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EXPLORING BINDING CHARACTERISTICS AND RELATED COMPETITION OF DIFFERENT PROTEIN-BOUND URAEMIC TOXINS

Introduction

Little is known about the possible difference in binding characteristics of **protein-bound uraemic toxins** (PBUTs) in patients with chronic kidney disease *versus* healthy controls [1-5] and whether this eventual difference can be attributed to (i) the **elevated levels** of competing uraemic toxins and/or (ii) **post-translational modifications** of albumin. Most previous binding studies used solutions containing commercially available human serum albumin and **lacked thus the uraemic conditions** [6-8]. Only a few studies discussed the competitive behaviour of PBUTs [1,9-11] and in most binding studies, no saturation of the binding sites was reached [1,5].

Methodology

A **binding curve** was constructed for hippuric acid (HA), indole-3-acetic acid (IAA), indoxyl sulphate (IS), and *p*-cresylsulphate (*p*CS) in 3 conditions: (i) healthy serum, (ii) blank haemodialysis (HD) serum, and (iii) non-treated HD serum. The mutual **binding competition** was studied in blank HD serum in pairs.

Equilibrium dialysis (37 °C, 5h) was used to separate the free fraction. Free (C_F) and total (C_T) PBUT concentrations were determined by UPLC-MS/MS and the albumin concentration (C_{Alb}) in each pool was determined by the bromocresol green method. The percentage protein binding (%PB) and binding coefficient (B) were calculated as follows:

$$\%PB = \left(1 - \frac{C_F}{C_T}\right) \qquad B = \frac{C_T - C_F}{C_{Alb}}$$

The one and two site binding models were fit to the experimental data B and C_F:

$$B = \frac{B_{max}\, C_F}{K_d + C_F} \quad \text{(one site)} \qquad B = \frac{B_{max1}\, C_F}{K_{d1} + C_F} + \frac{B_{max2}\, C_F}{K_{d2} + C_F} \quad \text{(two site)}$$

	Binding curves			Competition
	Healthy serum	Blank HD serum	HD serum	Blank HD serum
Proteins (g/L)				
Total protein	74.2	49.4	65.3	54.2
Albumin	48.1	29.4	40.1	33.6
Total PBUT (µM)				
HA	5.00	< LOD[a]	190.07	< LOD[a]
IAA	2.59	0.18	8.26	0.40
IS	4.54	< LOD[b]	111.35	< LOQ[d]
pCS	11.63	< LOQ[c]	196.16	0.38

LOD: limit of detection; LOQ: limit of quantification; [a]LOD (HA) = 0.5 µM; [b]LOD (IS) = 0.03 µM; [c]LOQ (*p*CS) = 0.3 µM; [d]LOQ (IS) = 0.2 µM

