Targeting the vessel wall in cardiovascular prevention

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Chapter 1

The endothelial glycocalyx: a potential barrier between health and vascular disease

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ABSTRACT

Purpose of review: Although cardiovascular prevention has improved substantially, we still face the challenge of finding new targets to reduce the sequelae of atherosclerosis further. In this regard, optimizing the vasculoprotective effects of the vessel wall itself warrants intensive research. In particular, the endothelial glycocalyx, consisting of proteoglycans, glycoproteins and adsorbed plasma proteins, may play an essential role in protecting the vessel wall from atherosclerosis.

Recent developments: In this review, we will discuss the different vasculoprotective effects exerted by the endothelial glycocalyx, the factors that damage it, and the first preliminary data on the glycocalyx dimension in humans. Whereas most glycocalyx research has traditionally focused on the microvasculature, more recent data have underscored the importance of the glycocalyx in protecting the macrovasculature against pro-atherogenic insults. It has been shown that glycocalyx loss is accompanied by a wide array of unfavorable changes in both small and larger vessels. Pro-atherogenic stimuli increase the shedding of glycocalyx constituents into the circulation, contributing to the progressive loss of the vasculoprotective properties of the vessel wall. Novel techniques have facilitated reproducible measurements of systemic glycocalyx volume in humans. Consistent with experimental data, the volume of the human glycocalyx is also severely perturbed by exposure to atherogenic risk factors.

Summary: Cumulating evidence suggests that an intact glycocalyx protects the vessel wall, whereas disruption of the glycocalyx upon atherogenic stimuli increases vascular vulnerability for atherogenesis.

Keywords: atherosclerosis, endothelial glycocalyx, hyaluronan, thrombosis

Abbreviations: NO, nitric oxide; SOD, superoxide dismutase

INTRODUCTION

Cardiovascular disease is the major worldwide cause of mortality. Although a plethora of interventions has attempted to reduce the burden of cardiovascular disease, current strategies aimed at lowering systemic risk factors have only achieved a 20-30% reduction in the cardiovascular event rate (1). Therefore, novel strategies to improve cardiovascular outcomes are overdue.

Attention has recently shifted from treating systemic risk factors, such as hypercholesterolemia and hypertension, towards increasing the vasculoprotective properties of the vessel wall itself. As the endothelium constitutes the first-line defense against atherosclerosis, research has focused predominantly on strategies to improve endothelial function. Up to now, it has proved to be a major challenge to unravel the components of the anti-atherogenic arsenal of the vessel wall.

In recent years, it has been recognized that the endothelial glycocalyx may contribute to the vasculoprotective effects of the vessel wall. The glycocalyx is a negatively charged, organized mesh of membranous glycoproteins, proteoglycans (e.g. syndecan-1), glycosaminoglycans and associated plasma proteins. Hyaluronic acid and the negatively charged heparan sulphate proteoglycans are its major constituents. The glycocalyx is situated at the luminal side of all blood vessels (2). The volume of the glycocalyx depends on the balance between biosynthesis and the enzymatic or shear-dependent shedding of its components (3). Historically, this layer was thought to be confined to a thickness of only several nanometers. More recently, it has been demonstrated to reach up to 0.5-3 μm intraluminally (4). This dimension of the glycocalyx by far exceeds the size of the endothelium and adhering leukocyte adhesion molecules (Figure 1) and has triggered researchers to study more closely the role of this layer in atherogenesis (5).

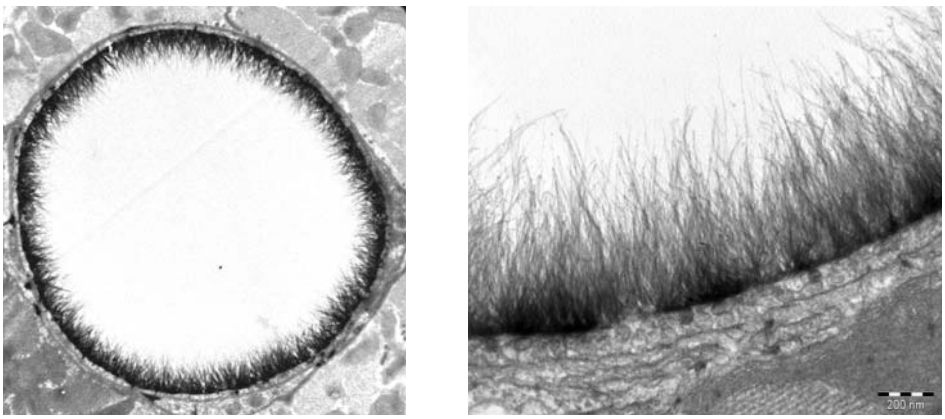

Figure 1. Electron microscopy image of the endothelial glycocalyx in a rat coronary capillary
The bush-like structure covering the endothelial cell is the glycocalyx.

In the present review, we will discuss the vasculoprotective effects exerted by the endothelial glycocalyx, the factors that damage it, and present some preliminary data on systemic glycocalyx measurements in humans.

