Angpt-2 mediates breakdown of the endothelial glycocalyx

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Introduction

Angiopoietin-2 (Angpt-2), an antagonist of the endothelium-stabilizing receptor Tie2 secreted by endothelial cells, promotes vascular permeability. We have previously shown that administration of Tie2 agonistic molecules, which counteract the devastating effects of Angpt-2, prevent ischemic and septic acute kidney injury (AKI). Angpt-2 probably mediates AKI through endothelial-cell contraction and junctional disintegration. Here we hypothesized that Angpt-2 might mediate the breakdown of the endothelial glycocalyx (eGC), a carbohydrate-rich vasoprotective layer lining the luminal surface of the endothelium, as well.

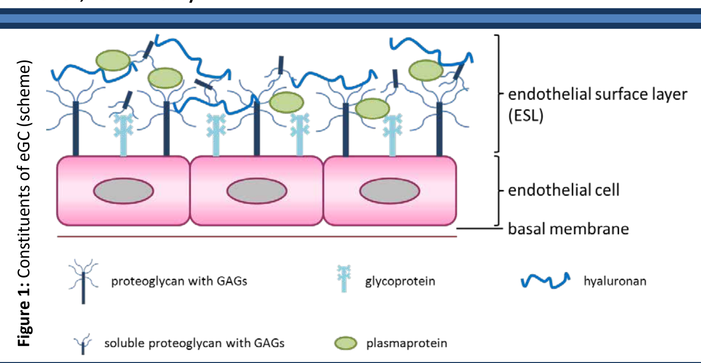


Figure 1: Constituents of eGC (scheme)

Methods

- Confocal and atomic force microscopy (figure 2) were used to visualize and analyze the thickness of the eGC on living endothelial cells.
- An i*n vivo* permeability assay was used to quantify vascular permeability in murine back skins (miles assay).

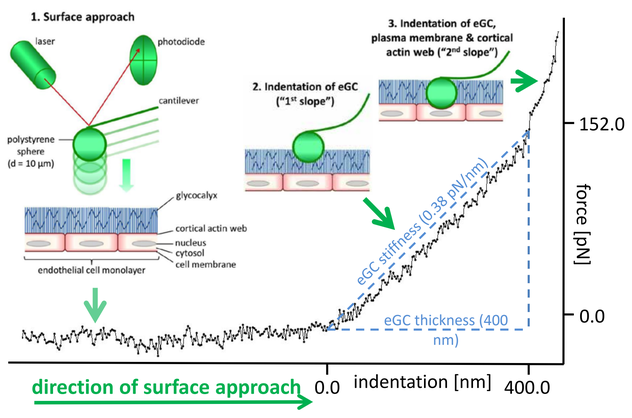
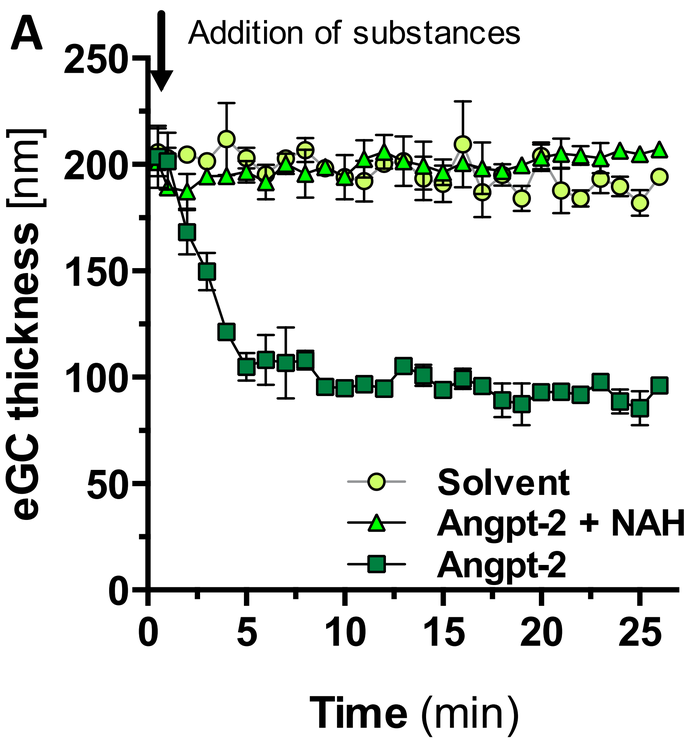
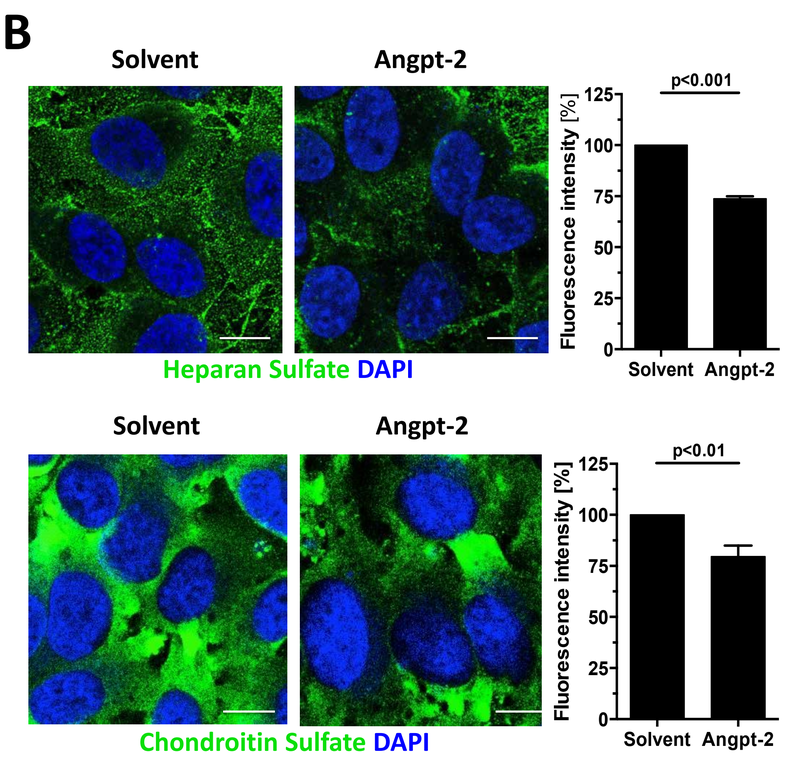


