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Impaired glycocalyx barrier properties and increased capillary tube haematocrit

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A healthy vascular endothelium is coated by a variety of transmembrane and membrane-bound molecules which, together, constitute the endothelial glycocalyx. The principal proteins on the endothelial cell surface are syndecans and glypicans, both equipped with large numbers of side chains composed of heparan and chondroitin sulphates. Together with bound and intercalated plasma proteins and hyaluronan the glycocalyx forms the endothelial surface layer with a thickness of over 1 μm, an exclusion zone for erythrocytes and labelled macromolecules (Pries & Kuebler, 2006).

Recent estimations, which are based on the exclusion of circulating red blood cells by the glycocalyx domain, as originally observed in intravital microscopy studies, suggest that the glycocalyx may occupy over 1000 ml of the systemic vasculature in healthy human subjects (Nieuwdorp et al. 2006). This amount of non-circulating plasma, confirmed to be about 800 ml by direct blood volume measurements (Rehm et al. 2001), allows the glycocalyx to reduce the functionally perfused capillary volume. Thus, the glycocalyx influences microvascular resistance and contributes to the low tube haematocrit (i.e. 20–50% of systemic values) that is found in capillaries.

In a recent issue of The Journal of Physiology, VanTeeffelen and coworkers determined the potential of vasodilators to modulate the extension and penetrability of the glycocalyx. Using the endothelium-dependent bradykinin (10^{-5} M) and the endothelium-independent sodium nitroprusside (10^{-6} M) to increase capillary perfusion, they evaluated the effects on both capillary tube haematocrit and exclusion properties of the glycocalyx (VanTeeffelen et al. 2008). Both vasodilators were found to substantially increase tube haematocrit in capillaries of mouse cremaster muscle and to decrease the 0.37 μm exclusion zone, representing the glycocalyx, for the 70 kDa colloid dextran by over 50%. The authors logically inferred that these effects were due to a modulation in glycocalyx volume, all the more so, since no such actions of bradykinin and nitroprusside were found in hyperlipidaemic ApoE3-Leiden mice, presumed to have a perturbed glycocalyx to begin with.

These very elegant in vivo studies on microvessels of the exteriorized cremaster muscle show that something besides mere vasodilatation is being incurred by two familiar vasoactive substances, independent of whether they induce flow increase predominantly via the endothelium or not. Obviously, an issue of paramount interest is how (at least some) vasoactive agents rapidly and apparently reversibly alter the dimensions of the endothelial glycocalyx. What the authors were unable to study was whether the change in dimension and – possibly – integrity of the glycocalyx has implications for vascular hydraulic conductance.

In studies on the intact coronary bed of isolated guinea pig hearts we have been able to demonstrate that another vasoactive substance, namely atrial natriuretic peptide (ANP), initiates degradation of the glycocalyx with concomitant increases in vascular leak and colloid permeability (Bruegger et al. 2005). Pertinently, not only ANP but also SNP (sodium nitroprusside) leads to increased transudate formation in such heart preparations, but not so the prostacyclin mimetic iloprost. Transudate, fluid appearing on the epicardial surface of the isolated hearts, is a direct measure of net fluid filtration in the intact coronary system and, thus, of hydraulic conductivity. These effects on coronary leak were clearly independent of coronary flow: on the one hand, ANP caused no coronary dilatation whatsoever (Bruegger et al. 2005), whereas, on the other hand, SNP and iloprost elevated coronary flow. A possible interpretation of these results could be that redistribution of coronary flow enhances the permeability surface area or leads to perfusion of zones of higher hydraulic conductance in the coronary bed without there being any real change in hydraulic conductance at the original sites. However, such a perfectly balanced redistribution is difficult to imagine and there is still the discrepancy between SNP and iloprost.

