Science in brief: The role of the glycocalyx in critical ill patients with reference to the horse

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Abstract

The glycocalyx, a gel-like layer covering the luminal surface of vascular endothelial cells has several key functions in maintaining homeostasis in the vasculature. Interest in the glycocalyx has significantly increased in the last decades and damage to this vital layer appears to influence progression and recovery in many disease processes. Pathological injury to the glycocalyx occurs in ischaemia–reperfusion, inflammation, sepsis, shock, burns and excessive shear stress. There is increasing evidence that iatrogenic interventions such as fluid therapy influence the integrity of the glycocalyx and can even worsen the initial injury from pathological events. Rapid infusion of crystalloids and colloids, hypervolaemia, hypernatraemia and hyperglycaemia have all been associated with increased glycocalyx shedding. Current treatment recommendations aim to minimise iatrogenic damage. In the future, interventions aimed at restoring glycocalyx integrity and function might become valuable tools in critical care.

The glycocalyx is a gel-like layer covering the luminal surface of vascular endothelial cells. It is comprised of membrane-attached proteoglycans, glycosaminoglycan chains, glycoproteins synthesised by the endothelial cells and adherent plasma proteins. The glycocalyx is essential for maintaining homeostasis in the vasculature[1]. In the healthy state, the glycocalyx regulates vascular permeability, provides anti-coagulant and anti-adhesive effects on the surface of endothelial cells and can shield endothelial cells from oxidative stress[1; 2]. Additionally, the glycocalyx senses fluid shear forces and transmits these forces to endothelial cells, initiating nitric oxide-mediated vasorelaxation. Considering these key functions, interest in the glycocalyx has significantly increased in the last decades and damage to this vital layer appears to influence progression and recovery in many disease processes. To the author's knowledge, the only reports on glycocalyx in horses relate to the reproductive tract[3-5], mesenchymal stem cells[6] and equine pulmonary intravascular macrophages[7]. Until species-specific information is available, findings form other species therefore have to be extrapolated to equine patients. Unfortunately, research in any species is hampered by the fragile nature of the layer and the fact that structure, thickness and composition vary between vascular beds and methods used to investigate the layer's characteristics[2]. The glycocalyx is thinner where it covers the microcirculation (as little as 0.2 µm) and thicker in larger vessels (up to 8 µm)[8]. In addition, glycocalyx grown on epithelial cells *in vitro* is not equivalent to its *in vivo* counterpart[9]. Research is further complicated by the fact that the effects of damage not only depend on the glycocalyx but also the nature of the underlying epithelium, making findings from one vascular bed not necessarily transferable to other areas of the body. A popular method to evaluate glycocalyx damage that is widely used in clinical studies is measuring circulating glycocalyx components, often syndecan-1 and hyaluronan, in plasma or urine of patients. While the degree of glycocalyx shedding correlates well with disease severity, the reliability of circulating glycocalyx components as endothelial damage biomarkers has not been widely accepted. There

is insufficient direct evidence showing that circulating concentrations of glycocalyx components correlate with endothelial injury[2]. Many proposed concepts about glycocalyx function in health and disease are therefore still speculative and controversial and not always backed up by clinical evidence[2; 10; 11].

Vascular permeability and transvascular fluid flux

In the healthy state, the glycocalyx serves as a barrier opposing vascular permeability through a negatively charged molecular meshwork by limiting transvascular movement of molecules that are negatively charged, larger than 70-kDa, or both. By establishing a transvascular albumin gradient, it has been hypothesised that the intact glycocalyx regulates transvascular fluid flux following the so-called revised Starling equation or revised Starling principle[8]. The revised Starling principle states that transvascular exchange of fluid no longer occurs according to a simple gradient of hydrostatic and oncotic pressures across the capillary wall. Rather, the endothelial glycocalyx layer generates a virtually protein-free space between the glycocalyx and the endothelial cell membrane. Thereby, the oncotic gradient is moved from being between the plasma and the interstitium to being between the plasma and the protein-free space between glycocalyx and endothelial cell membrane. The 'transglycocalyx' oncotic pressure replaces the interstitial oncotic pressure as the key force opposing fluid filtration in the revised equation[12]. A further major change to the Starling principle is that, according to the revised principle, the oncotic pressure difference across the endothelial glycocalyx layer can oppose, but not reverse, the filtration rate[8]. This is referred to as the 'no absorption' rule and states that raising the oncotic pressure in the plasma cannot recruit fluid from the interstitial space. However, not only the glycocalyx but also the underlying endothelium influence permeability. The double-barrier concept stipulates that simultaneous disruption of the endothelial cell barrier and glycocalyx is needed to readily increase vascular permeability. While damage to

