Therapeutic strategies targeting the endothelial glycocalyx: acute deficits, but great potential†

Bernhard F. Becker1*, Daniel Chappell2, Dirk Bruegger2, Thorsten Annecke1,2, and Matthias Jacob2

1Department of Physiology, Walter-Brendel-Centre of Experimental Medicine, Ludwig-Maximilians-University, Schillerstrasse 44, 80336 Munich, Germany; and 2Clinic of Anesthesiology, Ludwig-Maximilians-University, Munich, Germany

Received 27 November 2009; revised 6 May 2010; accepted 7 May 2010; online publish-ahead-of-print 11 May 2010

Damage of the endothelial glycocalyx, which ranges from 200 to 2000 nm in thickness, decreases vascular barrier function and leads to protein extravasation and tissue oedema, loss of nutritional blood flow, and an increase in platelet and leucocyte adhesion. Thus, its protection or the restoration of an already damaged glycocalyx seems to be a promising therapeutic target both in an acute critical care setting and in the treatment of chronic vascular disease. Drugs that can specifically increase the synthesis of glycocalyx components, refurbish it, or selectively prevent its enzymatic degradation do not seem to be available. Pharmacological blockers of radical production may be useful to diminish the oxygen radical stress on the glycocalyx. Tenable options are the application of hydrocortisone (inhibiting mast-cell degranulation), use of antithrombin III (lowering susceptibility to enzymatic attack), direct inhibition of the cytokine tumour necrosis factor-α, and avoidance of the liberation of natriuretic peptides (as in volume loading and heart surgery). Infusion of human plasma albumin (to maintain mechanical and chemical stability of the endothelial surface layer) seems the easiest treatment to implement.

Keywords

Albunin • Hydrocortisone • Ischaemia • Lipopolysaccharide • Permeability

This article is part of the Spotlight Issue on: Microvascular Permeability

1. Introduction

About 70 years ago, the existence of a thin layer of proteinaceous material at the endothelial surface, most likely in vessels, was postulated for the first time in conjunction with the regulation of vascular filtration phenomena. Mainly according to histochemical and then chemical analyses, this layer has since been termed the endothelial glycocalyx, and its primary composition has been quite well characterized. Foremost, one finds core proteoglycans of the syndecan and glypican families carrying highly sulfated, linear glycosaminoglycan attachments (chiefly heparan, chondroitin, and dermatan sulfates), as well as receptor-bound hyaluronan. Together, these constituents form a tight and negatively charged meshwork. However, for many decades, any physiological importance of this structure was deemed to be unlikely, partly due to the fact that it is largely destroyed upon conventional tissue fixation and optically transparent in most light microscopic examinations in vivo and, thus, at best noticeable only as an ‘exclusion’ zone for erythrocytes in blood-perfused vessels. Furthermore, an anatomical width of merely some tens of nanometres was suggested in first electron microscopic visualizations relying on traditional fixation modalities. Though the binding of lectins, antibodies, or cationized ferritin demonstrates the presence of surface molecules, this does not suffice to preserve the structure and is, moreover, generally performed after fixation, i.e. after the collapse of the glycocalyx.

A modern technique based on the stabilization of the glycocalyx with lanthanum ions during fixation with glutaraldehyde recently showed this structure at a dimension of 100–750 nm (Figure 1A and B). This revelation was in line with increasing evidence attributing a considerable physiological role to the apical endothelial glycocalyx, especially in relation to vascular permeability, adhesion of leucocytes and platelets, mediation of shear stress, and modulation of inflammatory processes.

In this regard, one must take into account that the endothelial glycocalyx represents just a basal skeleton, in vivo interacting intensely and dynamically with all manner of plasma constituents and, in effect, forming an endothelial surface layer (ESL). This represents the real physiological principle existing at the interface between flowing blood and the vessel wall, and some investigators have reported the ESL to attain a thickness of ≥1 μm in certain vessels.
The distinction between a non-circulating intravascular volume and a circulating volume, which is also shared, e.g. by the red blood cells, has allowed for a quantitative assessment of the size of the ESL by tracer dilution methods and for first indications of changes associated with pathophysiological conditions in man in vivo: Physiologically, the ESL has been estimated to comprise as much as 25% of the total intravascular space. However, the techniques of measurement are still subject to controversy.

An optical method to assess the thickness of the ESL indirectly in capillaries in vivo has been applied to human subjects by Nieuwdorp et al. This technique has also provided first evidence that there are significant reductions in the extent of the ESL during acute and chronic inflammatory challenge in man. Indeed, destruction of the glyocalyx has been directly and indirectly evidenced in several studies, e.g. after ischaemic challenge, during redox stress, by enzymatic attack, and after inflammation.

