ENZYMATIC DIGESTION OF THE ENDOTHELIAL GLYCOCALYX IS REGULATED BY THE TIE2 RECEPTOR AND ITS LIGANDS ANGIOPOIETIN-1 AND -2

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Introduction

Angiopoietin-1 and -2, two antagonistic ligands of the endothelium-stabilizing **receptor Tie2**, regulate **vascular permeability** and leukocyte transmigration through the endothelium. We have previously shown that administration of Tie2 agonistic molecules acting like Angpt-1, which counteract the devastating effects of Angpt-2, prevent ischemic and septic acute kidney injury. This effect is probably mediated 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, and that Angpt-1 can prevent this process..

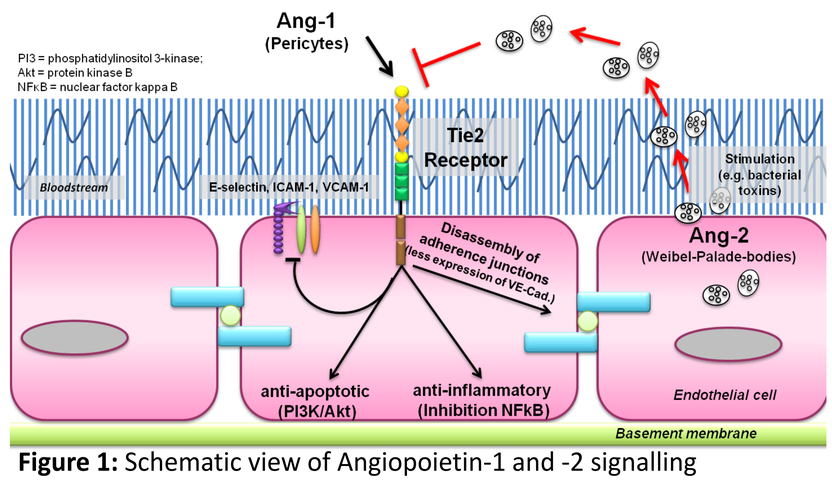

Figure 1: Schematic view of Angiopoietin-1 and -2 signalling

Methods

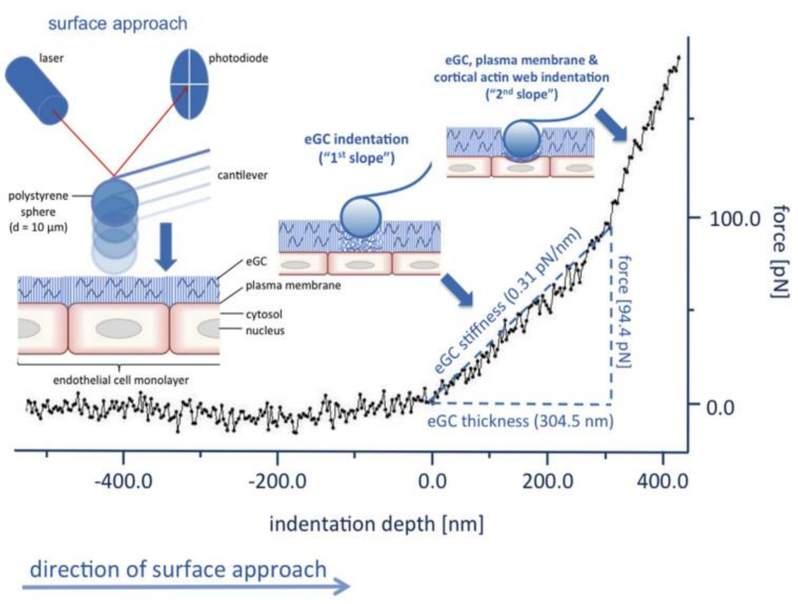

Figure 2: Atomic Force Microscopy (AFM)
The AFM tip (cantilever) travels vertically towards the endothelial surface and deflects upon contact to the endothelial glycocalyx (eGC). The deflection is measured as a laser beam reflected from the back of the cantilever. The resulting curve is transformed into a force-versus-distance curve where the slope directly reflects the stiffness (expressed in pN/nm). The first slope indicates the very first layer, the (eGC) and the second indicates the plasma membrane and the cortical actin web.

Figure 3: Immunofluorescence staining
Heparan sulfates (HS) were stained with a mouse monocloncal anti-HS antibody after Angpt-2 treatment.

