tissue factor pathway inhibitor, are bound within the glycocalyx structure [56].

Finally, the endothelial glycocalyx can also bind cytokines, which have profound effects on glycocalyx compound synthesis, or modulate inflammatory response by attenuating the binding of cytokines to cell surface receptors [56].

Endothelial glycocalyx in the critically ill and perioperative patients

The actual composition of the glycocalyx results from the balance between biosynthesis and shedding.

New perspectives in transfusion therapy

Hypervolemia

Hypervolemia was found to be a pathogenic factor that alters the ESL, and consequently, the competence of vascular barrier. Traditionally, the intact vascular barrier was proposed to retain colloids and proteins, whereas water and small solutes are able to freely move within the entire extracellular compartment [57]. In fact, this occurs only in the context of acute normovolemic blood replacement. However, it was found that in the context of acute hypervolemic hemodilution, 60 % of the infused volume shifted towards the interstitial space within minutes [58]. This unexpected effect occurred both with hydroxy ethyl starch of any generation and 5 % human albumin [59]. It was suggested that, according to the context, this different behavior of colloidal volume is related to the disruption of the ESL due to hypervolemia. Moreover; it was demonstrated quantitatively in human patients that the non-circulating part of the total plasma volume decreased significantly by two-thirds during volume loading [59]. Hypervolemia stimulates the heart to release atrial natriuretic peptide (ANP) into the circulation. This hormone leads to the shedding of glycocalyx constituents through a cGMP-linked proteolytic pathway [60].

The third space

For decades, perioperative fluid therapy was based on a generous replacement of the third space loss, deficits due to insensible perspiration and fasting. It was looked upon as an actively consuming compartment, but an extremely positive fluid balance during major surgery was the only consequence. The insensible perspiration and the preoperative deficits are in fact often negligible. After revising Starling forces, the third space appeared to be only a myth. The excess fluid most likely accumulates in the interstitial compartment, due to perioperative destruction of the EG due to traumatic inflammation and iatrogenic hypervolemia. Thus, in patients undergoing major surgical interventions, it is recommended to design an infusion regimen that does not substitute, but avoids, interstitial "third-space" shifting [57].

To replace the myth of third space loss, large volume fluid transfusions should be prohibited, because the resulting hypervolemia causes ANP release and damage to the EG with a resulting interstitial fluid shifting [52].

Ischemia–reperfusion (I/R) injury

Shedding of the endothelial glycocalyx due to ischemia was demonstrated in human and animal models after perioperative ischemia–reperfusion (I/R) and experimental ischemia, respectively [29, 61]. I\R during aortic clamping and cardiopulmonary bypass was complicated by vascular leakage with edema formation that can even progress to postoperative multiple organ failure [62, 63]. Hepatic I\R injury is an inevitable scenario in major liver surgery. Hepatic sinusoids were found to have a functional glycocalyx that is destroyed in murine hepatic I\R injury and major hepatic surgery [64].

The effects of I/R injury appear to be mediated by the rapid production of reactive oxygen species [65]. It appears

that circulating components of the endothelial glycocalyx will be more sensitive markers of early endothelial cell distress. Syndecan-1 and heparan sulfate are increased multifold in patients with perioperative global or regional ischemia, whereas no change took place in levels of integral membrane proteins (ICAM-1 and VCAM-1) [35].

Hyperglycemia and diabetes

Hyperglycemia, a common perioperative finding, was demonstrated to lead to damage to the glycocalyx [34].

Abdominal surgery and sepsis

The EG is shed after major abdominal surgery, as well as in patients with sepsis [66]. Derangements of EG play an important role in mortality following sepsis. During sepsis and after abdominal surgery, the plasma leaks out into the interstitial space and this has great impact on the development of edema and impaired oxygen and nutrient supply to tissues. The deterioration of the endothelial glycocalyx is one of the earliest steps within this scenario that triggers the loss of endothelial barrier function [67]. Therefore, the glycocalyx markers can be added to the widely used acute-phase proteins "C-reactive protein and procalcitonin" [68].

Hemorrhagic shock

Hemorrhagic shock is one of the conditions where degradation of the EG has been demonstrated [69]. Intravital microscopy of anesthetized rats subjected to hemorrhage showed 59 % loss in EG thickness in venules after hemorrhage. Furthermore, loss of EG was associated with maximal blood flow reduction [70].