In view of the emerging crucial physiological functions of the glyocalyx, especially in conjunction with the dynamically bound plasma proteins, and the obvious fragility of this layer, avoiding or at least attenuating destruction in critically ill patients might have the potential to influence outcome.

**Figure 1** (A and B) Exemplary electron microscopical image of the endothelial glyocalyx in the human umbilical vein, extension 300–750 nm (A), and a capillary of a guinea pig heart, extension 60–500 nm (B). For details, see Chappell et al. and Rehm et al. (C and D) Guinea pig heart perfused with human albumin for 3 min, prior to flushing for 1 min, fixation with formalin solution, and staining with antibody against human albumin (brown colour). Albumin concentrates at the luminal surface of capillaries (C); (D) Albumin arriving also at the adventitial side of a venule. (E and F) Guinea pig heart perfused for 1 min with fixation solution containing lanthanum. The trivalent cation does not get past the glyocalyx in the capillaries (E); the venule is leaky and allows staining also of the glyocalyx of adjacent cardiomyocytes (F). Adapted from Jacob et al. A, adventitial surfaces; ES, extracellular surface; IS, interstitial space; IV, intravascular space; M, cardiomyocyte.
2. Physiology of the glycocalyx

2.1 Regulation of vascular permeability in peripheral vessels

In 1896, the British physiologist Ernest Starling proposed filtration in common capillaries excluding those of the brain to be based on an inwardly directed oncotic pressure gradient between the interstitial and the circulatory space opposed to an outwardly directed hydrostatic one across the anatomical vessel wall.27 A regional heterogeneity schematically leading to high filtration in the arteriolar and high re-absorption in the venular capillary segment, respectively, formally complied with the low rates of lymphatic flow observed under physiological circumstances. Almost 90 years later, however, the postulated high resorption of interstitial fluid in the venular segments of the microcirculation was shown not to exist.28 Also, filtration across the vascular barrier surprisingly remains largely independent of the bulk colloid concentration surrounding the vessel.12

Neither the endothelial glycocalyx nor an ESL was part of the historical concept. In the peripheral circulation, a relevant barrier property of the endothelial glycocalyx was indicated for the first time by Curry and Michel in 1980.29 An importance of the negative charge of the glycocalyx for its barrier properties was reported, e.g. by Noble et al. in 1996.30 The more recent mechanical ‘double-barrier concept’ recognized it as a second competent barrier, in addition to that posed by the endothelial cell bodies as such.31 An important role of the glycocalyx has also been described in the glomerular filtration barrier32 and for mesenterial microvascular filtration.33 In 2004, Adamson et al.34 suggested a space beneath the protein-loaded endothelial glycocalyx to be almost protein-free, in contrast to the interstitial space, relocating the inwardly directed oncotic gradient suggested by Ernest Starling to the luminal side of the anatomical vessel wall, across the endothelial glycocalyx. On the basis of all these insights, one may expect two prerequisites for a functioning vascular barrier: (i) an intact endothelial glycocalyx (with its many charge-carrying sulfate residues) and (ii) a sufficiently high concentration of molecules which are able to bind (electrostatically) to the endothelial glycocalyx, thereby forming a tight meshwork hindering passage of colloids through the ensuing ESL.

There is meanwhile admissible experimental evidence that the type of the intravascular colloid available for interacting with the glycocalyx might be also very important in addition to providing the colloid osmotic pressure (COP). The artificial colloid hydroxyethylstarch, even when generating the four-fold intracoronary COP of albumin, sustained a far higher rate of hydraulic conductance and colloid permeability across the vascular barrier.35 One obvious explanation can be that the artificial colloid was inferior concerning the generation of an ESL. The tight, preferential binding of plasma albumin to the glycocalyx is illustrated in Figure 1C, showing an immunohistological stain with anti-albumin of the myocardial situs of a guinea pig heart perfused with human albumin and then briefly flushed with colloid-free, crystalloid buffer. Despite the wash-out phase, albumin remains strongly adherent on the endothelial surface.36

The question how colloids and plasma proteins found in the interstitial space of practically all organs get there in such amounts has recently been solved by looking at the intact coronary bed of isolated perfused hearts, i.e. at a complete microcirculatory system, including arterioles, capillaries, and venules.31 Especially, the latter are partly discontinuous, including large pores facilitating egress of macromolecules.37 Immunohistological examination suggested albumin to be homogeneously distributed over the whole interstitial space, but to leave the circulation via venular vessels (Figure 1D). Electron microscopy showed electron-dense fixation components to leave the intravascular space exclusively at venular large-pore sites while arterioles and capillaries are not permeable (Figure 1E and F).36 A schematic illustration of the new concept of microvascular fluid homeostasis is shown in Figure 2. The low filtration–low resorption concept of microvascular fluid homeostasis. An intact ESL, consisting of the endothelial glycocalyx and attached plasma protein molecules, oncotically limits fluid movement across the vascular wall. For details see text and Jacob et al.36 EC, endothelial cell; ESL, endothelial surface layer; IS, interstitial space; PC, e, g, t, and v, oncotic pressure in capillary plasma, ESL, below the ESL, in the tissue, and venular space, respectively; Pt, t, and v, hydrostatic pressure in the capillary, tissue, and venule, respectively.