Figure 2: Original tracing of a force–distance curve performed on the endothelial surface of living endothelial cells *in vitro*. Using a mechanical nanosensor (cantilever), mounted on an atomic force microscope, height (nm) and stiffness (pN/nm) of the eGC on the luminal surface of endothelial cells were quantified.

Results





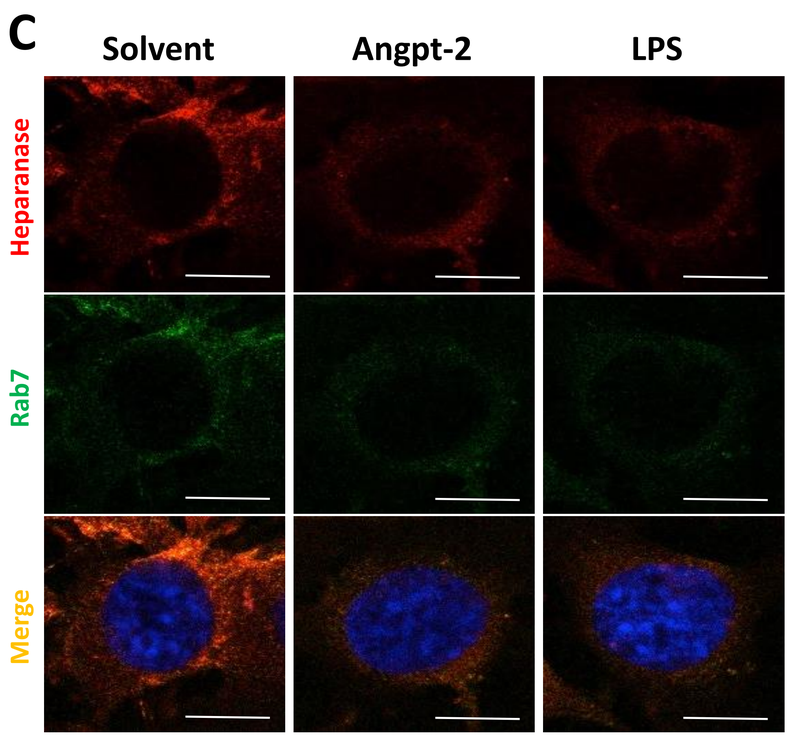
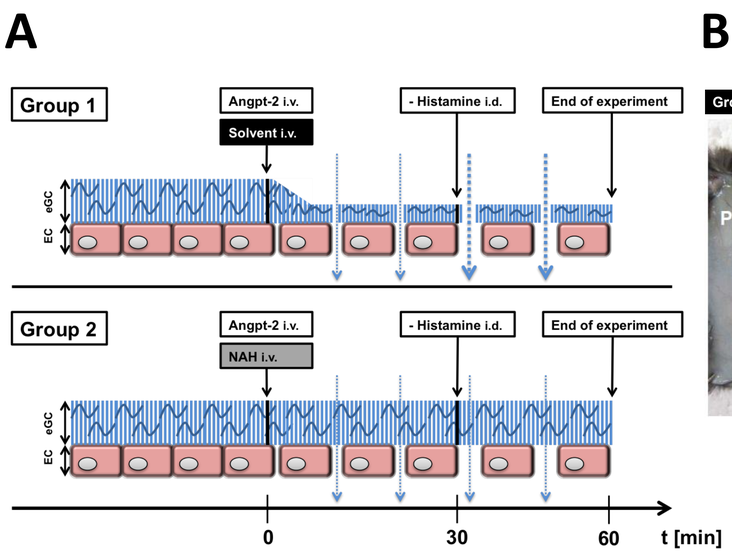
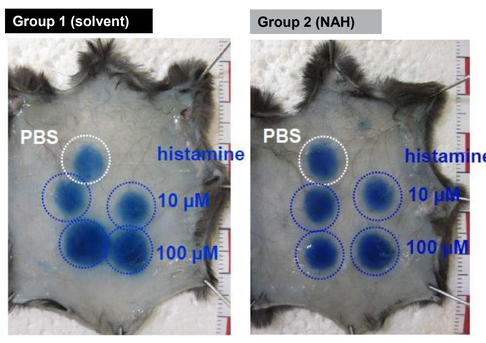
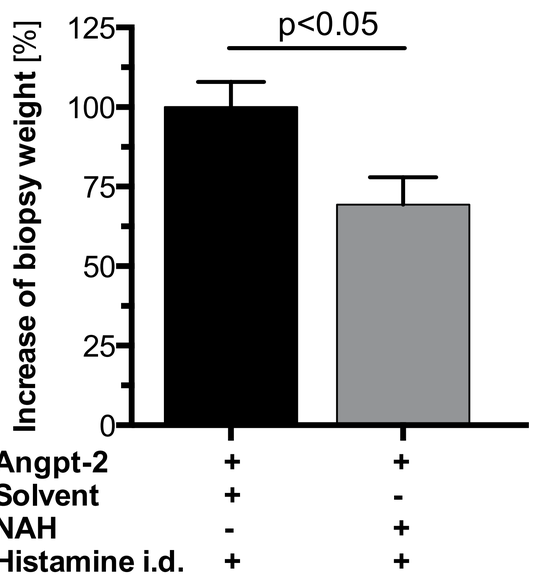