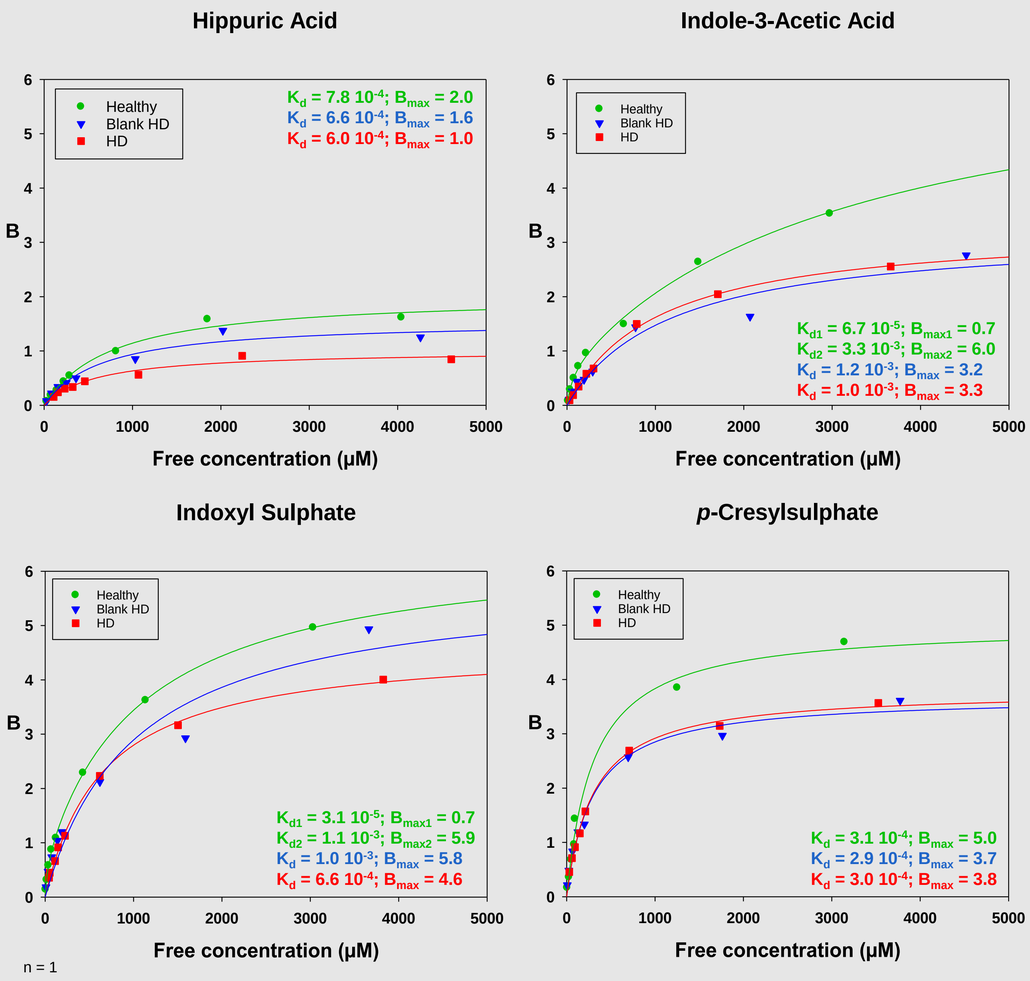
Results & Discussion

Binding curves

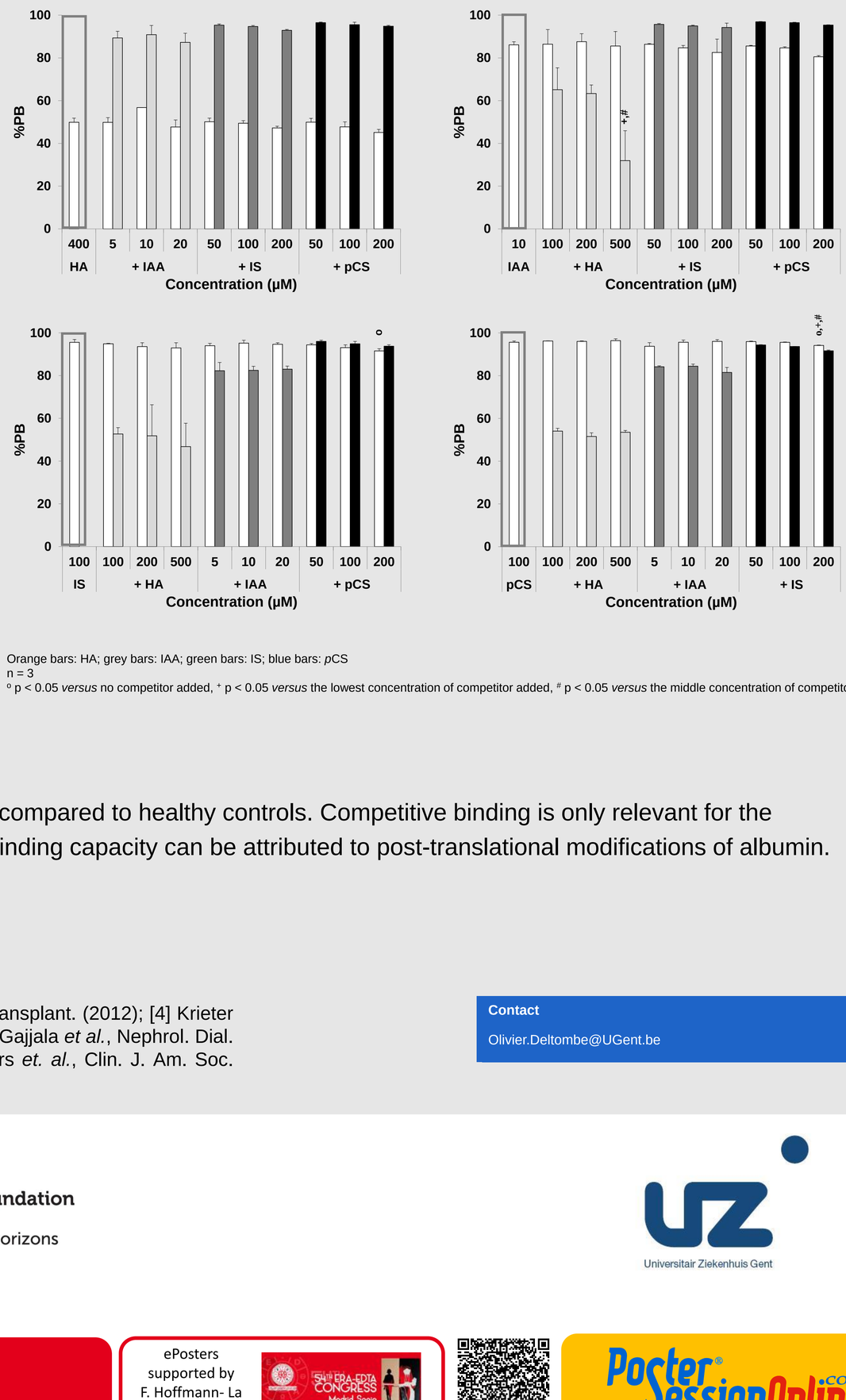
- Higher binding capacity in healthy serum *versus* (blank) HD serum
  - ➡ More solutes can be bound
- Binding capacity in HD serum = in blank HD serum, except for HA
  - ➡ Competition less important

Binding competition

- Mutual competition only for the strongly bound IS and *p*CS
- A trend of lower %PB of HA and IAA when added to serum containing IS or *p*CS *versus* when added to serum containing IAA or HA
  - ➡ Competition is present, though less important



n = 1



Orange bars: HA; grey bars: IAA; green bars: IS; blue bars: *p*CS
n = 3
° $p < 0.05$ *versus* no competitor added, + $p < 0.05$ *versus* the lowest concentration of competitor added, # $p < 0.05$ *versus* the middle concentration of competitor added

Conclusion

There is an intrinsic impact on protein binding in uraemia, revealing a lower binding capacity, as compared to healthy controls. Competitive binding is only relevant for the strongly bound PBUTs at high uraemic concentrations. In addition, at least part of the effect on binding capacity can be attributed to post-translational modifications of albumin.

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