![Figure 2](https://example.com/image2.png)
homeostasis proposed by us is presented in Figure 2. In regions with high intravascular pressure, the inwardly directed oncotic pressure gradient across the glycocalyx in conjunction with the high resistance to flow within the narrow strand gaps of the endothelium prevents flooding of the interstitial space. Within low-pressure sections, in contrast, free and easy access of plasma constituents towards the parenchymal cells allows a highly effective exchange of nutrients and waste products. Nonetheless, due to the low hydrostatic and oncotic pressure gradients pertaining in these segments, fluid shift is modest and the intra- and extravascular compartments remain in balance. Notably, all this works only as long as the ESL (glycocalyx plus bound proteins) is competent.

2.2 Mediation of shear stress

The endothelial release of nitric oxide (NO) due to shear stress is an important prerequisite of basal nutritive blood flow. Recent evidence suggested that shear transduction might not be related primarily to blood viscosity, but a result of selective interaction of plasma constituents with the endothelial glycocalyx. In isolated, spontaneously beating guinea pig hearts perfused at constant aortic pressure, only augmentation with albumin, but not artificial colloids, significantly increased coronary flow vs. a colloid-free perfusate (Figure 3), although albumin at the levels used (1.7 g%) provided the lowest viscosity of all colloidal perfusion solutions tested. This vasodilatation was experimentally related to the presence of an intact endothelial glycocalyx, and required an active nitric-oxide synthase, the two dependencies being mutually exclusive (Figure 3). Obviously, natural plasma proteins such as albumin are able to ‘hook’ into the endothelial glycocalyx, this specific interaction leading to a powerful vasodilatatory response enabling, e.g. a sufficient nutritive blood flow. As prolonged ischaemia has been shown to lead to the destruction of the glycocalyx, it is not at all astonishing that the ‘low-reflow’ phenomenon is a common occurrence during reperfusion.

2.3 Attenuation of leucocyte and platelet adhesion

Schematic presentations of the endothelial apical glycocalyx published to date generally convey a false impression in that the true dimension (200–2000 nm width) is misrepresented in relation to the cell body (30–100 nm thickness beyond the cell nucleus) and especially vs. most non-syndecan constituents. This mismatch applies particularly to membrane receptors and cellular adhesion molecules. In fact, selectins such as PECAM, VCAMs, and ICAMs and integrins such as CD11/CD18 span only ~10 nm. The glycocalyx core proteins, already much longer, carry chains of heparan and chondroitin sulfates and possess lectin-like structures. These may even extend the glycocalyx further by firmly binding saccharide-containing molecules in vivo. The immediate consequence of such steric proportions is that firm adhesion of leucocytes and blood platelets to the endothelial cells will be prevented as long as an intact glycocalyx is present (Figure 4). Indeed, damage to the glycocalyx substantially increases intravascular adhesion of leucocytes (polymorphonuclear granulocytes) and platelets. The repeatedly described elevation of adhesion of granulocytes and platelets in the acutely reperfused, post-ischaemic coronary bed can be easily explained based on this new assumption: the glycocalyx is generally degraded and shed during the reperfusion of the heart (Figure 4). Moreover, syndecan-1 knockout mice show increased vascular adhesion and emigration of leucocytes under both basal and inflammatory conditions in various contexts. Mechanical flattening of the glycocalyx, as observed in capillaries upon the passage of leucocytes, obviously does not suffice to bring enough adhesion molecules into contact, since leucocytes do not adhere at such sites physiologically. On the other hand, rolling of leucocytes, lymphocytes, and platelets along the vessel wall may be supported by an intact glycocalyx and cytokines and chemokines bound in the ESL. To complicate matters, binding of chemokines and cytokines may represent sequestration (= inactivation) or, alternatively, local concentration for presentation.
of the more loosely, receptor-bound hyaluronan chains, as incurred, e.g. by the action of hyaluronidase. Several scenarios have been found to associate with the deterioration or destruction of the ESL and glycocalyx, with pathophysiological sequelae such as capillary leak syndrome, oedema formation, accelerated inflammation, platelet hyperaggregation, hypercoagulation, and loss of vascular responsiveness. Some of the better characterized pathological states will be briefly outlined in the following.