Acute lung injury (ALI)

Acute lung injury (ALI) is a major health problem with no known specific treatment to prevent the onset of inflammatory lung injury, reflecting an incomplete understanding of its pathogenesis [71]. The EG has an important role in maintaining endothelial barrier function in the pulmonary microvasculature, and shedding of the EG could be a major factor contributing to the pathogenesis of ALI/ARDS [72, 73]. As early as the mid-1980s, a report indicated that disruption of the glycocalyx increased lung endothelial permeability to ferritin [74]. Degradation of EG has been shown to be a common pathology in a number of conditions associated with ALI, including sepsis, after major surgery, hemorrhagic shock and ischemia/reperfusion [35, 69, 75, 76]. Plasma resuscitation was found to restore the EG and also led to decreased lung injury [69].

Critically ill patients

Sublingual microvascular EGs were evaluated in healthy volunteers and critically ill patients. Special software was used for automatic analysis of the perfused boundary region (PBR), calculated as an index of glycocalyx damage. The PBR was increased in intensive care unit (ICU) patients compared to healthy controls, and tended to be higher in septic patients compared to non-septic patients, suggesting more severe glycocalyx alterations [77].

Perioperative tumor metastasis

An interesting and important role of the EG is that it acts as a barrier to prevent interactions between adhesion receptors on endothelial cells and ligands on the circulating tumor cells' surface. Factors causing shedding of the glycocalyx promote circulating tumor cell adhesion to the endothelium and promote metastasis [78].

Protection of the endothelial glycocalyx

Many perioperative factors lead to disruption of the EG. Five to seven days were required to endogenously restore EG thickness in vivo [79]. To date, the mechanisms of recovery of a destroyed EG in man are unknown. A general rule of thumb is that the prevention of damage should be preferred over a cure.

Inhalational anesthetics

In general, halogenated anesthetics offer protection against I\R injury in different organ systems [80]. Perischemic administration of sevoflurane provided a combination of pharmacologic pre-conditioning and post-conditioning in human volunteers after forearm I\R injury. It was suggested that this occurs through inhibition of leukocyte adhesion [80]. Maintaining the natural cover for endothelial adhesion molecules reduces cell adhesion [83]. A surprising and important finding was encountered in a study on isolated perfused heart model, where pre-conditioning and rapid post-conditioning with sevoflurane attenuated the negative effects of I\R on coronary leak and glycocalyx degradation. Ischemia led to a 70 % increase in transudate formation during reperfusion. This was accompanied by increases in heparan sulphate and syndecan, with electron microscopy revealing massive degradation of the glycocalyx. Also, histamine was released into transudate, and cathepsin B activity increased in effluent. Sevoflurane attenuated all these changes, except for histamine release [7]. Sevoflurane showed a potential

to be superior to propofol in protection against I\R injury [84, 85]. Anion gap elevation is predictive of negative outcome in the context of I\R injury, and sevoflurane attenuated a strong anion gap elevation more than propfol. Moreover, there was less heparan sulfate shedding over time in sevoflurane-anesthetized animals [86]. These might explain beneficial outcomes linked to clinical use of sevoflurane after an I\R suite.

Plasma proteins and plasma albumin

Theoretically, we may expect a protein-denuded glycocalyx to be less stable. Therefore, maintaining a sufficiently high concentration of plasma proteins is assumed to protect ESL. However, no precise target levels of plasma proteins are known [8]. For example, albumin availability provides an ESL that is mechanically stable enough to resist damage during the reperfusion phase of allografts. Furthermore, this attenuates enhanced adhesion of blood leucocytes in reperfused vessels [87]. In a rodent model of hemorrhagic shock, fresh plasma resuscitation demonstrated early signs of restoration of the EG, whereas lactated ringer (LR) resuscitation resulted in few signs of EG repair. In the same study, pulmonary syndecan-1 mRNA and alveolar cell surface syndecan-1 expression were lower in animals resuscitated with LR compared to plasma and correlated with more lung injury. Surprisingly, protective effects of plasma have been clinically demonstrated and were suggested to be due to its ability to restore the EG and preserve syndecan-1 after hemorrhagic shock, and not through replacement of coagulation proteins, as it has long been thought [69].

Hydrocortisone

Preliminary data show that hydrocortisone prevents the shedding of the glycocalyx [8]. In an isolated heart model, preconditioning with hydrocortisone significantly reduced glycocalyx shedding following both I/R and TNF-α–induced inflammation [33, 88]. Clinically preoperative hydrocortisone administration to cardiac surgical patients reduced inflammatory markers, in addition to reducing the use of circulatory and respiratory support as well as hospital stay [89]. There are many proposed mechanisms: blocking the synthesis of various chemokines and cytokines, thus preventing migration of inflammatory cells to tissues, changes in NO and prostacyclin levels, in addition to a decrease in paracellular permeability for macromolecules. Mast-cell stabilization by hydrocortisone and the prevention of degranulation should also prevent damage to the glycocalyx [90, 91].

