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Circulating proteoglycans and glycosaminoglycans during critical illness

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2014

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Citation for published version (APA):

Nelson, A. (2014). *Circulating proteoglycans and glycosaminoglycans during critical illness*. [Doctoral Thesis (compilation), Department of Clinical Sciences, Lund]. Anaesthesiology and Intensive Care.

Total number of authors:

1

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Circulating proteoglycans and glycosaminoglycans during critical illness

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Lund University
Faculty of Medicine
Department of Clinical Sciences
Anaesthesiology and Intensive Care
ISBN 978-91-7619-079-1
ISSN 1652-8220
Lund University, Faculty of Medicine Doctoral Dissertation Series 2014:150

Printed in Sweden by Media-Tryck, Lund University
Lund 2014



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List of papers

The thesis is based on the following papers. Additional data is presented in an Appendix.

- I. Axel Nelson, Ingrid Berkestedt, Artur Schmidtchen, Lennart Ljunggren, Mikael Bodelsson. 2008. Increased levels of glycosaminoglycans during septic shock: relation to mortality and the antibacterial actions of plasma. *Shock* 30, 623-637.
- II. Axel Nelson, Ingrid Berkestedt, Mikael Bodelsson. 2014. Circulating glycosaminoglycan species in septic shock. *Acta Anaesthesiol Scand* 58, 36-43.
- III. Axel Nelson, Svajunas Statkevicius, Ulf Schött, Pär I Johansson, Peter Bentzer. Effects of Fresh Frozen Plasma, Ringer's acetate and Albumin on plasma volume and on the endothelial glycocalyx following hemorrhagic shock in rats. In manuscript.
- IV. Axel Nelson, Joakim Johansson, Jonas Tydén, Mikael Bodelsson. Circulating syndecans during critical illness. In manuscript.

Abbreviations

| | |
|---------------|---|
| ALB | albumin |
| CLI | cecal ligation and incision |
| Da | Dalton |
| ELISA | enzyme-linked immunosorbent assay |
| FFP | fresh frozen plasma |
| GAG | glycosaminoglycan |
| HPLC | high-performance liquid chromatography |
| ICU | intensive care unit |
| RA | Ringer's acetate |
| SIRS | systemic inflammatory response syndrome |
| SOFA score | sequential organ failure assessment score |
| TNF- α | tumor necrosis factor alfa |
| TER | transcapillary escape rate |

Introduction

Background

Patients at the intensive care unit (ICU) represent the ultimate challenge for the physician due to the severity and often acute nature of the conditions. Major causes of admission are shock caused by trauma or a systemic inflammatory response syndrome (SIRS). These conditions are often accompanied with signs of damage to the vascular barrier (Reinhart et al 2002). It is today recognized that the endothelium participates in the development of the SIRS phenotype (Aird 2003).

Circulatory homeostasis involves regulation of vascular tone and transvascular fluid exchange, as well as regulation of coagulation and transport of migrating cells from the circulation (Aird 2003). The endothelium plays a fundamental role in these functions and involves the glycocalyx, the layer of protein and carbohydrates that covers its luminal surface.

The microscopic structure and molecular framework of the glycocalyx has been extensively studied during the past decades but the biological relevance is, however, not fully understood. This thesis investigates if measurement of glycocalyx molecules in the blood could provide insight into the pathophysiology of critical illness and/or be useful as markers of disease.

Glycocalyx

The luminal surface of the endothelial cells of the blood vessels is coated with a glycocalyx made out of membrane bound proteoglycans, proteins and carbohydrates as well as plasma proteins adsorbed into this matrix (Fig. 1, Weinbaum et al 2007). The glycocalyx represents the interface between blood and tissue and participates in key processes of homeostasis. Of particular interest is the membrane bound proteins with attached sulphated glycosaminoglycans (GAGs), the proteoglycans. On the vessel wall, these proteoglycans bind and present signal molecules including chemokines and cytokines to the circulating cells and thus take part in the recruitment of leucocytes to a damaged tissue (Wang et al 2005, Parish 2006). Furthermore,

specific GAG sequences (explained below) on the proteoglycans bind and activate antithrombin and thereby contribute to the anti-thrombotic properties of the vessel wall (Kjellén & Lindahl 1991, Lindahl 1999, Mertens et al 1992). The thickness of the glycocalyx gives rise to a significant reduction of microvessel cross area and contributes to the hemodynamic resistance of small vessels (Pries et al 1994 & 1997). The glycocalyx also constitute a resistance to transendothelial transport of water and macromolecules from the intravascular space to the interstitium (Weinbaum et al 2007, Singh et al 2007, Salmon & Satchell 2012) and plays an important role in a proposed revision of the starling principles that governs transvascular fluid exchange (Levick & Michel 2010, Woodcock & Woodcock 2012). All this data suggest that glycocalyx damage could be a player in the pathophysiology of SIRS but direct evidence for such a role is yet lacking.