Figure 3: Angpt-2 mediates eGC breakdown *in vitro*. In AFM online experiments Angpt-2 alone induces a rapid loss of the eGC in endothelial cells *in vitro* in comparison to Solvent or Angpt-2 and NAH, a substrate of the eGC digesting enzyme heparanase (**A**). Glycocalyx deterioration involved the specific loss of its main constituents heparan sulfate and chondroitin sulfate (**B**) paralleled by the secretion of the heparan sulfate-specific glucuronidase heparanase from late endosomal/lysosomal stores (**C**).

In-vivo miles assay – edema formation and plasma leakage







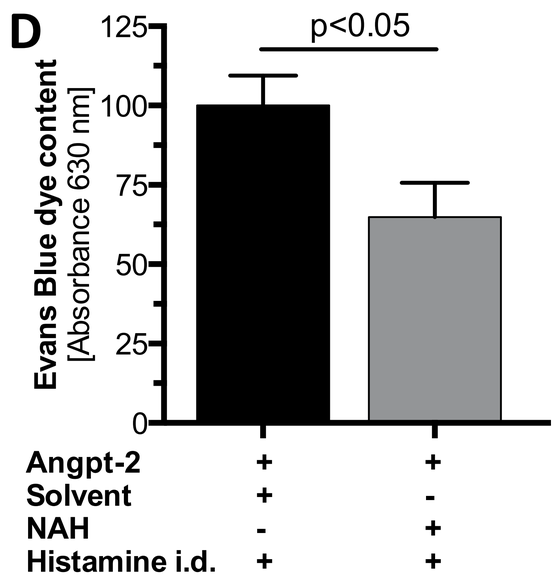


Figure 4: The principle of the *in vivo* miles assay is schematically shown in (**A**). Representative pictures of the murine back skin of both groups (left with solvent = control, right with NAH) are shown in (**B**). Miles assay revealed that exogenous Angpt-2 leads to heparanase-dependent eGC breakdown, which contributed to edema formation (**C**) and plasma leakage of large molecules (**D**)

Conclusion

- Breakdown of the endothelial glycocalyx is mediated by Angpt-2
- The Angpt/Tie2 ligand-receptor system is a concurrent gatekeeper of both layers of the vascular double barrier – the endothelial cell and the eGC
- The Tie2 axis might be a future treatment goal to protect the vascular double barrier to prevent vascular leakage in critical care nephrology

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