Direct inhibition of glycocalyx degradation

Etanercept, "a soluble bio-analogue of the TNF-α receptor," and rosuvastatin, a statin, prevented glycocalyx degradation in man [92, 93]. Doxycycline or ilomastat, two inhibitors of matrix metalloprotease activity, attenuated the shedding of the glycocalyx in an animal study [94].

Antithrombin III

Antithrombin III is a physiological inhibitor of serine proteases such as thrombin, plasmin, protease-3, and elastase. Serine proteases participate in a wide range of functions in the body, including blood clotting, immunity, and inflammation [95]. Supplementation of antithrombin has alleviated I/R injury in various organs [96]. Antithrombin has been shown to protect the glycocalyx following I/R or infusion of TNF-alpha [35, 97]. In addition, antithrombin has been found to bind tightly to the glycocalyx [97]. Therefore, a sufficient level within the glycocalyx may obstruct attack by proteases, heparanase, and hyaluronidase, thereby preventing shedding. These promising results warrant further assessing antithrombin III for protecting the glycocalyx in different clinical settings [8].

Antioxidants

Again, the protective action of the antioxidants from I/R injury might involve protection of the glycocalyx. NO applied during reperfusion was found to maintain the glycocalyx in the face of redox stress [98]. Therefore, enhancing antioxidant seems advantageous in glycocalyx protection, but there is no proof that this will preserve vascular permeability.

Avoiding hypervolemia

As mentioned earlier, acute hypervolemic hemodilution, as well as routine generous IV infusions for patients before intraoperative reperfusion procedures or before general or neuraxial anesthesia, are still practiced by anesthesiologists. Such strategies will cause damage to the EG, and about 60 % of the infused volume will be lost into the interstitial space within minutes [58]. Therefore, avoiding hypervolemia, especially in patients undergoing major abdominal surgeries, will improve outcome. By reduction of interstitial edema, better wound healing and improved pulmonary function are expected [99].

Avoiding the release of atrial natriuretic peptide

ANP release from the heart may occur, due not only to volume loading, but also to mechanical manipulation of the

heart, especially wall stress to the atria. ANP-induced shedding of the glycocalyx will consequently occur. In coronary artery surgery with and without cardio-pulmonary bypass, unexpectedly, identical elevations of syndecan-1 and heparan sulfate levels occurred in the circulation of both groups of patients [100]. Therefore, it was the rise in ANP and not ischemic stress to the heart and lungs that cause EG shedding.

Conclusion

A healthy endothelial surface layer in all body parts is important in preventing perioperative morbidity. Arrangements should be adopted by an anesthesiologist to avoid perioperative shedding of the endothelial glycocalyx. No specific drug can cause regeneration of the glycocalyx, so avoiding its shedding is important. A number of protective strategies can be adopted to prevent shedding of the EG; these are fields for future research in the perioperative setting.