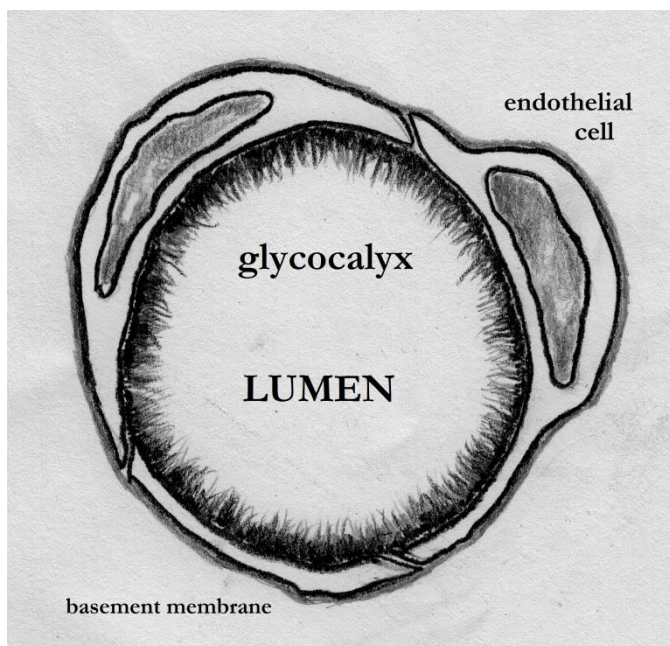


Figure 1.

A continuous microvessel. The hairy structures protruding into the lumen is the glycocalyx made out of proteoglycans, glycosaminoglycans, membrane-bound protein and adsorbed plasma proteins. Illustration by Ellinor Nelson.

Proteoglycans

Proteoglycans are proteins with covalently bound chains of GAG (Fig. 2). In cultured endothelial cells surface proteoglycans are dominated by syndecans and glypicans (Mertens et al 1992, David et al 1995). Several proteoglycans are present in the subendothelial matrix, including perlecan, collagen XVIII, versican, biglycan and agrin (Tran Lundmark 2008). It should be noted, however, that the structure and function of the endothelium varies in different vascular beds (Aird 2005). Furthermore, experiments shows that the glycocalyx examined with electron microscopy is about ten times thicker in intact vessels ex vivo compared to in culture (Chappell et al 2009). This indicates that current knowledge on endothelial proteoglycans is not fully understood.

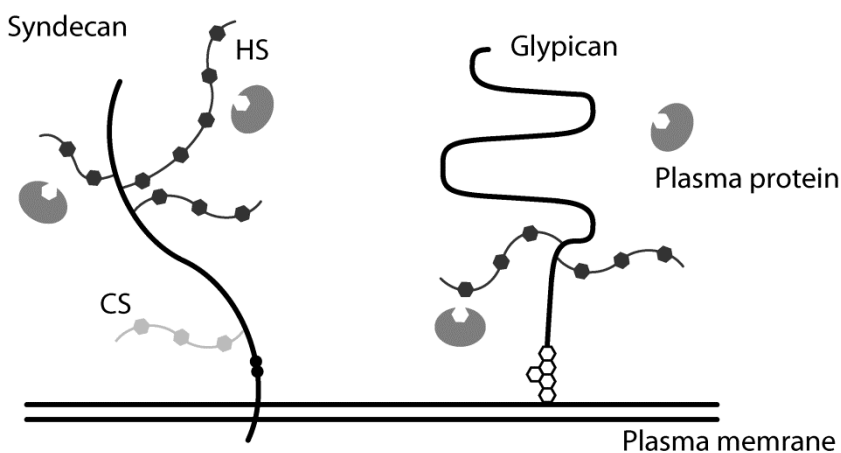


Figure 2. Cell surface bound proteoglycans. HS – Heparan sulphate, CS – Chondroitin sulphate. Illustration by the author.