Vascular permeability, nitric oxide release and the redox state

In recent years, several research groups have put forward the concept that the endothelial glycocalyx contributes to the vasculoprotective effects of the vessel wall. Numerous studies in both the micro and macrovasculature have demonstrated that constituents of the glycocalyx, such as hyaluronan, are involved in regulating nitric oxide (NO) release by serving as a mechano-shear sensor (6-8, 9••). This layer has also been shown to be involved in maintaining vascular permeability (10). In addition, the glycocalyx harbors a wide array of enzymes that might contribute to its vasculoprotective effect. Extracellular superoxide dismutase (SOD), an enzyme that converts oxygen radicals to hydrogen peroxide, is bound to heparan sulphate proteoglycans within the glycocalyx (11). Damage to the glycocalyx is accompanied by the increased shedding of extracellular SOD, which is probably related to the decreased availability of heparan sulphate binding sites. The latter shifts balance towards a pro-oxidant state (12). Collectively, these observations are of particular interest because altered vascular permeability, attenuated NO bioavailability and redox dysregulation are among the earliest characteristics of atherogenesis (13).

Endothelial glycocalyx disruption and coagulation

The endothelium is intimately involved in the regulation of coagulation pathways, such as thrombin generation and the inhibition of fibrinolysis (14, 15•). Under physiological conditions, the generation of thrombin is carefully minimized by a wide array of coagulation inhibition factors, comprising antithrombin, the protein C system and tissue factor pathway inhibitor, all located within the endothelial glycocalyx layer (16). It has recently been shown that specific disruption of the glycocalyx results in thrombin generation as well as platelet adhesion within a few minutes (17). Furthermore, various procoagulant stimulants such as pro-inflammatory cytokines have profound effects on glycocalyx compound synthesis (e.g. heparan sulphates and hyaluronan) and are thus likely to contribute to the impaired availability of the main anticoagulatory systems (18, 19).

Glycocalyx as a barrier for leukocyte adhesion

Exposure of adhesion molecules on endothelial cells and subsequent leukocyte rolling, tethering and transmigration are critical events in the course of atherogenesis (13). As the dimension of the glycocalyx by far exceeds that of adhesion molecules (i.e. intercellular adhesion molecule 1, P and L-selectin), the glycocalyx is likely to serve as a barrier for leukocyte adhesion (20). Inflammation and ischemia-induced shedding of the endothelial glycocalyx has been suggested to be an essential component in the inflammatory response of the

vasculature (21•). In line with this, restoration of the glycocalyx upon infusion of glycocalyx constituents has been shown to attenuate leukocyte rolling on the endothelial surface during pro-atherogenic/inflammatory stimuli (22). Collectively, these data underscore the relevance of an intact glycocalyx in preventing leukocyte adhesion to the vessel wall.

Glycocalyx in micro- versus macrovasculature

In spite of these observations, it has proved difficult to show the direct relevance of the glycocalyx as a vasculoprotective barrier in larger vessels. Whereas glycocalyx research has traditionally focused on the microvasculature, atherosclerosis does not occur within microvessels. Several studies have, however, emphasized that the relevance of the glycocalyx is not confined to smaller vessels (23). The glycocalyx has thus recently been visualized in large arteries in different animal models (5, 24, 25). The glycocalyx in larger vessels (i.e. arterioles) has also been shown to decrease the extravasation of LDL particles into the subendothelial space (26, 27). These data therefore imply that the glycocalyx could add to the vasculoprotective properties of the vessel wall in the microvasculature as well as the macrovasculature (Figure 2).

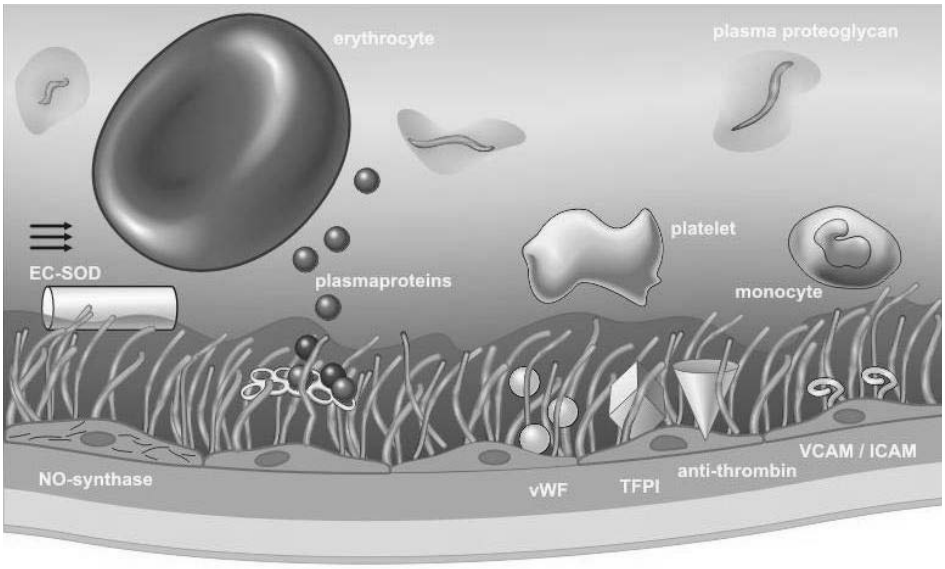

Figure 2. Schematic representation of the glycocalyx

Figure 2a. Physiological role of the glycocalyx

Endothelial glycocalyx regulates nitric oxide synthase activity, harbors superoxide dismutase, serves as a physical barrier for macromolecules, including plasma proteins and lipoproteins. In addition, the glycocalyx attenuates platelet as well as leukocyte adhesion.