A feature common to both ANP and SNP and a distinction to iloprost concerns the generation of cyclic GMP in endothelial cells. In fact, coronary venous release of cGMP in hearts treated with ANP and SNP have previously showed an identical linear and highly significant correlation with the increase in transudate flow (Becker et al. 1993). Therefore, signalling events within endothelial cells linked to the generation of cGMP, be it by the action of particulate or soluble guanylyl cyclase, could be responsible. How such mechanisms may rapidly influence hydraulic conductivity and permeability of the vascular bed has gained some attention in the past. VASP, calpains and aquaporins are candidates currently being pursued, to name just a few. However, changing the structure or composition of the endothelial glycocalyx has not been linked to any of these.

In contrast to the experiments of VanTeeffelen and coworkers ANP has previously been applied directly into the coronary system and not superficially (Bruegger et al. 2005). The effects of ANP and SNP on hydraulic conductivity had not disappeared completely by 20 min of wash-out, though barrier function did show considerable recovery. Thus, the reversable increase in capillary tube haematocrit noted in the present paper of VanTeeffelen and coworkers may well result from a perturbation of the endothelial glycocalyx allowing extravasation of intravascular fluid in their particular preparation (VanTeeffelen et al. 2008), just as in the intact coronary bed. Since more extravasation will occur at a given perfusion pressure the slower the blood flow, such a mechanism complies with the inverse relationship found between capillary tube haematocrit and red blood cell velocity in the cremaster studies. The authors discuss causal involvement of nitric oxide in the mediation of the effects of bradykinin and nitroprusside. We would like to extend this appealing concept

to generally encompass mechanisms mediated by the second messenger cGMP. Indeed, infusion of a membrane-permeable analogue of cGMP evoked a 20% increase in hydraulic conductivity in the isolated heart preparation (Becker *et al.* 1993). Iloprost, in contrast, causes coronary dilatation mainly via generation of cAMP, a signalling pathway presumed to reduce capillary permeability.

Previous intravital microscopy studies have showed that capillary tube haematocrit may increase during agonist and metabolic stimulation, suggesting that a reduction in glycocalyx exclusion was involved (Klitzman & Duling, 1979). Whereas tube haematocrit increased fourfold after degradation of the glycocalyx initiated by adenosine or muscle activity, no significant changes were seen with these interventions after pretreatment with heparinase. Heparinase is known to deteriorate the glycocalyx and had already elevated capillary tube haematocrit.

Degradation of the glycocalyx in cremaster capillaries has been shown after intravenous bolus administration of oxidized lipoproteins. In this study VanTeffeelen *et al.* used hyperlipidaemic ApoE3-Leiden mice as an experimental model of atherogenic degradation of the glycocalyx. Interestingly, they observed both an impairment of vascular barrier properties in the vicinity of subendothelial lipid deposits and endothelial dysfunction, the latter evidenced by impaired arteriolar dilatation to bradykinin and to reactive hyperaemia. Since dilatation by such stimuli is normally augmented by shear-stress-induced generation of nitric oxide by the endothelium, mediated in turn by the endothelial glycocalyx, the poorer response complies fully with only a rudimentary glycocalyx in ApoE3-Leiden mice.

The majority of capillaries in these mice demonstrated reduced red blood cell (RBC) exclusion already under baseline conditions, presumably reflecting a degraded glycocalyx. In addition, this decrease was paralleled by a 30–35% increase in capillary tube haematocrit and an increase in RBC flux of 150–200% without a change in RBC velocity. Thus, the hyperlipidaemic conditions might have influenced microcirculatory perfusion characteristics by increasing plasma viscosity.

Glycocalyx perturbation has been demonstrated during hyperglycaemic, ischaemic and atherogenic conditions in patients and may contribute to dysregulation of microvascular perfusion. A new aspect concerns the effects on the glycocalyx of stimulators of endothelial production of cGMP.

Most puzzling, however, is how such rapid and at least partly reversible changes in barrier function of the glycocalyx come to pass, particularly if this should entail actual shedding of components of the glycocalyx. In our opinion, here lies the biggest challenge raised by the studies of VanTeeffelen and colleagues and our observations on the isolated coronary system. The time has clearly come to advance and test hypotheses as to which cell-signalling mechanisms facilitate receptor-mediated changes of a fast and reversible nature on the proteins coating the endothelial cell surface.

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