This thesis evaluates circulating syndecans and the following text is therefore restricted to syndecans. Mammalian syndecans constitute a family of four transmembrane proteoglycans that always have heparan sulphate chains and sometimes chondroitin sulphate chains attached during synthesis (Saunders et al 1989, Pierce et al 1992, Carey et al 1992, Kojima et al 1992). The syndecans are products of four different genes that share amino acid sequence homology in the transmembrane and cytoplasmic domains, while the extracellular domains show no more than 10-20% sequence similarity. The core proteins of the syndecans vary between 20 and 45 kDa in size and each syndecan attach 3-5 heparan sulphate or chondroitin sulphate chains (David et al 1995, Manon-Jensen et al 2010). Syndecans are expressed throughout the

body but the relative distribution of the different types varies between tissues as well in response to external stimuli (Kim et al 1994, Okuyama et al 2013, Kainulainen et al 1996). Experiments to determine the basal expression of syndecans are mainly performed in murine cultured cells and tissues. These experiments show that syndecan-1 is expressed in endothelial, epithelial and differentiated B-cells. Other syndecans are less studied but syndecan-2 has been found to be expressed in endothelium and fibroblasts while syndecan-3 is expressed in high concentration in neuronal tissue. Syndecan-4 is present on several cell types but the expression is generally lower compared to the other syndecans (Kim et al 1994).

The syndecans share a protease cleavage site near the transmembrane domain that facilitates the shedding of the ectodomain of the molecule (Fig. 3). This does not only reduce the amount of the molecule bound to the cell surface but also generates soluble syndecan fragments constituting the ectodomains that can function as effectors in the local microenvironment (Teng et al 2012).

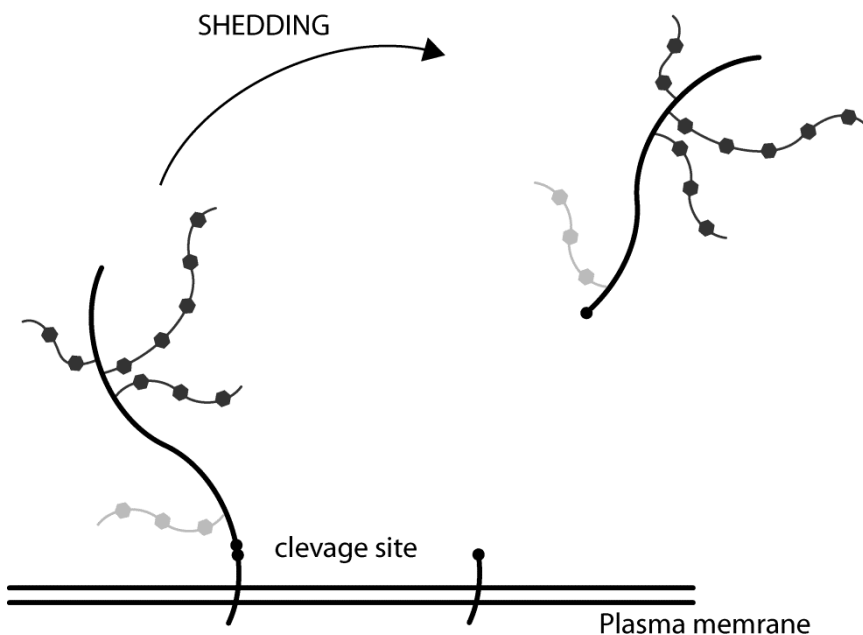


Figure 3. Illustration of shedding of a syndecan ectodomain from the cell surface. Illustration by the author.

Shedding of syndecans from the cell surface and subsequent detection in the surrounding media can be induced by various stimuli including thrombin (Subramanian et al 1997), *Staphylococcus aureus* toxins (Park et al 2004) and

Chemokine ligand 5 (Charnaux et al 2005). Cleavage seems to be mediated via metalloproteinases (Fitzgerald et al 2000).

This data show that syndecan presence on the cell surface is variable and depending on local stimuli. They also suggest that shedding of vascular syndecan should be detected in blood.

Glycosaminoglycans

GAGs are polymers of disaccharide subunits consisting of hexosamine (D-glycosamine or D-galactoseamine) and either hexuronic acid (D-glucuronic acid, L-iduronic acid) or galactose units. Depending on this basic chemical structure of the glycan backbone the GAGs can be classified into heparan sulphate, heparin, chondroitin sulphate, keratan sulphate and hyaluronic acid (Fig. 1). The GAG polymer is arranged in an unbranched alternating sequence and carries sulphate groups at varying positions along the chain. The presence of the sulphate groups gives the molecules a high negative charge. Importantly, these modifications are incomplete and partly stochastically iterated resulting in considerable heterogeneity between as well as within GAG chains (Lindahl 2014, Chi et al 2008). Moreover, the level of diversity varies between the different GAGs where heparan sulphate is the most diverse while hyaluronic acid is fully homogenous. This structural heterogeneity provides the foundation for the ability of GAGs to bind and interact with a multitude of proteins, including growth factors, chemokines, extra-cellular matrix proteins and antimicrobial peptides (Kreuger et al 2006, Taylor & Gallo 2006, Barańska-Rybak et al 2006). The GAG is generally attached to a serine residue on the core protein (proteoglycan) and the GAG chains can differ considerably in length, from one to more than 100 disaccharide subunits (Kjellén & Lindahl 1991).