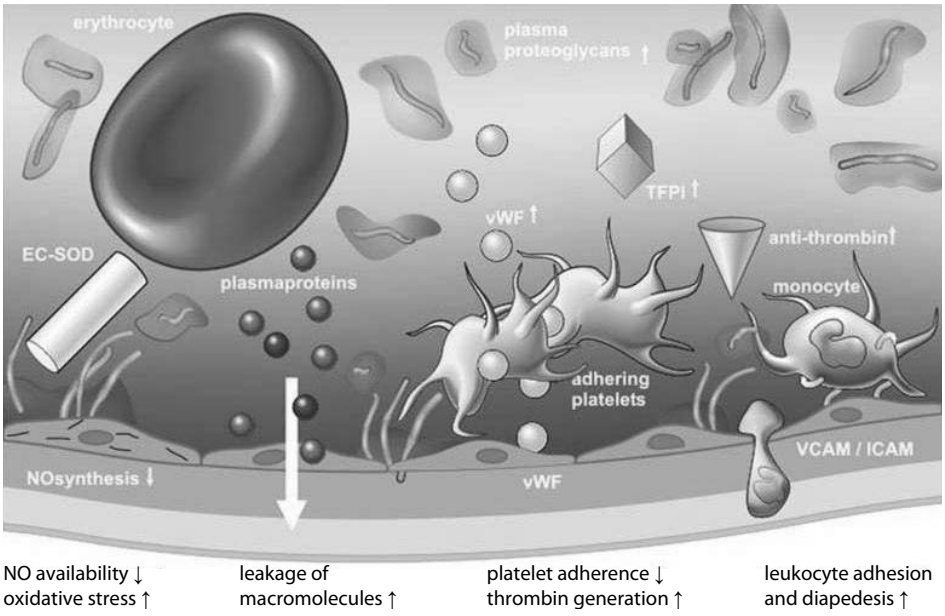

Figure 2b. Consequences of glycocalyx perturbation

Glycocalyx perturbation results in a pro-atherogenic state, characterized by endothelial dysfunction, increased vascular permeability, as well as the activation of coagulation and cellular adhesion/migration.

Glycocalyx disrupted by atherogenic stimuli

Many experimental findings underline the importance of an intact endothelial glycocalyx. Disruption of the glycocalyx can be caused by numerous factors. For example, inflammation and ischemia can induce shedding of the glycocalyx (19, 21•). Interestingly, the thickness of the glycocalyx is decreased at high compared with low-risk regions of the murine carotid artery supporting a potential role of glycocalyx disruption in rendering disturbed flows regions more susceptible to atherogenesis (25). In addition, exposure of endothelial cells to oxidized LDL in vitro decreases the amount of heparan sulphate proteoglycans associated with the luminal cell surface (28). Similarly, the infusion of oxidized LDL reduced the endothelial glycocalyx thickness in an animal model (17).

Glycocalyx volume assessment in humans

To date, direct visualization of endothelial glycocalyx in humans has been unsuccessful, mainly because the endothelial glycocalyx is a very delicate structure depending critically on the presence of flowing plasma (2). As a consequence, the best way to measure the endothelial glycocalyx in humans is to compare intravascular volumes using a glycocalyx permeable versus a glycocalyx impermeable tracer, thus providing an estimate of the glycocalyx volume

upon subtraction of these two volumes (29). At present, we found that in patients with type 1 diabetes, who are characterized by disturbances in proteoglycan synthesis (30,_31), systemic glycocalyx was profoundly reduced compared with healthy age and sex-matched controls (32). Other risk groups are currently being evaluated.

CONCLUSION

Evidence in experimental models has revealed that the glycocalyx exerts a wide array of anti-atherogenic effects, which include acting as a vascular permeability barrier, mediating NO release, and inhibiting coagulation as well as leukocyte and platelet adhesion. In line with this, glycocalyx disruption is accompanied by enhanced sensitivity of the vasculature towards atherogenic stimuli, and glycocalyx restoration can (at least partly) reverse this increased vulnerability. Data on glycocalyx changes in humans and its potential consequences for atherogenesis are, as yet, scarce. Novel options to estimate glycocalyx volume in vivo will allow us to address these issues in patient groups. It will be a challenge to determine the validity of systemic glycocalyx measurement as a marker of cardiovascular disease, as well as to assess whether and to what extent interventions aimed at glycocalyx restoration have the capacity to modulate the atherogenic vulnerability of the vasculature.

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