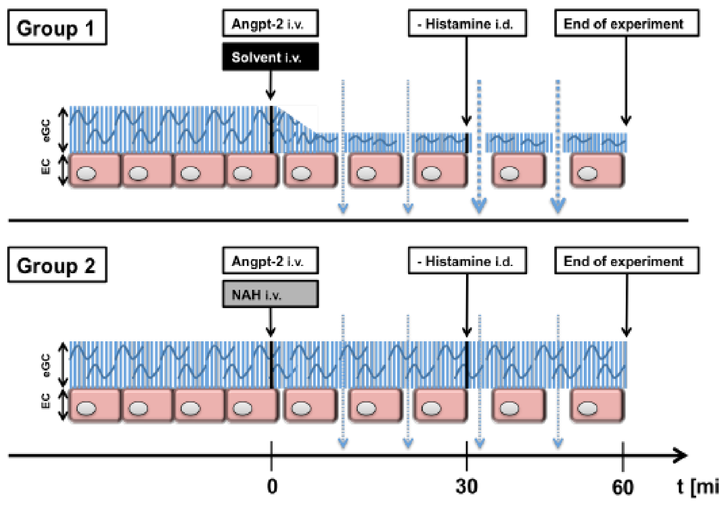

Figure 4: Schematic view of *in vivo* permeability assay (modified Miles assay)
Mice received Angpt-2 (65ng/g mouse), Evans Blue (150 µl, 0,5% in PBS) and 150 µg of the Heparanase inhibitor NAH or an equivalent volume of solvent (PBS) intravenously. Evans Blue facilitates visualization and quantification of vascular leakage.

Results

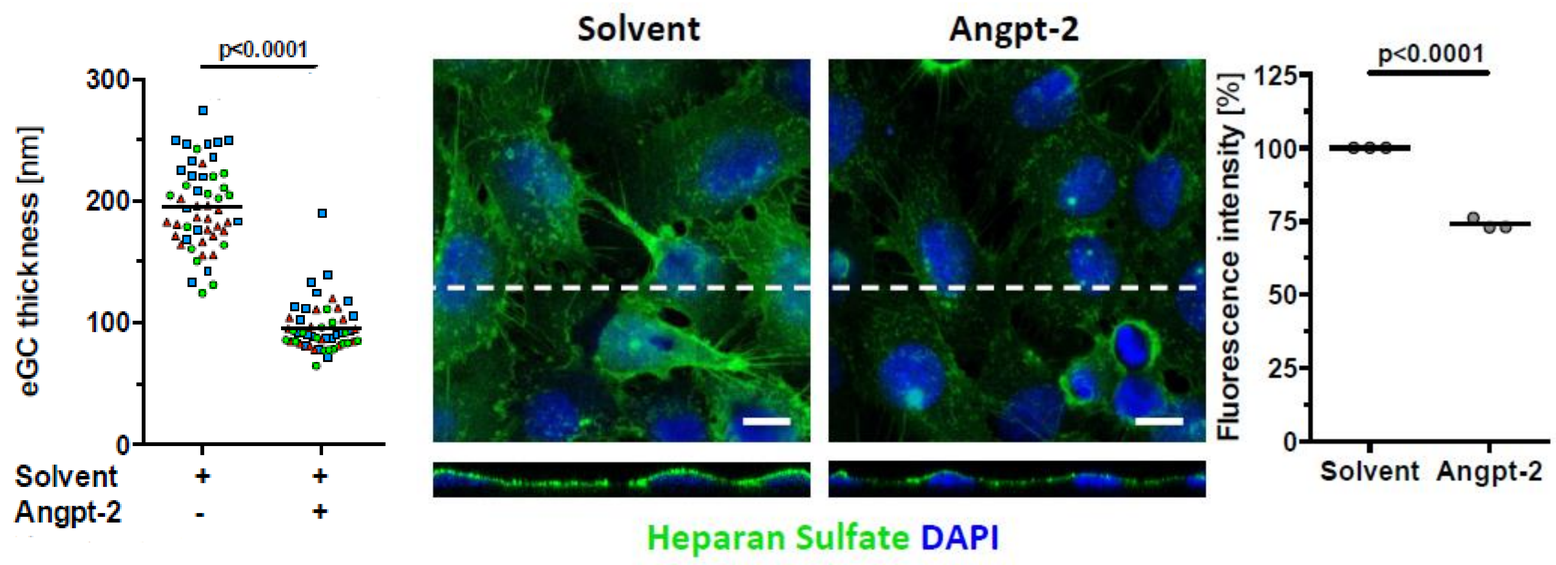

Figure 5: Angpt-2 is a negative regulator of the eGC *in vitro*
Glycocalyx thickness of living cells measured via AFM, immunofluorescence images and **fluorescence intensity** analysis of heparan sulfate (major constituent of eGC) staining after incubation with Angpt-2 (100 ng/ml)