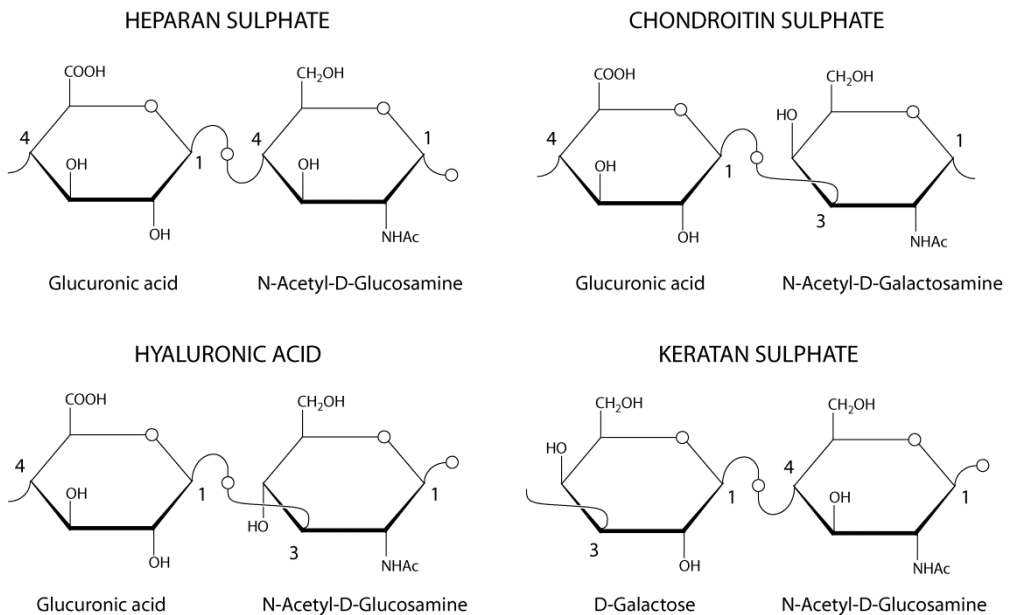


Figure 4.

The biochemical structure of the disaccharide building blocks of the different GAGs. The principle for classification is based on the chemical structure of the monosaccharide components as well as the linkage between them. The disaccharides depicted are unmodified. Modifications include sulphate groups attached to various OH groups. Illustration by the author.

Experiments on cultured bovine capillary and large vessel endothelial cells as well as human umbilical endothelial cells shows that cell surface and excreted GAG is 40-70% made out of heparan sulphate while 20-40% is chondroitin sulphate and hyaluronic acid (Bar et al 1985). Microscopy on rat post-capillary venules treated with depolymerizing enzymes that recognizes different GAG subtypes shows that heparan sulphate contributes to about 50% of the volume of the endothelial glycocalyx, hyaluronic acid to about 40%, and chondroitin sulphate to about 10% (Gao & Lipowsky 2010). Exposure of cultured endothelial cells to TNF- α or endotoxin is associated with a decrease in GAGs bound to the cells and an increase in the release of GAGs to the culture supernatant (Klein et al 1992).

Isolation and analysis of GAG in plasma of healthy individuals shows that chondroitin sulphate is the quantitatively dominating circulating GAG while lower quantities of heparan and keratan sulphate are detected (Staprans & Felts 1985, Calatroni et al 1969). Moreover, the circulating GAG is present both as large sized GAGs (~100 kDa) and in smaller fragments (~5 kDa, Staprans & Felts 1985).

Leucocytes contain chondroitin sulphate and small amounts of hyaluronic acid that can be released after degranulation (Olsson & Gardell 1967, Lewitt et al 1986, Steward et al 1990). Platelets contain chondroitin sulphate that is released during aggregation and small amounts of heparan sulphate that does not seem to be released during platelet activation (Nader 1991, Ambrosius et al 2008).

The metabolism and excretion of GAGs in the plasma depends on the GAG species and the molecular size. Hyaluronic acid is almost exclusively metabolized in the liver (Frazier et al 1997), an observation that has been utilized for measurement of hepatic blood flow/function (Wang et al 1997). Large sized chondroitin and heparan sulphate molecules are metabolized in the liver while urinary excretion is restricted to small sized fragments (Wood et al 1973, Pecly et al 2006, Perry et al 1977, Boneu et al 1990).