3.1 Reperfusion injury

Tissue damage during partial or complete ischaemia is accentuated by the re-established perfusion, known to cause disturbances in its own right. Although the degree of damage depends on the area subjected to ischaemia and can vary considerably, microvascular dysfunction is a common pathophysiological aspect, along with the enhanced adhesion of blood leucocytes and platelets and the activation of blood coagulation pathways. The endothelial cells play a central role following ischaemia/reperfusion (I/R), as oedematous swelling can detach them from the basal membrane. Endothelial cells suffer oxidative stress, resulting in the adhesion and migration of leucocytes, especially in post-capillary venules, and in increased vascular permeability. Recent studies have shown in isolated heart models that 20 min warm (37°C) no-flow ischaemia with subsequent reperfusion is sufficient to initiate near-complete degradation of the glycocalyx in this sensitive (because plasma-free) setting (Figure 4). In an organ less sensitive to ischaemia, the bowel, ischaemia of 60 min is necessary to induce a significant reduction of the glycocalyx volume. These experimental findings have already been successfully transferred to clinical settings. Rehm et al. showed an increase of the main components of the glycocalyx, syndecan-1 and heparan sulfate, in the plasma of vascular surgical patients with global or regional ischaemia. The intraoperative damage was proportional to the duration of ischaemia. Bruegger et al. similarly described increased levels of syndecan-1 and heparan sulfate in the arterial blood of patients undergoing coronary artery bypass surgery. In both investigations, shedding of the glycocalyx occurred concomitant with reperfusion.

3.2 Inflammation and trauma

A systemic inflammatory response can be triggered classically by microbial invasion, a state that is called sepsis, but also by various non-specific insults including surgical trauma. In this pathological state, capillary leakage is one of the major clinical problems compromising cardiac pre-load and causing interstitial oedema, finally leading to a severely disturbed microcirculation. The vascular endothelium is one of the earliest sites of injury during inflammation. Signs of insult include cell membrane damage with accompanying loss of cell contents, swelling, conversion to a pro-aggregatory and pro-coagulatory phenotype, and necrosis. Degradation of the endothelial glycocalyx initiated by inflammatory mediators might play a crucial role here. Tumour necrosis factor (TNF)-alpha and bacterial lipopolysaccharide (LPS) have been experimentally demonstrated to cause shedding of the glycocalyx, the latter actually even shown in humans. TNF-alpha, in particular, activates mast cells, themselves a rich source of substances like cytokines, proteases, hantamine, and heparanase, additionally degrading the endothelial glycocalyx. Pertinently, glycocalyx constituents were detected in the blood of patients in septic shock, the level being positively related to overall mortality. Surgical trauma has also been

---

**Figure 4** The glycocalyx is shown for guinea pig hearts under normal perfusion (top), after I/R (middle), and after I/R with protection by antithrombin (bottom). The green arrows represent the estimated maximal extension (10 nm) of the bonds between membrane molecules for firm endothelial adhesion of leucocytes and platelets. Adhesion molecules in question are the ICAMs, VCAMs, PECAM, integrins, etc. For details, see Chappell et al. (modified from Chappell et al.).

to rolling cells. The absolute outcome of damage and shedding of the glycocalyx with respect to the adhesion of blood-borne cells (including tumour cells) is, therefore, difficult to prognosticate at the present level of understanding.

3. Scenarios of damage

Considering the relatively short time span of attention that has been granted elucidation of the physiological role of the glycocalyx, a surprisingly large amount of evidence has accrued concerning processes of its damage. Perturbation can range from deterioration of just the ESL to fundamental destruction of the glycocalyx itself. Loss of constituents of the endothelial glycocalyx, termed shedding, can encompass removal of entire syndecan and glypican core proteins plus attached glycosaminoglycan side chains. Minor disturbances include selective cleavage of heparan and chondroitin sulfate side groups, e.g. by the action of heparanases and heparinases, or just shedding of the more loosely, receptor-bound hyaluronan chains, as incurred, e.g. by the action of hyaluronidase. Several scenarios have been found to associate with the deterioration or destruction of the ESL and glycocalyx, with pathophysiological sequelae such as capillary leak syndrome, oedema formation, accelerated inflammation, platelet hyperaggregation, hypercoagulation, and loss of vascular responsiveness. Some of the better characterized pathological states will be briefly outlined in the following.