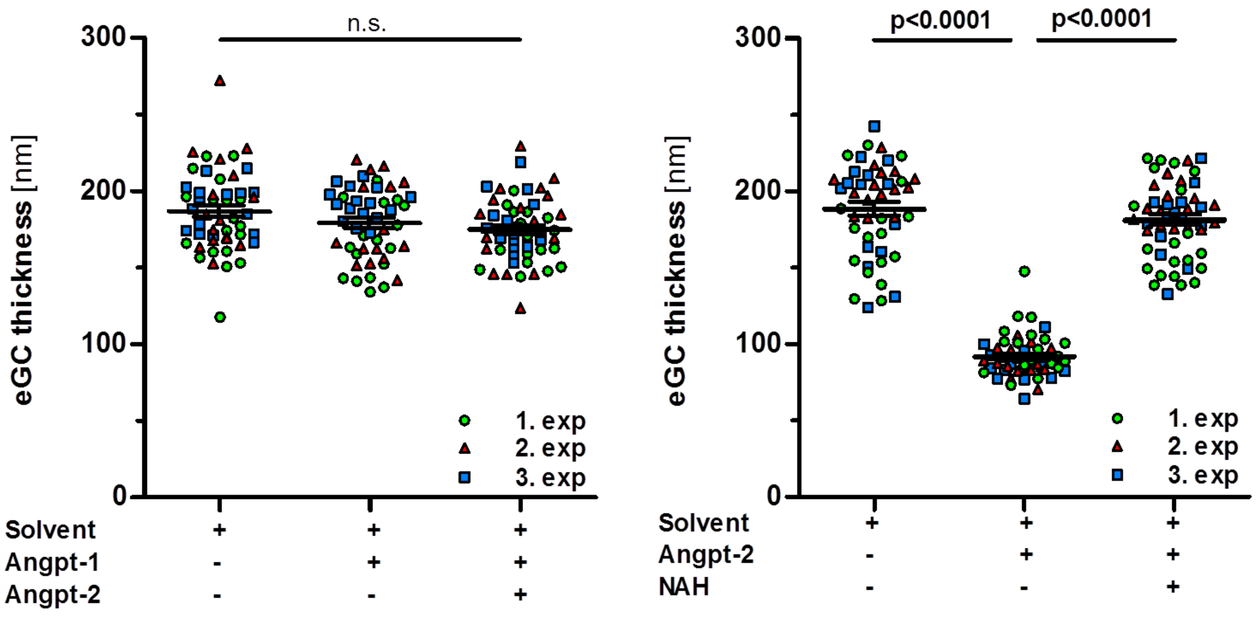

Figure 6: Angpt-2-mediated breakdown of eGC thickness depends on heparanase and is abolished via Angpt-1
Glycocalyx thickness of living cells were measured via AFM after incubation with Angpt-1 (400 ng/ml) and Angpt-2 (100 ng/ml); Angpt-2 and NAH (heparanase inhibitor; absolut 150 µg)

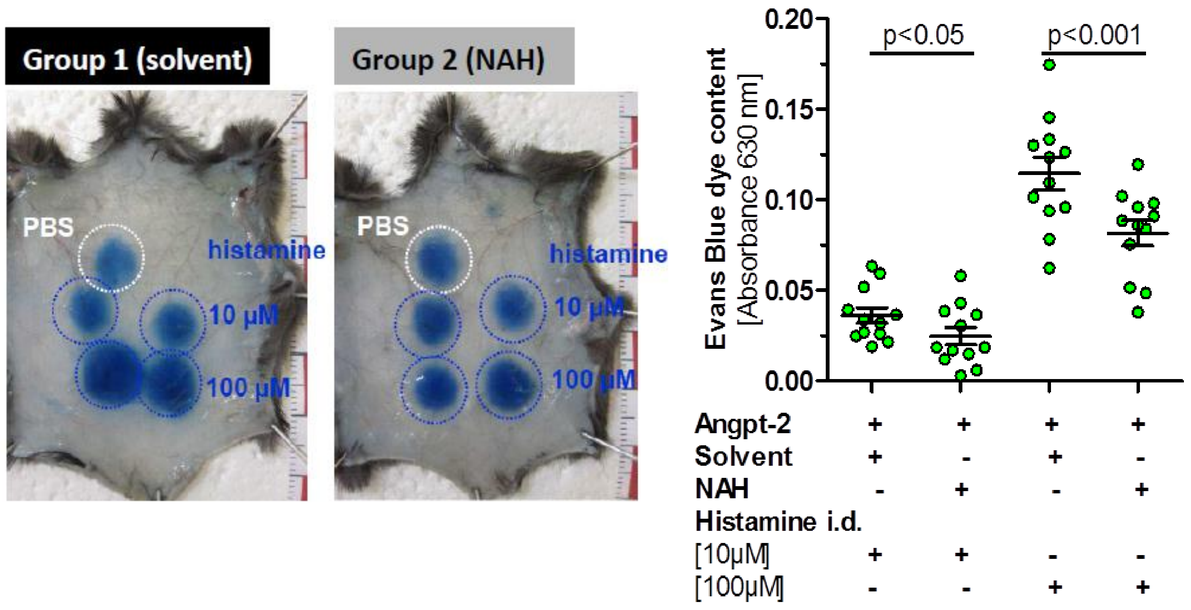

Figure 7: Heparanase inhibition with NAH prevents Angpt-2 mediated increase of permeability and oedema formation *in vivo*
Representative images of murine back skin with Evans Blue leakage at injection sites of PBS (control) or histamine (10 and 100 µM). Bars showing **Evans blue dye content** of back skin biopsies after mediator treatment.

Conclusion

→ **Angiopoietin-2 contributes to eGC breakdown via release of eGC-digesting heparanase**

→ **Angiopoietin-2 mediated eGC breakdown contributes to vascular leakage *in vivo***

→ **Protection of the eGC might become an important treatment goal to prevent vascular leakage in critical care nephrology**

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