These data suggest that cleavage of GAG from the endothelium would in the circulation primarily be detected as heparan sulphate and hyaluronic acid. Thus increased plasma levels of these GAGs could possibly reflect endothelial damage.

Aims of the thesis

Hallmarks of severe inflammatory conditions include dysregulated leucocyte adhesion and transmigration, impaired regulation of coagulation, decreased vascular resistance and increased vascular permeability. All these abnormalities relate to impaired functions of the blood vessel wall in which proteoglycans and their GAGs have been suggested to contribute.

The overall purpose of the thesis project was to determine if concentrations of GAGs and/or proteoglycans are increased in the blood of sepsis patients and to investigate the clinical relevance of measuring the concentration in critically ill patients.

Methodological considerations

Ethics

The participation of humans consisted of blood sampling and registration of clinical data only and did not include any interventions. The studies on septic shock patients at the intensive care unit in Lund, Sweden (paper I, II) was approved by the regional research ethics committee in Lund, Sweden (dnr LU-915-02) and on critically ill patients at the intensive care unit in Östersund, Sweden (paper IV) was approved by the regional research ethics committee in Linköping, Sweden (dnr 2010/427-31). Informed consent was obtained from the patients or the next kin of unconscious patients.

All animal experiments were performed on anaesthetized rats, which were euthanized before regaining consciousness (paper III, Appendix) and were approved by the Ethical committee on animal experiments in Lund, Sweden (dnr M103-09). The animals were treated in accordance with the guidelines of the National Institutes of Health and Care for use of laboratory animals.

Patients

The Lund septic shock patients were recruited from the ICU and inclusion criteria were suspicious infection and fulfilling two or more criteria for SIRS (American College of Chest Physicians 1992). Retrospectively, the majority of patients was found to be refractory to fluid treatment and hence fulfilled the criteria for septic shock. Blood samples were withdrawn within 24 h of admission to the ICU. In the Östersund ICU material, all patients admitted to the ICU were included and classification was performed at discharge based on journal notes. The validity of diagnoses was verified by an investigator blinded to the results of the study.

Analysis of glycosaminoglycans

Due to the remarkable structural diversity within the GAG chains, the analysis and quantification of GAGs was a major challenge. Based on the different purposes in the different papers, a variety of methods was utilized.

In paper I a dye binding assay that takes advantage of the interaction between sulphate groups on the GAG polymers and the multivalent cationic dye Alcian blue was used (Björnsson 1998, Frazier et al 2008). At low pH carboxylic and phosphoric acid groups are neutralized leaving sulphate groups of the GAG practically the only negative residues to interact with Alcian blue. A high ionic strength and presence of detergents dissociates binding to proteins and facilitates adequate size of the formed complexes to be captured on a membrane for detection by reflectance. This method provides a crude but robust measure of the total quantity of sulphated GAG in a sample. Since hyaluronic acid is devoid of sulphate groups it is not measured with this method. Pilot experiments showed that the sensitivity for the assay declines for GAG chains smaller than 5 kDa in size.

In paper II, we wanted to refine the observations in paper I. For this purpose, we quantified the different types of GAG, i.e. hyaluronic acid and heparan, chondroitin and keratan sulphate. In addition, severe sepsis may be accompanied with dysfunction of the liver, the metabolizing organ, and/or the kidneys that excrete small fragments of GAG (Staprans & Felts 1985). Based on this consideration we wanted the method to also have a high sensitivity in the analysis of small GAG fragments. For this purpose a tandem high-performance liquid chromatography (HPLC) mass spectrometry method was developed for analysis of chondroitin sulphate and hyaluronic acid disaccharides using an approach suggested by Oguma and colleagues (Oguma et al 2007). The method uses a sample preparation based on digestion of endogenous chondroitin sulphate and hyaluronic acid with chondroitinase ABC, an enzyme that depolymerizes the GAG chains into disaccharide elements. The mixture was then passed through a 5kDa centrifugal filter, and the filtrate was used for analysis. Due to the highly polar properties of the digested GAG molecules a graphite column was used for separation of the disaccharides in the HPLC step. The disaccharides were then directly injected into the mass spectrometer via an electrospray interface. For quantification, quadropole mass detection in two steps with fragmentation in between was used resulting in a high level of specificity. Due to differences in sample matrix composition that might affect the sensitivity of the electrospray ionization, each plasma sample was normalized to a spiked aliquot not treated with chondroitinase ABC.

