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Development of the damaged glycocalyx hypothesis - A review

AJ Drake-Holland[1] and MIM Noble[2]*

[1]Robert Gordon University, Aberdeen, UK

[2]University of Aberdeen, UK

Abstract

We have reviewed the literature from 2008 up to 2018 that is relevant to our 2008 postulate that arterial glycocalyx dysfunction is the first step in the atherothombotic process. It would appear from this survey that the hypothesis is still a reasonable one. Vascular glycocalyx dysfunction has been shown to alter fluid exchange with tissue and is involved in oedema and iflammation formation, leading to revised equations for the Starling principle. Interactions between anticoagulant factors bound to the vascular glycocalyx have been postulated to protect vessels from intravascular thombosis. The glycocalyx, according to all authors that we have reviewd, is negatively charged, but we found no measurement of glycocalyx electrical potential. This negativity is suggested as a mechanism for repulsion of adhesion molecules. We suggest that future work should focus on the electrophysiology, e.g., endothelial cells are highly negatively charged, as are leukocytes and platelets. We postulate that electrostatic repulsion of like charges may play an important role in the protective function of the vascular glycocalyx, and its failure during dysfunction may contribute to the initiation of arterial disease.

Introduction

In 2008, inspired by the work on vascular glycocalyx by Vink, we collaborated with him to postulate that arterial glycocalyx dysfunction is the first step in the atherothombotic process [1]. Our previous experience of the glycocalyx had been the finding that high luminal blood glucose concentration in an artery inhibited shear stress induced arterial dilatation for which the endothelial glycocalyx acts as mechanotransducer [2]. As this function is mediated by nitric oxide (NO) release, we reasoned that its inhibition by hyperglycemia might lead to the reduced protection of arteries by the NO and contribute to the predilection to arterial disease in diabetes mellitus. The result [2] also provided an explanation for the predilection for the disease to be sited in those parts of the arterial tree with low shear stress [3]. That the vascular glycocalyx (delineated by Vink & Duling [4]) was necessary for shear stress induced arterial dilatation had been established by the fact of its abolition by hyaluronidase, hyaluron being a vital component of the gel [5]. The endothelial glycocalyx is a gel including a network of membrane-bound proteoglycans and glycoproteins, covering the endothelium luminally. Both endothelium- and plasma-derived soluble molecules integrate into this gel mesh. Insight has been gained into the role of the glycocalyx in vascular physiology and pathology, including mechanotransduction, hemostasis, signaling, and interactions between blood cells and the vessel wall. There had also been hints of a possible role of degradation of the glycocalyx by oxidized low density lipoprotein [6, 7], and hyperglycemia [8]. There have also been postulates that endothelial cell glycocalyx modulates immobilization of leukocytes at the endothelial surface [9] and that the glycocalyx had vasculoprotective properties [10]. The purpose of the present review is to indicate evidence since 2008 that is relevant to the hypothesis that arterial glycocalyx dysfunction is the first step in the atherothombotic process [1].

Vascular permeability aspects

The discovery of the vascular glycocalyx has led to a modification of the Starling principle [11] governing the balance between opposing oncotic and hydrostatic pressures. This is determined particularly by the properties of the vascular barrier. Endothelial glycocalyx, located with a thickness of at least 200 nm on the luminal side of healthy vasculature, plays a vital role in vascular permeability by constituting the vascular barrier together with the endothelial cells themselves [12]. Experiments confirm that the effect of plasma and interstitial fluid colloid osmotic pressure on micro vascular fluid exchange is much less than predicted by the conventional Starling principle, and is in agreement with modern models [13]. A more recent proposal is that in arterioles and capillaries, there is very little fluid and colloid extravasation due to the endothelial surface layer formed by the glycocalyx and albumin [14]. Woodcock & Woodcock [15] found that a revised equation for the Starling principle led to improved composition of resucitation fluid, of which the main feature was the inclusion of albumen.

Biochemical and molecular aspects

Albumen and heperan sulphate in the glycocalyx are negatively charged and repel adhesion molecules such as th platelet-endothelial-cell-adhesion molecule (PECAM), the vascular cell adhesion molecule (VCAM) and the intercellular adhesion molecule (ICAM). Thrombosis within vessels can be a side effect of the mechanisms to inhibit bleeding from wounds by the classic coagulation cascade and through platelet action. The glycocalyx has an important function in that it protects

Correspondence to: MIM Noble, Department of Medicine and Therapeutics, Polwarth Building, Foresterhill, Aberdeen AB25 2ZH, UK. E-mail: mimnoble@mac.com

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vessels from intravascular thrombosis. Antithrombin III, an inhibitor of thrombin and activated factors IX and X, bind to the glycocalyx. Heparan sulphate and heparin cofactor II are present in the glycocalyx. This anticoagulant action is reinforced by the endothelial cells which produce thrombomodulin and chondroitin sulphate, which interacts with thrombin to activate the protein C. The tissue factor pathway inhibitor binds via heparin sulphates to inhibit factors VIIa and Xa [16].