3.1 Reperfusion injury

Tissue damage during partial or complete ischaemia is accentuated by the re-established perfusion, known to cause disturbances in its own right. Although the degree of damage depends on the area subjected to ischaemia and can vary considerably, microvascular dysfunction is a common pathophysiological aspect, along with the enhanced adhesion of blood leucocytes and platelets and the activation of blood coagulation pathways. The endothelial cells play a central role following ischaemia/reperfusion (I/R), as oedematous swelling can detach them from the basal membrane. Endothelial cells suffer oxidative stress, resulting in the adhesion and migration of leucocytes, especially in post-capillary venules, and in increased vascular permeability. Recent studies have shown in isolated heart models that 20 min warm (37°C) no-flow ischaemia with subsequent reperfusion is sufficient to initiate near-complete degradation of the glycocalyx in this sensitive (because plasma-free) setting (Figure 4). In an organ less sensitive to ischaemia, the bowel, ischaemia of 60 min is necessary to induce a significant reduction of the glycocalyx volume. These experimental findings have already been successfully transferred to clinical settings. Rehm et al. showed an increase of the main components of the glycocalyx, syndecan-1 and heparan sulfate, in the plasma of vascular surgical patients with global or regional ischaemia. The intraoperative damage was proportional to the duration of ischaemia. Bruegger et al. similarly described increased levels of syndecan-1 and heparan sulfate in the arterial blood of patients undergoing coronary artery bypass surgery. In both investigations, shedding of the glycocalyx occurred concomitant with reperfusion.

3.2 Inflammation and trauma

A systemic inflammatory response can be triggered classically by microbial invasion, a state that is called sepsis, but also by various non-specific insults including surgical trauma. In this pathological state, capillary leakage is one of the major clinical problems compromising cardiac pre-load and causing interstitial oedema, finally leading to a severely disturbed microcirculation. The vascular endothelium is one of the earliest sites of injury during inflammation. Signs of insult include cell membrane damage with accompanying loss of cell contents, swelling, conversion to a pro-aggregatory and pro-coagulatory phenotype, and necrosis. Degradation of the endothelial glycocalyx initiated by inflammatory mediators might play a crucial role here. Tumour necrosis factor (TNF)-alpha and bacterial lipopolysaccharide (LPS) have been experimentally demonstrated to cause shedding of the glycocalyx, the latter actually even shown in humans. TNF-alpha, in particular, activates mast cells, themselves a rich source of substances like cytokines, proteases, histamine, and heparanase, additionally degrading the endothelial glycocalyx. Pertinently, glycocalyx constituents were detected in the blood of patients in septic shock, the level being positively related to overall mortality. Surgical trauma has also been...
demonstrated to be related to an impressive shedding of glyocalyx in human patients, at least when associated with transient ischaemia. It must be appreciated that degradation of the glyocalyx by inflammatory mediators is but the trigger of further inflammatory processes, starting and maintaining a potentially destructive feed-back mechanism. For example, loss of the glyocalyx uncovers membrane surface adhesion molecules for immunocompetent cells. Shed heparan sulfates additionally attract leucocytes chemotactically, increasing their presence at the site of inflammation. Originally, these mechanisms are designed to sanitize a potentially harmful focus locally. However, they may become the illness itself, overwhelming the organism. Such a self-destructive state independent of the original focus is then termed the ‘systemic inflammatory response syndrome’, with mortality rising up to 80%.

3.3 Atherosclerosis and diabetes

Atherosclerosis is a vascular disease of the larger arterial vessels and typically requires increased levels of low-density lipoprotein (LDL) to develop. Subendothelial accumulation of lipoproteins leads to inflammatory processes and finally to plaque formation. Though the role of the glyocalyx in this process is unclear, there have been several interesting findings, both in vitro and in vivo. Vink et al.

analysed the effect of clinically relevant concentrations of oxidized LDL on the cremaster muscle of the hamster. High concentrations not only deteriorated the glyocalyx but also increased platelet adhesion rates. A further study revealed a reduction of the glyocalyx thickness following a cholesterol-rich diet. Additionally, the authors found an inverse relation between glyocalyx thickness and the intima-media ratio. The thickness of the glyocalyx is not homogeneous anyway, but varies even within a single vessel (Figure 1). In healthy mice, the glyocalyx thickness in the sinus region of the internal carotid artery was much lower than in the common carotid artery. This indicates a reduction of the vascular protective capacity of the glyocalyx in areas with an increased atherosclerotic risk. In man, arteriosclerosis was associated with a fall in the total body volume of the ESL. In summary, these results suggest that an alteration of the glyocalyx plays a pivotal role in the initiation and progression of atherosclerosis.

Diabetes mellitus is characterized by a profound vascular disorder comprising both macro- and microvascular complications. Prevalent signs are an increased permeability of the vessel wall for macromolecules and a reduction in vasodilatory responsiveness. The exact mechanisms contributing to the generalized ‘vascular’ dysfunction in diabetes have not been fully elucidated. Recently, Nieuwdorp et al.

showed that acute hyperglycaemia resulted in a reduction of the volume of the glyocalyx with a concomitant increase in vascular permeability. Both hyaluronan and hyaluronidase plasma levels were found to be increased in diabetic patients with a reduced glyocalyx volume. Several pathways were postulated to contribute to this loss of glyocalyx: oxygen radicals produced during hyperglycaemia could directly damage the glyocalyx structure, or hyperglycaemia could activate glyocalyx degrading enzymes. Indeed, the decrease of glyocalyx thickness was most pronounced in type 1 diabetes mellitus patients with pre-existing microalbuminuria. Albuminuria is a sign of a deteriorated vascular barrier function within the glomeruli and could arise directly from the degradation of the glyocalyx. Chronic systemic glyocalyx injury would then account for the increased permeability for macromolecules such as albumin or LDL. Glyocalyx loss would also concomitantly activate the inflammatory and coagulatory cascades, known to play a vital part in the development of cardiovascular symptoms in patients with diabetes mellitus. Accordingly, the endothelial glyocalyx has been proposed as a possible target for therapy in certain nephrotic syndromes.