Originally, the purpose was to apply the same mass spectrometric approach for analysis of heparan sulphate and keratan sulphate but unfortunately the sensitivity of

the instruments available was insufficient. Instead, we used commercially available ELISA kits with antibodies targeting the N-acetylated and N-sulphated motifs in heparan sulphate, found primarily on cell surface-derived heparan sulphate (van den Born et al 2005). Importantly, this method provides a measure of the relative abundance of specific heparan sulphate motifs in the plasma sample, while this not necessarily reflects the absolute quantity.

Analysis of proteoglycan core proteins

Plasma levels of syndecans were determined using commercially available ELISA kits or antibody sets. These methods rely on the combined specificity obtained by two sets of antibodies raised against the ectodomain of the corresponding core protein. Due to the fact that plasma composition may vary considerably among critically ill patients, care was taken to evaluate and modify the assays in order to establish that the recovery was above 70% and that serial dilutions were linear.

Animal experiments

In Appendix, a cecal ligation and incision (CLI) procedure was performed to mimic the abdominal sepsis phenotype (Scheiermann et al 2009). In this model a 1 cm long incision is made in cecum leading to leakage of faeces into the abdominal cavity. This provides a fast and dynamic time course and results in development of a septic phenotype within a couple of hours. However, since most severe infections seen in clinical practice have progressed over more than one day it could be argued that the model is too acute.

Haemorrhagic shock was induced by bleeding the animals 30 ml/kg during 30 min corresponding to approximately 50% of the total blood volume (paper III). The volume was chosen to mimic a severe haemorrhage. One hundred and fifty min after completion of haemorrhage the animals were resuscitated with fresh frozen plasma (FFP, 37.5 ml/kg), 5% albumin (ALB, 30ml/kg) or Ringer's acetate (RA, 75 ml/kg or 135ml/kg) during a resuscitation period of 30 min. The resuscitation volumes of the colloids and the larger volume of RA was chosen with the objective to give equal plasma volume expansion. The lower volume of RA was used for comparison based on previous studies (Peng et al 2013, Haywood-Watson et al 2011).

Plasma volume and transcapillary escape rate

In paper III plasma volume was determined by a dilution technique. A bolus of a known amount of radioactively labelled albumin was injected and after 5 min equilibration the radioactivity of a withdrawn sample was measured from which the distribution volume can be deduced. By measuring the decline of labelled albumin in blood during one hour the transcapillary escape rate for albumin (TER) can be assessed (Bansch et al 2010).

Inhibition of antimicrobial activity

GAGs are known to modify the action of a multitude of substances. To assess their effect on the endogenous antimicrobial activity of plasma or individual antimicrobial peptides a radial diffusion assay was utilized (Lehrer et al 1991). Cavities were punched in agarose agar plates with small isolates of *Escherichia coli*. Plasma or antimicrobial peptides with or without GAG was pipetted into the cavities. Depending on the effective antibacterial activity of the pipetted sample a larger or smaller clear zone without bacterial growth can be seen after incubation and growth of the bacteria into visible colonies. The diameter of the zone is measured with a calliper.

Results and discussion

Median plasma level of sulphated GAG was two-fold increased in septic shock patients compared to neurosurgical controls and the median level was higher in the patients that did not survive (Fig. 1, Paper I). This initial observation formed the foundation for the present thesis and indicates that presence and/or distribution of GAGs as a group is altered in severe infection. The relation to mortality suggests that GAG could be valuable as a prognostic marker. Median plasma concentration of syndecan-1 was 10-fold increased compared to the controls (Fig. 2, Paper I) suggesting that the increase in circulating GAG, at least partly, originates from heparan sulphate containing proteoglycans released from the endothelial surface. Our observation of increased syndecan-1 levels in sepsis has since been confirmed by several investigators, supporting the accuracy of our data (Steppan et al 2011, Sallisalmi et al 2012). We also found that GAG, at concentrations found in some of the septic patients, at least in vitro inhibits the endogenous antimicrobial activity against *E. coli* (Fig 4, Paper I). It is possible that this contributes to an impaired clearance of microbes in the circulation.

In order to refine the results in paper I, analysis of the different GAG subtypes was performed. Median plasma levels of heparan sulphate and hyaluronic acid were 4-fold higher in septic shock patients than in controls while no significant change in the levels of chondroitin sulphate was detected (Fig. 1 and 2, paper II). Median plasma levels of keratan sulphate was slightly decreased (Fig. 1, paper II). GAG levels did not relate to the sequential organ failure assessment score (SOFA, Vincent et al 1998) of GAG metabolizing organs, liver and kidney (Table 3, paper II). These results in combination with the recognition that heparan sulphate and hyaluronic acid are the major GAG species on the endothelium, while chondroitin sulphate are normally found in the plasma, indicates that the altered GAG levels of the septic patients originate from spillage of the endothelial glycocalyx. A similar pattern of released GAG was recently found in patients with severe pneumonia (Schmidt et al 2014).