References

1. Henrich M, Gruss M, Weigand MA. Sepsis-induced degradation of endothelial glycocalix. Scientific World J. 2010;10:917–23.
2. Pries AR, Kuebler WM. Normal endothelium. Handb Exp Pharmacol. 2006;1–40
3. Weinbaum S, Tarbell JM, Damiano ER. The structure and function of the endothelial glycocalyx layer. Annu Rev Biomed Eng. 2007;9:121–67.
4. Lipowsky HH. The endothelial glycocalyx as a barrier to leukocyte adhesion and its mediation by extracellular proteases. Ann Biomed Eng. 2012;40:840–8.
5. Desjardins C, Duling BR. Heparinase treatment suggests a role for the endothelial cell glycocalyx in regulation of capillary hematocrit. Am J Physiol. 1990;258:H647–54.
6. Chappell D, Dörfler N, Jacob M, Rehm M, Welsch U, Conzen P, Becker BF. Glycocalyx protection reduces leukocyte adhesion after ischemia/reperfusion. Shock. 2010;34:133–9.
7. Annecke T, Chappell D, Chen C, Jacob M, Welsch U, Sommerhoff CP, Rehm M, Conzen PF, Becker BF. Sevoflurane preserves the endothelial glycocalyx against ischaemia–reperfusion injury. Br J Anaesth. 2010;104:414–21.
8. Becker BF, Chappell D, Bruegger D, Annecke T, Jacob M. Therapeutic strategies targeting the endothelial glycocalyx: acute deficits, but great potential. Cardiovasc Res. 2010;87:300–10.
9. Danielli JF. Capillary permeability and oedema in the perfused frog. J Physiol. 1940;98:109–29.
10. Bennett HS, Luft JH, Hampton JC. Morphological classifications of vertebrate blood capillaries. Am J Physiol. 1959;196:381–90.
11. Luft JH. Fine structures of capillary and endocapillary layer as revealed by ruthenium red. Fed proc. 1966;25:1773–83.
12. Reitsma S, Slaaf DW, Vink H, van Zandvoort MA, oude Egbrink MG. The endothelial glycocalyx: composition, functions, and visualization. Pflugers Arch. 2007;454:345–59.
13. Tarbell JM, Weinbaum S, Kamm RD. Cellular fluid mechanics and mechanotransduction. Ann Biomed Eng. 2005;33:1719–23.
14. Fransson LA, Belting M, Cheng F, Jönsson M, Mani K, Sandgren S. Novel aspects of glypican glycobiology. Cell Mol Life Sci. 2004;61:1016–24.
15. Esko JD, Selleck SB. Order out of chaos: assembly of ligand binding sites in heparan sulfate. Annu Rev Biochem. 2002;71:435–71.
16. Funderburgh JL. Keratan sulfate: structure, biosynthesis, and function. Glycobiology. 2000;10:951–8.
17. Laurent TC, Fraser JR. Hyaluronan. FASEB J. 1992;6:2397–404.
18. Lee JY, Spicer AP. Hyaluronan: a multifunctional, megaDalton, stealth molecule. Curr Opin Cell Biol. 2000;12:581–6.
19. Sugahara K, Mikami T, Uyama T, Mizuguchi S, Nomura K, Kitagawa H. Recent advances in the structural biology of chondroitin sulfate and dermatan sulfate. Curr Opin Struct Biol. 2003;13:612–20.
20. Nandi A, Estess P, Siegelman MH. Hyaluronan anchoring and regulation on the surface of vascular endothelial cells is mediated through the functionally active form of CD44. J Biol Chem. 2000;275:14939–48.
21. Pries AR, Secomb TW, Gaehtgens P. The endothelial surface layer. Pflugers Arch. 2000;440:653–66.
22. Kinsella MG, Bressler SL, Wight TN. The regulated synthesis of versican, decorin, and biglycan: extracellular matrix proteoglycans that influence cellular phenotype. Crit Rev Eukaryot Gene Expr. 2004;14:203–34.
23. Sperandio M. Selectins and glycosyltransferases in leukocyte rolling in vivo. FEBS J. 2006;273:4377–89.
24. Rüegg C, Mariotti A. Vascular integrins: pleiotropic adhesion and signaling molecules in vascular homeostasis and angiogenesis. Cell Mol Life Sci. 2003;60:1135–57.
25. Müller AM, Hermanns MI, Cronen C, Kirkpatrick CJ. Comparative study of adhesion molecule expression in cultured human macro- and microvascular endothelial cells. Exp Mol Pathol. 2002;73:171–80.
26. Berndt MC, Shen Y, Dopheide SM, Gardiner EE, Andrews RK. The vascular biology of the glycoprotein Ib-IX-V complex. Thromb Haemost. 2001;86:178–88.
27. Huxley VH, Curry FE. Differential actions of albumin and plasma on capillary solute permeability. Am J Physiol. 1991;260:H1645–54.
28. Klitzman B, Duling BR. Microvascular hematocrit and red cell flow in resting and contracting striated muscle. Am J Physiol. 1979;237:H481–90.
29. van den Berg BM, Vink H, Spaan JA. The endothelial glycocalyx protects against myocardial edema. Circ Res. 2003;92:592–4.
30. Hjalmarsson C, Johansson BR, Haraldsson B. Electron microscopic evaluation of the endothelial surface layer of glomerular capillaries. Microvasc Res. 2004;67:9–17.
31. Ueda A, Shimomura M, Ikeda M, Yamaguchi R, Tanishita K. Effect of glycocalyx on shear-dependent albumin uptake in endothelial cells. Am J Physiol Heart Circ Physiol. 2004;287:H2287–94.
32. Rehm M, Zahler S, Lötsch M, Welsch U, Conzen P, Jacob M, Becker BF. Endothelial glycocalyx as an additional barrier determining extravasation of 6 % hydroxyethyl starch or 5 % albumin solutions in the coronary vascular bed. Anesthesiology. 2004;100:1211–23.
33. Chappell D, Jacob M, Hofmann-Kiefer K, Bruegger D, Rehm M, Conzen P, Welsch U, Becker BF. Hydrocortisone preserves the vascular barrier by protecting the endothelial glycocalyx. Anesthesiology. 2007;107:776–84.