3.4 Hypervolaemia

According to the traditional clinical concept of exchange between the human fluid compartments, the intact vascular barrier retains colloids and proteins, whereas water and small solutes are able to freely move within the whole extracellular compartment. Indeed, the volume effect of iso-oncotic colloids is almost 100% when applied to human subjects under conditions of acute normovolaemic blood replacement. However, in patients receiving acute hypervolaemic haemodilution as a pre-emptive blood-saving measure in the context of major surgery, ~60% of the infused volume was discovered to shift towards the interstitial space within minutes (Figure 5A). This unexpected and clinically undesirable escape of fluid occurred independently of whether 6% hydroxyethylstarch of any generation or 5% human albumin was employed (Figure 5A). Possibly, this context-sensitivity of colloid volume effects is related to the alteration of the ESL due to hypervolaemia. As demonstrated quantitatively in human patients by a double-tracer method, the absolute volume of the non-circulating part of the total plasma volume decreased significantly by two-thirds during volume loading (Figure 5B). Obviously, hypervolaemia is a pathogenetic factor that alters the ESL and, consequently, a substantial part of vascular barrier competence.

The underlying cause for the detrimental action of volume loading is presumably to be found in a volume-sensitive regulatory system. The heart releases atrial natriuretic peptide (ANP) into the circulation in the face of mechanical wall stress and, thus, also in hypervolaemia. This hormone is known to induce rapid shifts of intravascular fluid into the interstitial space, and it also has a parallel effect on the endothelial glyocalyx. In an isolated heart preparation, the intracoronary infusion of physiological concentrations of ANP led to the shedding of glyocalyx constituents (Figure 6). Various types of endothelial receptors for natriuretic peptides are all connected to the intracellular cGMP cascade. Interestingly, the elevation of endothelial cGMP levels generally seems to induce microvascular fluid leakage, and this could be based on the shedding of the glyocalyx.

The exact proteolytic pathway(s) being activated via cGMP remain(s) to be clarified.

4. Strategies of protection

In the rat, a relatively rapid regeneration of microvascular luminal glyocalyx was observed after spinal cord injury, but after 3 days this glyocalyx still evidenced altered composition and charge properties. In the mouse, full restitution of the microvascular glyocalyx after damage by infusion of either hyaluronidase or TNF-alpha requires 5–7 days. Although the dynamics of the restoration of a destroyed endothelial glyocalyx in man are unknown so far, it would seem clear that the prevention of damage should be preferred over cure. Unfortunately, there are no established clinical trials showing protection of the glyocalyx, or any benefit resulting from such an intervention in patients. Hopefully, this situation will be remedied within the next few years. Principally, it is possible to differentiate between levels of protection, strategies being aimed at preservation or resurrection of the ESL or, in the face of more severe attack, at fundamental protection of the glyocalyx proper.
4.1 Preservation of the ESL

The simplest way to achieve protection of the ESL is to maintain a sufficiently high concentration of plasma proteins. Although precise knowledge of the required levels is not available, studies with albumin suggest that concentrations far below the physiological value may be adequate. This assumption complies with the everyday clinical experience that considerable dilution of plasma with non-natural colloids is tolerated well by most patients. From a theoretical standpoint, one may expect poorer mechanical stability of a protein-denuded glycocalyx, heightened susceptibility towards attack by proteases and other enzymes, as well as secondary damage to the vessel wall incurred by greater adherence of inflammatory cells.

4.2 Plasma albumin in organ transplantation

In end effect, protection of the ESL means protection of the fragile structure of the glycocalyx. The idea to protect the endothelium of allografts by the presence of albumin during cold ischaemia follows the theory that only a complete ESL might be mechanically stable enough to resist a reperfusion phase, besides possible enzymatic digestion. Furthermore, enhanced adhesion of blood leucocytes in reperfused vessels, causally facilitated by the shedding of the glycocalyx, would contribute to acute and chronic reperfusion damage, and should, thus, be attenuated. This was tested in a model of heart transplantation, coronary vasculopathy being one of the most important obstacles of long-term survival in this collective of organ
Therapeutic strategies targeting the glycocalyx

4.3 Hydrocortisone and the mast cell

Hydrocortisone exhibits protective effects against I/R injury and inflammation in general. Acutely, it prevents the migration of inflammatory cells from circulation to tissues by blocking the synthesis of various chemokines and cytokines. The mechanisms include changes in the expression and activity of enzymes producing vasodilatory agents such as NO and prostacyclin. Furthermore, glucocorticoids are known to achieve a decrease in paracellular permeability for macromolecules. Although glucocorticoids are routinely applied in the prevention of interstitial oedema and swelling, the exact mode of action remains unclear.