To study GAG and proteoglycan release in a standardized model and evaluate potential treatments, a cecal ligation and incision model of acute peritonitis in the rat was evaluated. No change in plasma levels or urinary excretion of sulphated GAG or heparan sulphate compared to sham was detected over the 4.5 h observation time (Fig. 3 and 4, Appendix). Previous studies have found increased levels of GAG and syndecan-1 in models of systemic inflammation (Hofmann-Kiefer et al 2009,

Hayashida et al 2008, Torres et al 2013, Bansch et al 2011, Wang et al 1997). Differences in study design including longer observation time after intervention might be responsible for the differences in results. Nonetheless, the fact that our CLI animals, having a survival of 50% after 6.5h (Scheiermann et al 2009), did not show any release of GAG or heparan sulphate to the circulation during the observation time points shows that a large release of glycocalyx components is not necessary for development of the septic phenotype.

It is recognized that trauma triggers a systemic inflammation (Lee et al 2007) and increased levels of syndecan-1 have been found in patients with severe trauma (Haywood-Watson et al 2011). In a model of severe haemorrhage in the rat, we observed an increase in circulating heparan sulphate and syndecan-1 levels after bleeding. The animals were then resuscitated with fresh frozen plasma, albumin or Ringer's acetate in volumes outlined previously. At the end of the experiment the plasma concentration of heparan sulphate was higher in animals resuscitated with Ringer's acetate compared to colloids (Fig. 4, paper III). After compensating for differences in plasma volume the difference was, however, markedly reduced and no longer statistically significant (Fig. 4, paper III). These results show that modification of conventional resuscitation therapy does not alter the release of glycocalyx components to the circulation. Furthermore, there were no difference in TER between resuscitated animals and SHAM (Fig. 5, paper III), indicating that the observed release of endothelial glycocalyx components was not sufficient for the animals to develop substantial changes in microvascular permeability. A recent study found no difference in permeability in hamsters subjected to enzymatic degradation of the glycocalyx (Landsverk et al 2012). There are, however, numerous data showing that glycocalyx degradation coincides with increased vascular permeability (Salmon & Satchell 2012, Bansch et al 2011, Peng et al 2013) but direct experimental support is lacking.

Several investigators have found increased levels of syndecan-1 among different isolated patients groups (sepsis, haemorrhage due to trauma, abdominal surgery, ischemia-reperfusion surgery) compared to control patients (Sallialmi et al 2012, Haywood-Watson et al 2011, Steppan et al 2011, Rehm et al 2007) while differences between groups of patients with different underlying causes to critical illness have not been studied. Furthermore, syndecan-1 is only one of several syndecans present on the endothelial surface and release of other proteoglycans than syndecan-1 is poorly explored. In 137 consecutive ICU patients with sepsis, trauma, gastrointestinal tract bleeding, cardiac arrest and intoxication, we found no statistically significant difference in median plasma level of syndecan-1, syndecan-2, syndecan-3 or syndecan-4 between the groups (Fig 2, paper IV). Syndecan-1 and syndecan-3 but not syndecan-2 or syndecan-4 levels were higher among the ICU patients compared to controls. These results show that syndecan-1 and syndecan-3 release is a general consequence of critical illness while the usefulness as markers of different underlying

conditions such as infection is poor. Median levels of all syndecans were different in ICU patients that died compared to survivors supporting a prognostic relevance of syndecan release. We found no relation between the concentrations of syndecan-1 and cardiovascular SOFA score, which shows that syndecan-1 cannot be used as a marker of vascular damage in clinical practice. A pathophysiological role of glycocalyx damage cannot be excluded based on the present results but routine measurement of syndecans in the circulation probably has limited value to the clinician.

Conclusions

The levels of glycosaminoglycans are higher in septic shock patients compared to controls and the increased levels are made out of heparan sulphate and hyaluronic acid, GAG subtypes present in high concentration on the endothelium.

A similar release of heparan sulphate is seen in an animal model of haemorrhagic shock but there are no detrimental effects on vascular permeability and heparan sulphate or syndecan-1 release is not affected by modification of conventional resuscitation therapy.

Release of glyocalyx components is not necessary for development of the septic phenotype in a rat abdominal sepsis model.

Syndecan-1 is a marker of critical illness but is not selective between underlying conditions.