34. Nieuwdorp M, van Haeften TW, Gouverneur MCLG, Mooij HL, van Lieshout MHP, Levi M, Meijers JCM, Holleman F, Hoekstra JBL, Vink H, Kastelein JJP, Stroes ESG. Loss of endothelial glycocalyx during acute hyperglycemia coincides with endothelial dysfunction and coagulation activation in vivo. Diabetes. 2006;55:480–6.
35. Rehm M, Bruegger D, Christ F, Conzen P, Thiel M, Jacob M, Chappell D, Stoeckelhuber M, Welsch U, Reichart B, Peter K, Becker BF. Shedding of the endothelial glycocalyx in patients undergoing major vascular surgery with global and regional ischemia. Circulation. 2007;116:1896–906.
36. Vink H, Duling BR. Identification of distinct luminal domains for macromolecules, erythrocytes, and leukocytes within mammalian capillaries. Circ Res. 1996;79:581–9.
37. Yang Y, Yang G, Schmidt EP, In vivo measurement of the mouse pulmonary endothelial surface layer. J Vis Exp. 2013; e50322. doi:10.3791/50322
38. Liuhanen S, Sallisalmi M, Pettilä V, Oksala N, Tenhunen J. Indirect measurement of the vascular endothelial glycocalyx layer thickness in human submucosal capillaries with a plug-in for image. J Comput Methods Programs Biomed. 2013;110:38–47.
39. Nieuwdorp M, Meuwese MC, Mooij HL, Ince C, Broekhuizen LN, Kastelein JJP, Stroes ESG, Vink H. Measuring endothelial glycocalyx dimensions in humans: a potential novel tool to monitor vascular vulnerability. J Appl Physiol. 2008;104:845–52.
40. Djaberi R, Schuijf JD, de Koning EJ, Wijewickrama DC, Pereira AM, Smit JW, Kroft LJ, de Roos A, Bax JJ, Rabelink TJ, Jukema JW. Non-invasive assessment of microcirculation by sidestream dark field imaging as a marker of coronary artery disease in diabetes. Diab Vasc Dis Res. 2013;10:123–34.
41. Barker AL, Konopatskaya O, Neal CR, Macpherson J V, Whatmore JL, Winlove CP, Unwin PR, Shore AC. Observation and characterisation of the glycocalyx of viable human endothelial cells using confocal laser scanning microscopy presented at the biophysical chemistry conference 2003, Warwick, UK, July 21–23, 2003. Phys Chem Chem Phys. 2004;6:1006.
42. Megens RTA, Reitsma S, Schiffers PHM, Hilgers RHP, De Mey JGR, Slaaf DW, oude Egbrink MGA, van Zandvoort MAMJ. Two-photon microscopy of vital murine elastic and muscular arteries. Combined structural and functional imaging with subcellular resolution. J Vasc Res. 2007;44:87–98.
43. Rehm M, Haller M, Brechtelsbauer H, Akbulut C, Finsterer U. Extra protein loss not caused by surgical bleeding in patients with ovarian cancer. Acta Anaesthesiol Scand. 1998;42:39–46.
44. Starling EH. On the absorption of fluids from the connective tissue spaces. J Physiol. 1896;19:312–26.
45. Becker BF, Chappell D, Jacob M. Endothelial glycocalyx and coronary vascular permeability: the fringe benefit. Basic Res Cardiol. 2010;105:687–701.
46. Levick JR. Revision of the Starling principle: new views of tissue fluid balance. J Physiol. 2004;557:704.
47. Adamson RH, Lenz JF, Zhang X, Adamson GN, Weinbaum S, Curry FE. Oncotic pressures opposing filtration across non-fenestrated rat microvessels. J Physiol. 2004;557:889–907.
48. Jacob M, Bruegger D, Rehm M, Stoeckelhuber M, Welsch U, Conzen P, Becker BF. The endothelial glycocalyx affords compatibility of Starling's principle and high cardiac interstitial albumin levels. Cardiovasc Res. 2007;73:575–86.
49. Levick JR, Michel CC. Microvascular fluid exchange and the revised Starling principle. Cardiovasc Res. 2010;87:198–210.
50. Hu X, Weinbaum S. A new view of Starling's hypothesis at the microstructural level. Microvasc Res. 1999;58:281–304.
51. Adamson RH, Clough G. Plasma proteins modify the endothelial cell glycocalyx of frog mesenteric microvessels. J Physiol. 1992;445:473–86.