4.4 Direct inhibition of factors involved in degradation

There are few cases of experimentally proven protection of the glycocalyx in man by a specific drug. One concerns the use of etanercept, a soluble bio-analog of the TNF-alpha receptor, to significantly antagonize glycocalyx degradation and functional disturbances induced by the injection of LPS in human volunteers. Another relates to a partial recovery of the systemic glycocalyx volume (significantly reduced vs. healthy controls) of 13 patients with familial hypercholesterolaemia after 8 weeks of treatment with rosuvastatin. However, it is unclear whether this was a direct statin effect, or more related to the improved lipid status.

In an animal study, shedding of the glycocalyx induced by the inflammatory stimulus formyl-Met-Leu-Phe was attenuated by the application of doxycycline or ilomastat, two inhibitors of matrix metalloprotease activity. However, there was no conclusive proof that metalloproteases were directly involved in the cleavage of constituents of the glycocalyx in this setting, although such has been proposed for the shedding of syndecans. Because thrombin has also been reported to support the cleavage of syndecan ectodomains, there could be an opening for the therapeutic use of antithrombin.

4.5 Antithrombin III and protease inhibition

Antithrombin III is a physiological inhibitor of serine proteases such as thrombin and elastase. However, it not only inhibits coagulation abnormalities, but also reduces inflammatory responses. One mechanism of action involves promoting endothelial production of prostacyclin by interacting with heparin-like glycosaminoglycans, a vital part of the endothelial glycocalyx. This binding is also pivotal for accentuating its inhibition of thrombin. At this time, antithrombin is not recommended for the treatment of sepsis. This is presumably because it has generally been administered with heparin or heparinoids, a combination unfavourable for the binding of antithrombin to the glycocalyx. The fact that activated protein C behaves well in the treatment of septic patients suggests that the intention, i.e. inhibition of inflammation and proteolytic activation, is correct. Recent studies in humans revealed the degree of glycocalyx shedding to depend on the extent of an ischaemic impact and on the intensity of septic shock. The latter even correlates with mortality. Thus, there are causal correlations between the severity of disease and glycocalyx integrity. In guinea pig hearts, antithrombin has also been shown to protect the glycocalyx following I/R or infusion of TNF-alpha (Figure 4). Vascular leakage is ameliorated by the administration of antithrombin to pigs suffering from LPS-induced sepsis. Antithrombin inhibits numerous serine proteases such as...
thrombin, plasmin, protease-3, and elastase so that its application could directly abrogate proteolytic damage. Serine proteases participate in a wide range of functions in the body, including blood clotting, immunity, and inflammation. Inflammation and the acute inflammatory response to I/R are associated with enhanced thrombin formation. Thrombin, in turn, activates platelets, endothelium, macrophages, and leucocytes and forms fibrin, linking the inflammatory and coagulation systems. Escalation of thrombin formation may significantly contribute to the adverse functional consequences of ischaemia on myocardium, whereby inhibition by antithrombin III should be protective. Indeed, supplementation of antithrombin has alleviated I/R injury in various organs. However, this protection has been largely attributed to the augmented release of prostacyclin.

In addition to the above, antithrombin has been found to bind tightly to the glycocalyx. Conceivably, a sufficient level within the glycocalyx may obstruct attack by proteases, heparanase, and hyaluronidase, thereby preventing shedding. These promising results clearly warrant further assessing the glycocalyx as a therapeutic target benefiting from antithrombin III in clinical settings.

4.6 Avoidance strategies (antioxidants, normovolaemia, atrial placidity)

Antioxidants have been found to protect tissues and organs from I/R damage in innumerable studies. That this action might critically involve protection of the glycocalyx has received attention only recently. An experimental study by Bruegger et al. described a protective action of NO, applied only during reperfusion, on maintaining the glycocalyx and the permeability barrier in the face of redox stress. This latter action was exerted only if the glycocalyx was not destroyed enzymatically beforehand, so that a direct radical scavenging action of NO was held responsible by the authors. All in all, enhancing antioxidant defence of the glycocalyx seems intuitively advantageous, but no clinical studies focused on preserving vascular permeability have yet used this approach convincingly.

