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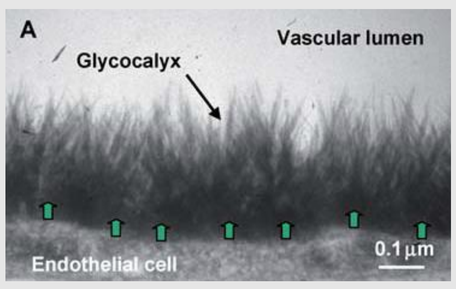
EXPLORING THE MECHANISMS LINKING URAEMIC TOXINS TO ENDOTHELIAL GLYCOCALYX DEGRADATION

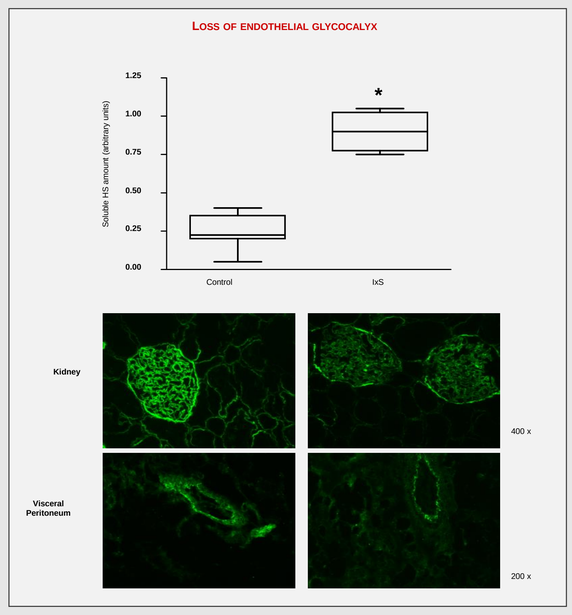
Introduction

A **damaged endothelial glycocalyx (eGC)** is critical in the development of cardiovascular disease.



Becker, Cardiovasc Res: 300-310, 2014

By using a rat model, our group linked the protein-bound **uraemic toxin indoxyl sulfate (IxS)** to endothelial glycocalyx disruption as indicated by **shedding of heparan sulfate** - one of its major constituents - into the circulation. In addition, strong leukocyte adhesion and a perturbed capillary blood flow were observed.



The amount of soluble heparan sulfate (HS) in the serum was significantly higher in the indoxyl sulfate (IxS) rats (n=6) versus the control rats (n=8) (P<0.01). Staining of HS in the glycocalyx was consistently less pronounced in the tissue exposed to IxS compared to control.

(Pletinck A, JASN 2013 (24): 1981-1994)

Objective

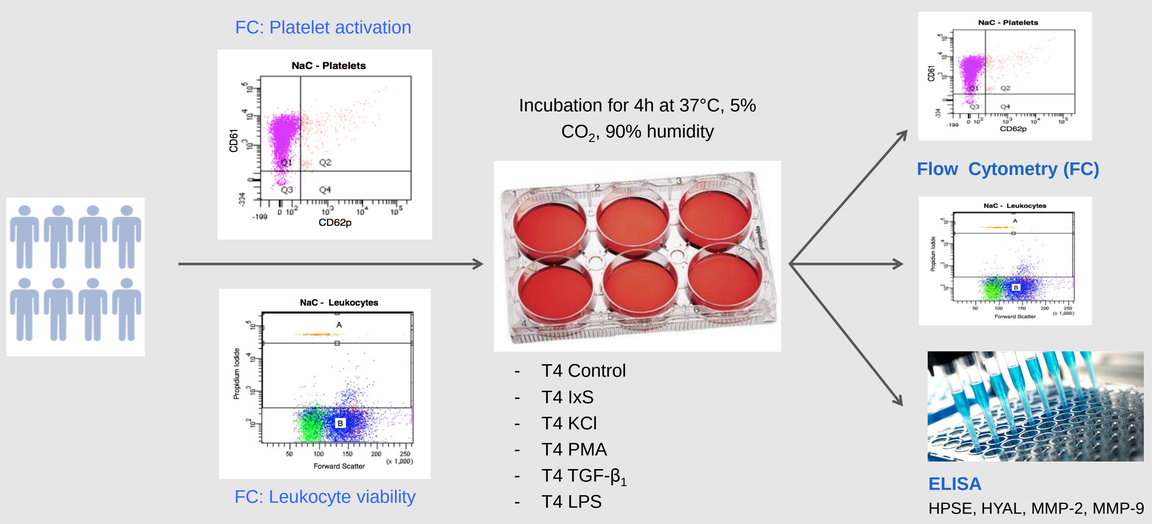
Does IxS induce expression of eGC degrading enzymes [heparanase (HPSE), hyaluronidase (HYAL), matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9)] **by healthy leukocytes and/or platelets?**

Methods

- **Incubation of sodium citrate (NaC)** blood* from 8 healthy donors for 4h with:
  - IxS (0,2096 mmol/L)
  - KCl (0,2096 mmol/L), a control for the salt in the assay

  Stimuli for the eGC degrading enzymes:
  - phorbol 12-myristate 13-acetate (PMA, 1.35 µM) for HPSE and HYAL
  - transforming growth factor-β1 (TGF-β1, 10 µg/mL) for MMP-2
  - lipopolysaccharide (LPS, 100 ng/mL) for MMP-9

  **To avoid platelet activation, the phlebotomy was performed with a 20G needle and the first tube was discarded.*



- **Flow Cytometry:** Evaluation of Cell Viability (propidium iodide exclusion assay) and platelet activation (CD62P labelling)
- The concentration of eGC degrading enzymes in the blood plasma was analysed with commercial available enzyme-linked immunosorbent assays (**ELISA**, LifeSpan BioSciences, USA for HPSE and HYAL; R&D Systems Europe, UK for MMP-2 and MMP-9).

Results

Results ELISAs				
		median	IQR	P-value vs control
HPSE (pg/ml)	T4 control	27,61	34,34	
	T4 IxS	42,71	45,86	0,075
	T4 PMA	42,19	34,65	0,075
HYAL (ng/ml)	T4 control	0,52	0,54	
	T4 IxS	0,96	0,77	0,67
	T4 PMA	3,94	5,83	0,01
MMP-9 (ng/ml)	T4 control	48,02	23,14	0,093
	T4 IxS	44,33	46,80	0,208
	T4 LPS	852,64	417,42	0,012
		mean	SD	P-value vs control
MMP-2 (ng/ml)	T4 control	217,60	32,66	
	T4 IxS	210,25	25,96	0,11
	T4 TGF-β1	216,25	23,17	0,83

- **IxS induced a 20% increase in heparanase (HPSE) expression**
- IxS caused a small decrease in the percentage of viable leukocytes, compared to the control after 4 hours (85.6 ± 9.4 vs 87.3 ± 9.2%; p=0.043)
- No effect of IxS on platelet activation
- No effect of IxS on aggregation of platelets with monocytes (16.5 ± 5.9 vs 14.3 ± 5.4 %; p=0.134), granulocytes (17.3 ± 6.9 vs 18.2 ± 8.4 %; p=0.401) or lymphocytes (15.9 ± 6.3 vs 17.3 ± 7.4 %; p=0.231)
- TGF-β_1 is no adequate stimulus for MMP-2 in this setting. FGF-2 will be tested as an alternative.

Conclusion

These results suggest that **indoxyl sulfate plays a role in degradation of the endothelial glycocalyx by increasing heparanase expression**. This could be one of the mechanisms by which indoxyl sulfate contributes to an increased risk of cardiovascular disease in chronic kidney disease patients.

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