52. Doherty M, Buggy DJ. Intraoperative fluids: how much is too much? Br J Anaesth. 2012;109:69–79.
53. Jacob M, Rehm M, Loetsch M, Paul JO, Bruegger D, Welsch U, Conzen P, Becker BF. The endothelial glycocalyx prefers albumin for evoking shear stress-induced, nitric oxide-mediated coronary dilatation. J Vasc Res. 2007;44:435–43.
54. Constantinescu AA, Vink H, Spaan JAE. Endothelial cell glycocalyx modulates immobilization of leukocytes at the endothelial surface. Arterioscler Thromb Vasc Biol. 2003;23:1541–7.
55. Nieuwdorp M, Meuwese MC, Vink H, Hoekstra JBL, Kastelein JJP, Stroes ESG. The endothelial glycocalyx: a potential barrier between health and vascular disease. Curr Opin Lipidol. 2005;16:507–11.
56. Broekhuizen LN, Mooij HL, Kastelein JJP, Stroes ESG, Vink H, Nieuwdorp M. Endothelial glycocalyx as potential diagnostic and therapeutic target in cardiovascular disease. Curr Opin Lipidol. 2009;20:57–62.
57. Jacob M, Chappell D, Rehm M. The "third space"–fact or fiction? Best Pr res Clin anaesthesiol. 2009;23:145–57.
58. Rehm M, Haller M, Orth V, Kreimeier U, Jacob M, Dressel H, Mayer S, Brechtelsbauer H, Finsterer U. Changes in blood volume and hematocrit during acute preoperative volume loading with 5 % albumin or 6 % hetastarch solutions in patients before radical hysterectomy. Anesthesiology. 2001;95:849–56.
59. Jacob M, Chappell D, Rehm M. Clinical update: perioperative fluid management. Lancet. 2007;369:1984–6.
60. Bruegger D, Jacob M, Rehm M, Loetsch M, Welsch U, Conzen P, Becker BF. Atrial natriuretic peptide induces shedding of endothelial glycocalyx in coronary vascular bed of guinea pig hearts. Am J Physiol Heart Circ Physiol. 2005;289:H1993–9.
61. Kurzelewski M, Czarnowska E, Beresewicz A. Superoxide- and nitric oxide-derived species mediate endothelial dysfunction, endothelial glycocalyx disruption, and enhanced neutrophil adhesion in the post-ischemic guinea-pig heart. J Physiol Pharmacol. 2005;56:163–78.
62. Bown MJ, Nicholson ML, Bell PR, Sayers RD. Cytokines and inflammatory pathways in the pathogenesis of multiple organ failure following abdominal aortic aneurysm repair. Eur J Vasc Endovasc Surg. 2001;22:485–95.
63. Paparella D, Yau TM, Young E. Cardiopulmonary bypass induced inflammation: pathophysiology and treatment. An update Eur J Cardiothorac Surg. 2002;21:232–44.
64. Golden R Van, Reinier M, Vrisekoop N, Al E: The Mechanism and Physiological Relevance of Glycocalyx Degradation in Hepatic Ischemia\Reperfusion Injury. Antioxid Redox Signal 2014:Epub ahead of print.
65. Rubio-Gayosso I, Platts SH, Duling BR. Reactive oxygen species mediate modification of glycocalyx during ischemia-reperfusion injury. Am J Physiol Heart Circ Physiol. 2006;290: H2247–56.
66. Steppan J, Hofer S, Funke B, Brenner T, Henrich M, Martin E, Weitz J, Hofmann U, Weigand MA, Steppan J, Hofer S, Funke B, Brenner T, Henrich M, Martin E, Weitz J, Hofmann U, Weigand MA. Sepsis and major abdominal surgery lead to flaking of the endothelial glycocalix. J Surg Res. 2011;165: 136–41.
67. Noble MIM, Drake-Holland AJ, Vink H. Hypothesis: arterial glycocalyx dysfunction is the first step in the atherothrombotic process. QJM. 2008;101:513–8.
68. Lichtenstern C, Brenner T, Bardenheuer HJ, Weigand MA. Predictors of survival in sepsis: what is the best inflammatory marker to measure? Curr Opin Infect Dis. 2012;25:328–36.
69. Kozar RA, Peng Z, Zhang R, Holcomb JB, Pati S, Park P, Ko TC, Paredes A. Plasma restoration of endothelial glycocalyx in a rodent model of hemorrhagic shock. Anesth Analg. 2011;112: 1289–95.