On the other hand, generations of anaesthetists have performed acute hypervolaemia haemodilution as a blood-saving measure and, still today, patients are routinely infused generously before intraoperative reperfusion procedures or before general or neuraxial anaesthesia. However, the traditional conclusion that iso-osmotic colloids have a constant and clinically usable volume effect of 100% is increasingly subject to doubt. Owing to the damage to the ESL, ~60% of the infused volume shifts into the interstitial space within minutes (Figure 5). This argues for avoiding hypervolaemia as far as possible. A requirement-adapted fluid approach improves outcome, especially in patients undergoing major abdominal surgery. One may presume that avoiding volume overload and the associated release of natriuretic peptides protects the endothelial glycocalyx, helping to maintain the barrier properties of the ESL. This will lead to a reduction of interstitial oedema with all positive effects described recently, including less disturbance of wound healing, improved pulmonary function, etc. Besides this, nutritive microvascular blood flow should be enhanced on the basis of a preserved dilatatory shear stress response.

Avoiding the release of ANP from the heart seems to be a good idea not only in conjunctión with volume loading. In a very recent study comparing coronary artery surgery with and without cardiopulmonary bypass (‘off-pump’), Bruegger et al. found that respectively identical elevations of syndecan-1 and heparan sulfate levels occurred in the circulation of both groups of patients. This was totally unexpected from the viewpoint of ischaemic stress to the heart and lungs in the two groups. However, mechanical manipulation of the heart, especially wall stress to the atria, is excessive in both situations. Thus, one may expect ANP release to peak and ANP-induced shedding of the glycocalyx to occur at similar extents, independent of the surgical procedure.

4.7 Supply of ‘prefab’ components

Henry and Duling performed one of the first successful attempts at the restitution of the glycocalyx in vivo in anaesthetized hamsters. Capillary glycocalyx damaged by hyaluronidase was partially regenerated by acute i.v. infusion of a combination of hyaluronan and chondroitin sulfate. Recently, Potter and Damiano applied the same two constituents to cultured human umbilical vein endothelium. Although cultured endothelial cells express only a rudimentary glycocalyx, supra-physiological levels of hyaluronan together with chondroitin sulfate did lead to a detectable increase in the viscometrically effective thickness of the surface layer. Elevated expression of syndecan-1 improves post-infarction healing of myocardium in mice. Culture media of cells have variously been augmented with syndecans, whereupon both anti- and pro-inflammatory effects were reported. However, these studies did not really check for a change in the composition or dimension of the endothelial glycocalyx.

Confounding these rather spurious results is the major drawback that there are no preparations of syndecans, hyaluronan, or chondroitin sulfate registered for human use. The situation may be different for heparan sulfate, because heparin and heparinoids are analogous structures and are pharmacologically tested drugs. Indeed, heparin and heparin derivatives such as enoxaparin have proved useful in moderating inflammation in syndecan knockout mice, especially with regard to the intestinal barrier function.

Whether these benefits resulted from the incorporation of the compounds into the endothelial glycocalyx or even an indirect improvement of its consistency was not examined.

5. Summary and perspectives

Benefits derived from numerous clinically established therapeutic agents, especially hydrocortisone and antithrombin, may be, at least partly, based on the protection of the endothelial glycocalyx from enzymatic destruction. Inflammatory mediators released due to trauma and critical illness, foremost TNF-alpha, as well as ANP, liberated in the course of volume expansion or heart surgery, are to be regarded as sure candidates initiating such destruction. TNF-alpha may be directly inhibited using pharmacologically available antagonists. Stabilization of resident tissue mast cells offers a convincing alternative, since mast cells are a prodigious source of TNF-alpha and many other cytokines and chemokines, as well as of numerous proteases and heparanase in man. Effects of ANP and other natriuretic peptides on the glycocalyx, elicited perhaps via the activation of metalloproteases, are best precluded by the avoidance or minimization of mechanical stress to the heart. Iatrogenic hypervolaemia has a negative impact on the integrity of the endothelial glycocalyx. In addition, its mechanical destruction, especially if protein-denuded, appears conceivable. Antithrombin seems to proffer protection from enzymatic attack, but here, as in all other proposed therapeutic options, there is no direct clinical proof for such a mode of action.
Selective inhibition of a given protease or group of proteases in order to prevent shedding of the glycocalyx is not regarded as a viable option by us, since too many enzymes seem capable of attack (unpublished personal work). In the near future, we may expect a choice of anaesthetic regimens as prophylactic aid in attenuating damage to the glycocalyx, augmenting the increasingly well-established avoidance of volume-loading procedures in clinical care. One of the simplest options to implement at this time is to maintain physiological levels of albumin in plasma and in solutions bathing isolated tissues.

**Conflict of interest:** none declared.

**Funding**

Institutional funding was provided by the Walter-Brendel-Centre of Experimental Medicine.

**References**