Svensk sammanfattning

Blodförgiftning är en spridd och svår infektion och har en hög dödlighet. Skador på blodkärlen är en viktig del i sjukdomsutvecklingen. Glykosaminoglykaner (GAG) är negativt laddade kolhydrater som bland annat förekommer på blodkärlens insida och här utgör gränsskiktet mot blodbanan. På denna plats är GAG betydelsefullt för blodkärlens blodproppshämmande effekt, för transport av vita blodkroppar ut i vävnaden samt bidrar till blodkärlens täthet – funktioner som är gravt störda vid svår blodförgiftning.

Denna avhandling undersöker om GAG bidrar till blodförgiftning och om det har någon nytta att mäta dessa ämnen i blodet på sjuka patienter i syfte att optimera vården.

Vi kunde visa att halten GAG är högre i blodet på patienter med svår blodförgiftning än hos kontrollpatienter. De patienter som sedermera avled hade de högsta GAG koncentrationerna. Detta talar för att en högre halt av GAG i blodet kan vara ett tecken på svår sjukdom. I syfte att ytterligare belysa denna upptäckt analyserades olika typer av GAG patienternas blod. Koncentrationerna av heparan sulfat och hyaluronsyra var förhöjda. Eftersom detta är de typer av GAG som normalt återfinns i högst halt på blodkärlens yta så tror vi att patienterna med blodförgiftning får en ökning av GAG i blodet via en frisättning av GAG från kärnväggen och att det därför är möjligt att detta återspeglar kärlskada.

För att studera GAG-frisättning på ett mer kontrollerat och isolerat sätt än man kan göra på patienter så mätte vi även GAG i en djurmodell på råttor. Vi kunde då inte se någon ökning av GAG-nivån, troligen på grund av att försökstiden var för kort. Försöket visar trots detta att försöksdjuren kan utveckla ett tillstånd som liknar blodförgiftning utan att GAG-nivåerna stiger. Detta talar emot att GAG i blodet aktivt påverkar själva sjukdomsutvecklingen vid blodförgiftning.

Stora blodförluster medför liksom blodförgiftning en aktivering av immunförsvaret. I en djurmodell för stor blodförlust på råttor såg vi att heparansulfatnivån var ökad vilket talar för att djuren fått skador på blodkärlen. Vi testade om olika typer av vätskeersättning kunde lindra denna förmodade kärlskada men tyvärr hade de olika typerna av behandling likvärdig påverkan på GAG-frisättningen.

Eftersom vi också kunde se en GAG-ökning i blodet på djur som fått blodförlust så undrade vi om GAG-stegring förekommer även vid andra typer av svår sjukdom. Vi

utnyttjade då att GAG i regel är bundet till en proteinmolekyl och kallas tillsammans för en proteoglykan. Syndekaner är exempel på proteoglykaner som förekommer på blodkärlen. Analys visade att blodnivån av syndekan-1 är stegrat vid blodförgiftning men tyvärr även vid flera andra svåra sjukdomstillstånd. Det går därför inte att använda syndekan-1 som en indikator på blodförgiftning

Sammantaget visar avhandlingen att glykosaminoglykaner och syndekan, komponenter som normalt sett finns på blodkälens insida, ökar i koncentration i blodet vid svår sepsis men att den direkta kliniska nyttan av analys på intensivvårdspatienter sannolikt är låg.

Acknowledgements

I wish to express my sincere gratitude to those who have helped, supported and encouraged me throughout this study. To name a few:

My supervisor and dear friend *Mikael Bodelsson* for his support, advises and genuine interest in science. Thank you for always saying “test it” when I had a new idea.

Peter Bentzer, my co-supervisor, for his enthusiasm, scientific experience and ultraprompt email corresponance.

My dear *father* for introducing me to science. Hunting ticks on the football pitch for analysis of *Borrelia* is a happy memory that probably generated acquired immunity. Thank you for your continuous help throughout this study.

Ingrid Berkestedt for having some absolutely fantastic moments in the lab together and for being such an inspiring friend in sciences and everything else.

Johan Törnebrandt for enduring my mutterings, help, and fruitful discussions.

Thorgerdur Sigurdadottir for sharing her enthusiasm and her ever failing patience.

Co-workers at Grupp Bodelsson; *Louise Walther-Sturesson*, *Viveka Björck*, *Pia Andersson*, *Sigurbjörg Rutardottir*.

Helén Axelberg for precision hands and for being a joy to work with.

Artur Schmidtchen for introducing the original idea on GAG release.

Joakim Johansson for assistance with specimens.

Thanks to all co-workers at the BMC B14 lab corridor and the staff members of the laboratory of clinical chemistry in Helsingborg for fruitful discussions and help in various matters.

Above all these my sincere gratitude to *Ellinor*. I love you.

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