70. Torres F, Torres L, Sondeen J, Polykratis I, Dubick M. In vivo evaluation of venular glycocalyx during hemorrhagic shock in rats using intravital microscopy. Microvasc Res. 2013;85:128–33.
71. Collins SR, Blank RS, Deatherage LS, Dull RO. Special article: the endothelial glycocalyx: emerging concepts in pulmonary edema and acute lung injury. Anesth Analg. 2013;117:664–74.
72. Singleton PA, Lennon FE: Acute Lung Injury Regulation by Hyaluronan. J. Allergy Ther. 2011; Suppl 4.
73. Strunden MS, Bornscheuer A, Schuster A, Kiefmann R, Goetz AE, Heckel K. Glycocalyx degradation causes microvascular perfusion failure in the ex vivo perfused mouse lung: hydroxyethyl starch 130/0.4 pretreatment attenuates this response. Shock. 2012;38:559–66.
74. Schneeberger EE, Hamelin M. Interaction of serum proteins with lung endothelial glycocalyx: its effect on endothelial permeability. Am J Physiol. 1984;247:H206–17.
75. Schmidt EP, Yang Y, Janssen WJ, Gandjeva A, Perez MJ, Barthel L, Zemans RL, Bowman JC, Koyanagi DE, Yunt ZX, Smith LP, Cheng SS, Overdier KH, Thompson KR, Geraci MW, Douglas IS, Pearse DB, Tuder RM. The pulmonary endothelial glycocalyx regulates neutrophil adhesion and lung injury during experimental sepsis. Nat Med. 2012;. doi:10.1038/nm.2843.
76. Annecke T, Fischer J, Hartmann H, Tschoep J, Rehm M, Conzen P, Sommerhoff CP, Becker BF. Shedding of the coronary endothelial glycocalyx: effects of hypoxia/reoxygenation vs ischaemia/reperfusion. Br J Anaesth. 2011;107:679–86.
77. Donati A, Damiani E, Domizi R, Romano R, Adrario E, Pelaia P, Ince C, Singer M. Alteration of the sublingual microvascular glycocalyx in critically ill patients. Microvasc Res. 2013;90:86–9.
78. Mitchell M, King M. Physical biology in cancer. 3. The role of cell glycocalyx in vascular transport of circulating tumor cells. Am J Physiol Cell Physiol. 2014;306:C89–97.
79. Potter DR, Jiang J, Damiano ER. The recovery time course of the endothelial cell glycocalyx in vivo and its implications in vitro. Circ Res. 2009;104:1318–25.
80. Lucchinetti E, Ambrosio S, Aguirre J, Herrmann P, Härter L, Keel M, Meier T, Zaugg M. Sevoflurane inhalation at sedative concentrations provides endothelial protection against ischemia-reperfusion injury in humans. Anesthesiology. 2007;106:262–8.
81. Lee HT, Ota-Setlik A, Fu Y, Nasr SH, Emala CW. Differential protective effects of volatile anesthetics against renal ischemia-reperfusion injury in vivo. Anesthesiology. 2004;101:1313–24.
82. Kersten JR, Schmeling TJ, Pagel PS, Gross GJ, Warltier DC. Isoflurane mimics ischemic preconditioning via activation of K(ATP) channels: reduction of myocardial infarct size with an acute memory phase. Anesthesiology. 1997;87:361–70.
83. Chappell D, Heindl B, Jacob M, Annecke T, Chen C, Rehm M, Conzen P, Becker BF. Sevoflurane reduces leukocyte and platelet adhesion after ischemia-reperfusion by protecting the endothelial glycocalyx. Anesthesiology. 2011;115:483–91.
84. Bruegger D, Bauer A, Finsterer U, Bernasconi P, Kreimeier U, Christ F. Microvascular changes during anesthesia: sevoflurane compared with propofol. Acta Anaesthesiol Scand. 2002;46:481–7.
85. Annecke T. Kubitz JC, Kahr S, Hilberath JM, Langer K, Kemming GI, Rehm M, Bittmann I, Conzen PF: Effects of sevoflurane and propofol on ischaemia-reperfusion injury after thoracic-aortic occlusion in pigs. Br. J. Anaesth. 2007;98: 581–90.