Electrophysiological aspects

The Bio-electric Law [17] has highlighted the hitherto neglected role of electrons in physiology. All living cells have a negative trans-membrane potential due to an excess of negative charge carried by electrons generated by mitochondria during oxidative phosphorylation. In the polarised endothelial cell, the trans membrane potential is approximately -80mV [18], and although depolarisation has been studied using high extracellular potassium ion concentrations, there do not seem to be any measurements of depolarisation during more physiological stimuli such as increased shear stress. This would appear to be an area requiring research focusing. Also, although all authors agree that the glycocalyx is negatively charged, there appear to be no measurements of its electrical potential.

The importance of this negative charge follows from the fact that the blood cells that are potentially damaging to the endothelium and the platelets and leukocytes. The membrane potential of platelets in physiological buffer was estimated to be -52 to -60 mV [18]. Estimates of membrane potential in white blood cells vary from -45 to -86mV [19]. These negatively charged cells are presumably subject to electrostatic repulsion with respect to the negatively charged glycocalyx (electrical potential unknown) and the endothelial cell with trans membrane potential of -80mV, when polarized at extracellular potassium ion (K^+) concentration of 4mM [20]. This could explain, at least in part, the modulation of immobilization of leukocytes at the endothelial surface [21]. Another apparently neglected area of research is related to the lack of measurements of possible depolarisation of endothelial cells during physiological events such as shear stress induced vasodilatation. Depolarisation, according to the Bio-electric theory is due to electron outflow from the cell. If so, it would be likely that electron gain by the glycocalyx would result, contributing to glycocalyx electrical negative charge.

Degradation of the glycocalyx

Some pathological conditions have been associated with glycocalyx degradation. The effect of diabetes mellitus has been the most explored of these conditions, in which endothelial glycocalyx damage coincides with microalbinuria in type 1 diabetes [22-24]. The loss of glycocalyx during hyperglycaemia can cause intravascular coagulation [25]. Microalbinuria may be a sign of general loss of control of permeability [26]; endothelial damage occurs in diabetic kidney disease [27]. This is consistent with the claim that endothelial glycocalyx protects against myocardial edema [28]. The possibility of glycocalyx degradation in other pathological *in vitro* and *in vivo* models and patients are underging or require exploration.

In of hepatic ischaemia and reperfusion injury, glycocalyx degradation has been postulated to follow from the effect of endothelial glycocalyx degredation by reactive species [29]. In the case of the inflammation, protein C depletion and fibrinolysis in trauma patients, high levels of syndican-1 are found, which is marker endothelial glycocalyx degradation [30]. Patients with severe injury and early traumatic coagulopathy display endothelial glycocalyx degradation, which induces endogenous heparinisation [31]. Even erythrocytes with a transmural potential of about -10mV [32], which normally stream clear of the glycocalyx can penetrate into the glycocalyx during impaired microvascular perfusion [33]

Evidence compatible with glycocalyx dysfuncion as the first step in the initiation of vascular disease.

Shredding of the glycocalyx appears as an essential initial step in the pathophysiology of atherosclerosis and microangiopathic complications of diabetes mellitus, as well as in chronic venous disease [34, 35]. Atherosclerosis risk factors are hypercholesterolemia low density lipoprotein (LDL), hyperglycemia, inflammation, and altered low shear stress, all of which can damage glycocalyx [36]. This may allow LDL and leukocytes to penetrate to the subendothelial space initiating inflammation [37] and atheroma plaque formation [34]. Leukocytes may also migrate into the venous wall and initiate inflammation leading to morphologic and functional venous changes of the chronic venous disease. It has been claimed that treatment with sulodexide (a glycosaminoglycan) improves peripheral arterial obstructive disease and diabetic nephropathy with albuminuria [34]. Physicians are now considering early treatment of vascular diseaase by targeting treatment to the glycocalyx [38], e.g., sulodexide[34]. Not only drugs but normalisation of high shear stress is being considered as therapy [36].

Conclusion

It appears that the original hypothesis that arterial glycocalyx dysfunction is the first step in the atherothrombotic process is still to be considered as reasonable. The latest expression of this appears from Zhanga et al [39]. As far as fundamental understanding of the underlying mechanisms of vascular endothelial glycocalyx physiology and pathophysiology are concerned, the main recommendations for future research ought to be the answering of the many unanswered questions on the electrostatic and electrophysiological aspects.

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