the glycocalyx alone overlying a tight cellular barrier has minimal effect on permeability, identical glycocalyx damage overlying a leaky cellular monolayer will rapidly increase fluid eflux[11]. The effects of glycocalyx damage therefore depend on the underlying endothelial cell phenotype and vary between and probably also within tissues[11]. When glycocalyx-dependent permeability changes within the systemic vasculature are studied, it is therefore important to consider how both, glycocalyx structural adaptations and the underlying endothelial cell phenotype, will influence detectable permeability changes[11]. The new concept of the revised Starling principle has possible clinical implications: According to the no absorption rule, use of colloids to prevent or treat oedema would be fruitless as fluid cannot be reabsorbed[8]. The clinical applicability of this theory has been questioned as infusions of hyperosmolar and hyperoncotic solutions seem to increase the intravascular volume well beyond the infused volume suggesting that recruitment of interstitial fluid into the intravascular space takes place[10; 13]. Anecdotally, a poor correlation between albumin concentration and the presence or absence of peripheral oedema is occasionally noted in sick horses. The revised Starling principle offers an attractive explanation why even with very low albumin concentrations minimal oedema might be observed, providing the glycocalyx layer is intact, maintaining the protein-free space above the endothelium. On the other end of the spectrum, massive interstitial fluid accumulation might be observed with severe damage to this internal barrier, as seen in states of systemic inflammation, despite relatively normal albumin concentrations[14].

Glycocalyx and fluid therapy

Intravenous fluid therapy is an important part of managing critically ill horses or horses undergoing general anaesthesia. Pathological injury to the glycocalyx occurs in ischaemia–reperfusion, inflammation, sepsis, shock, hyperglycaemia, burns and excessive shear

stress[12]. There is increasing evidence that iatrogenic interventions such as fluid therapy influence the integrity of the glycocalyx and can even worsen the injury from the initial pathological events[15]. Rapid crystalloid or colloid fluid administration induce endothelial glycocalyx shedding in people and dogs[16; 17]. Proposed mechanisms include hemodilution of plasma components and potential osmotic changes.

Elimination of infused fluid during surgery and anaesthesia decreases to approximately 10%–20% of that in a conscious state due to the effects of the procedure and anaesthetics on adrenergic activity and levels of aldosterone, antidiuretic hormone, and renin. The half-life of crystalloids can be up to 10 times longer under general anaesthesia augmenting plasma volume expansion and increasing the risk of hypervolaemia and peripheral fluid accumulation[14]. The effect of hypervolaemia on the glycocalyx is controversial[18]. Hypervolaemia has been postulated to damage the glycocalyx, at least partially by triggering the release of atrial natriuretic peptide (ANP) that caused an increase in vascular permeability and histological degradation of the glycocalyx in an animal model[19]. Findings in human patients with induced hypervolaemia (15-25ml/kg/h) during routine surgery are conflicting with some finding evidence of ANP release and glycocalyx shedding[20; 21] while others did not[22]. However, almost all studies showed proportionate fluid retention following high volume infusions under general anaesthesia. Use of such high fluid rates would be rare in routine equine surgeries but clinicians should still be aware of the increased risk of fluid accumulation during general anaesthesia and possible associated risks of hypervolaemia.