86. Annecke T, Rehm M, Bruegger D, Kubitz JC, Kemming GI, Stoekelhuber M, Becker BF, Conzen PF. Ischemia-reperfusion-induced unmeasured anion generation and glycocalyx shedding: sevoflurane versus propofol anesthesia. J Invest Surg. 2012;25: 162–8.
87. Jacob M, Paul O, Mehringer L, Chappell D, Rehm M, Welsch U, Kaczmarek I, Conzen P, Becker BF. Albumin augmentation improves condition of guinea pig hearts after 4 hr of cold ischemia. Transplantation. 2009;87:956–65.
88. Chappell D, Hofmann-Kiefer K, Jacob M, Rehm M, Briegel J, Welsch U, Conzen P, Becker BF. TNF-alpha induced shedding of the endothelial glycocalyx is prevented by hydrocortisone and antithrombin. Basic Res Cardiol. 2009;104:78–89.
89. Kilger E, Weis F, Briegel J, Frey L, Goetz AE, Reuter D, Nagy A, Schuetz A, Lamm P, Knoll A, Peter K. Stress doses of hydrocortisone reduce severe systemic inflammatory response syndrome and improve early outcome in a risk group of patients after cardiac surgery. Crit Care Med. 2003;31:1068–74.
90. Mukaida N. Zachariae CC, Gusella GL, Matsushima K: Dexamethasone inhibits the induction of monocyte chemotactic-activating factor production by IL-1 or tumor necrosis factor. J. Immunol. 1991;146:1212–5.
91. Hafezi-Moghadam A, Simoncini T, Yang Z, Limbourg FP, Plumier J-C, Rebsamen MC, Hsieh C-M, Chui D-S, Thomas KL, Prorock AJ, Laubach VE, Moskowitz MA, French BA, Ley K, Liao JK. Acute cardiovascular protective effects of corticosteroids are mediated by non-transcriptional activation of endothelial nitric oxide synthase. Nat Med. 2002;8:473–9.
92. Nieuwdorp M, Meuwese MC, Mooij HL, van Lieshout MHP, Hayden A, Levi M, Meijers JCM, Ince C, Kastelein JJP, Vink H, Stroes ESG. Tumor necrosis factor-alpha inhibition protects against endotoxin-induced endothelial glycocalyx perturbation. Atherosclerosis. 2009;202:296–303.
93. Meuwese MC, Mooij HL, Nieuwdorp M, van Lith B, Marck R, Vink H, Kastelein JJP, Stroes ESG. Partial recovery of the endothelial glycocalyx upon rosuvastatin therapy in patients with heterozygous familial hypercholesterolemia. J Lipid Res. 2009;50:148–53.
94. Mulivor AW, Lipowsky HH. Inhibition of glycan shedding and leukocyte-endothelial adhesion in postcapillary venules by suppression of matrixmetalloprotease activity with doxycycline. Microcirculation. 2009;16:657–66.
95. Afshari A, Wetterslev J, Brok J, Møller AM. Antithrombin III for critically ill patients. Cochrane Database Syst Rev. 2008; CD005370. doi:10.1002/14651858.CD005370.pub2
96. Mizutani A, Okajima K, Uchiba M, Isobe H, Harada N, Mizutani S, Noguchi T. Antithrombin reduces ischemia/reperfusion-induced renal injury in rats by inhibiting leukocyte activation through promotion of prostacyclin production. Blood. 2003;101:3029–36.
97. Chappell D, Jacob M, Hofmann-Kiefer K, Rehm M, Welsch U, Conzen P, Becker BF. Antithrombin reduces shedding of the endothelial glycocalyx following ischaemia/reperfusion. Cardiovasc Res. 2009;83:388–96.
98. Bruegger D, Rehm M, Jacob M, Chappell D, Stoeckelhuber M, Welsch U, Conzen P, Becker BF. Exogenous nitric oxide requires an endothelial glycocalyx to prevent postischemic coronary vascular leak in guinea pig hearts. Crit Care. 2008;12: R73.
99. Brandstrup B. Fluid therapy for the surgical patient. Best Pract Res Clin Anaesthesiol. 2006;20:265–83.
100. Bruegger D, Rehm M, Abicht J, Paul JO, Stoeckelhuber M, Pfirrmann M, Reichart B, Becker BF, Christ F. Shedding of the endothelial glycocalyx during cardiac surgery: on-pump versus off-pump coronary artery bypass graft surgery. J Thorac Cardiovasc Surg. 2009;138:1445–7.