Hypernatraemia, induced by infusion of hypertonic saline, is equally controversial, damaging the glycocalyx in some experimental studies[23] but not in others[24]. Acute and chronic hyperglycaemia also compromises the glycocalyx[2] and high glucose concentrations (11.1mmol/L) may exacerbate sepsis-induced vascular endothelial activation and injury and glycocalyx degradation[25]. Choice of resuscitation fluid might also influence glycocalyx

damage and recovery. Haemorrhagic shock is associated with glycocalyx shedding and increased vascular permeability. Compared to crystalloids, using hydroxyethyl starch (HES) or plasma for resuscitation might improve vascular barrier function and glycocalyx recovery[26; 27]. Different colloids might have different effects and not only the oncotic properties, but also the relative ability to protect and restore the endothelial glycocalyx could be relevant. Although HES increased colloid oncotic pressure more than albumin in a study using isolated guinea pig hearts, the reduction in capillary permeability was less compared to albumin. This was attributed to the ability of albumin to be partially incorporated into the glycocalyx layer[28]. *In vitro* and *in vivo* studies suggest that albumin and fresh frozen plasma might be superior to crystalloids and artificial colloids as resuscitation fluids, particularly during haemorrhagic shock, but prospective clinical studies are needed to confirm these findings[29]. Current recommendations for fluid therapy and resuscitation in people have not yet changed greatly in light of these findings but emphasise the importance of avoiding fluid overload, sever hypernatraemia and hyperglycaemia[12; 24]. It is probably prudent to heed this advice in in horses, too.

Intravascular inflammation and coagulation

The intact endothelium has multiple anticoagulant and profibrinolytic properties including the production and release of nitric oxide, prostacyclin and tissue factor pathway inhibitor[30]. Endothelial cells also secrete heparan sulfate, which augments the anticoagulant action of antithrombin through binding of antithrombin in plasma to heparan sulfate located on the luminal surface and in the basement membrane of the endothelium. Antithrombin's anticoagulant activity is known to increase dramatically when bound to heparan sulfate on the endothelial glycocalyx[31]. During inflammatory conditions, such as sepsis and acute respiratory distress syndrome, glucuronidases, including heparanases, reactive oxygen species

and other proteases, disrupt the glycocalyx, exposing adhesion molecules for leucocytes and platelets such as E-selectin and intercellular adhesion molecule 1 on the denuded endothelium[2; 32]. Exposure of the adhesion molecules and a decrease in the antithrombotic properties leads to leucocyte and platelet recruitment, and fibrin and thrombus formation. While this is initially a beneficial process, aiming to trap and eliminate invading microorganisms, excessive activation can lead to altered blood flow, impaired oxygen delivery and subsequent organ failure[33-35]. Circulating syndecan-1 and hyaluronan concentrations have repeatedly been associated with disease severity, particularly in sepsis, and development of disseminated intravascular coagulation (DIC)[36-38]. The search for effective treatments for sepsis and DIC has been ongoing for decades and currently some hope is placed in therapies that either prevent or limit damage to the glycocalyx or accelerate its regeneration. As mentioned, judicious fluid therapy and use of plasma or albumin have been proposed but require further investigation. Inhalation anaesthesia using sevoflurane has been suggested to have glycocalyx-protecting or regenerating effects in some studies[39; 40] but this was not confirmed by others[22; 41]. Other drugs with possible benefits include corticosteroids, antithrombin, heparin and heparinoids[42]. Large scale clinical trials for these drugs in mainly septic patients, used with a different rational, so far failed to demonstrate beneficial results consistently[43]. New treatment approaches include glycocalyx components and enzyme inhibitors to prevent shedding, factors to accelerate angiogenesis and nanomaterial and glycocalyx-mimetic biomaterials. Results of clinical trials are still outstanding[44].

While the endothelial glycocalyx is unquestionable of great physiological and pathophysiological importance, research is still in its infancy. Until targeted treatments are fully investigated, avoiding iatrogenic damage by using judicious fluid therapy and avoiding hypervolaemia, hypernatraemia and hyperglycaemia appears sensible.

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