PACAP deficiency as a cause of increased platelet aggregability in idiopathic nephrotic syndrome

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Introduction

Patients with nephrotic syndrome (NS) have an increased risk for thrombosis, including both deep venous and arterial thrombosis, which significantly increases morbidity and mortality rates. The pituitary adenylate cyclase-activating polypeptide (PACAP) was recently identified as an inhibitor of megakaryopoiesis and platelet aggregability. We recently demonstrated urinary losses of PACAP and its binding protein ceruloplasmin in children with congenital NS (CNS), resulting in plasma PACAP deficiency, associated with increased megakaryopoiesis, thrombocytosis and platelet hyperaggregability. We now studied PACAP levels in children with idiopathic NS (INS), and its role in thrombocytosis and platelet hyperaggregability.

Methods

Children between 1 and 16 years old with a first episode or relapse of idiopathic steroid sensitive NS were included in our study. Plasma and urine levels of PACAP and ceruloplasmin were measured, as well as platelet counts and platelet aggregation responses to collagen. All tests were performed 3 times: first during nephrotic state before start of corticosteroid treatment, second 4 weeks after the start of remission and third during later remission after stop of corticosteroid treatment (figure 1).

Results

24 children with idiopathic steroid sensitive NS were included and 29 nephrotic episodes were observed (table 1). Proteinuria and hypoalbuminemia disappeared when patients were in remission (table 2). Creatinine levels were normal at all time points. Mean platelet counts were not significantly altered after remission in comparison to during nephrotic state. However, in 11 of 29 observed episodes, a platelet count above the reference value of 450 x 10^3/µL was seen during nephrotic state, while only 2 of 18 after stop of treatment. No correlations between platelet counts and plasma PACAP or serum albumin levels were seen (data not shown). Plasma and urine PACAP levels and urine ceruloplasmin levels were measured at the 3 time points by western blot (figure 2). Urinary losses of PACAP and ceruloplasmin were documented during the nephrotic state, leading to plasma PACAP deficiency, and normalizing in the non-nephrotic state (figure 3). Significantly higher aggregation responses were found during nephrotic state than after remission and after stop of corticosteroid treatment (figure 4). When recombinant PACAP was added to the platelet-rich plasma sample during the nephrotic state, decreased aggregation responses were seen ($P < 0.05$ for 0.25 µg/mL collagen stimulation). Platelet aggregation correlated inversely with plasma PACAP levels, while not with serum albumin levels.

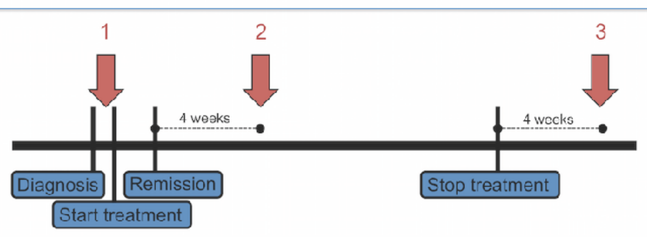


Figure 1: Overview of study time points
Samples were taken at three time points (red arrows) in INS patients: (1) immediately after diagnosis and before the start of corticosteroid treatment; (2) 4 weeks after the start of remission and (3) 4 weeks after stop of corticosteroid treatment.

Demographics of INS patients	
Total number of patients (n)	24
Total number of observed NS episodes (n)	29
Mean age at inclusion (years ± SD)	6.3 ± 4.2
Gender (male/female)	17/7
First episode versus relapse of NS	18/11
Histological type of NS (n)	
MCNS	10
FSGS	4
No biopsy	10

Table 1: Demographics

	Nephrotic	4 weeks after start of remission	4 weeks after stop of corticosteroids	*P1*	*P2*
Plasma albumin (g/L)	21.9 ± 6.0	42.0 ± 3.5	42.7 ± 3.3	<0.001	<0.001
Urine total proteins (g/L)	13.54 ± 11.08	0.09 ± 0.06	0.12 ± 0.08	<0.001	<0.001
Urine protein/creatinine (g/g)	17.81 ± 15.67	0.19 ± 0.15	0.13 ± 0.05	<0.001	<0.001
Serum creatinine (mg/dL)	0.35 ± 0.18	0.40 ± 0.18	0.40 ± 0.16	0.049	0.010
Platelet count (10*9/L)	384 ± 109	390 ± 96	350 ± 91	NS	NS

Table 2: Biochemical characteristics

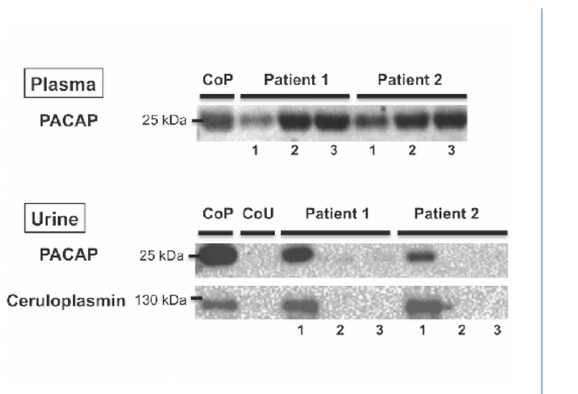


Figure 2: Western blot of PACAP levels in plasma and urine and ceruloplasmin levels in urine of 2 representative patients
Immunoblot analysis of PACAP in equal amounts of plasma and urine samples and of ceruloplasmin in equal amounts of urine, for 2 respresentative INS patients, during nephrotic state (1), 4 weeks after remission (2) and 4 weeks after stop of corticosteroids (3). Control plasma pool (CoP) and control urine pool (CoU) were loaded as a positive and negative control.

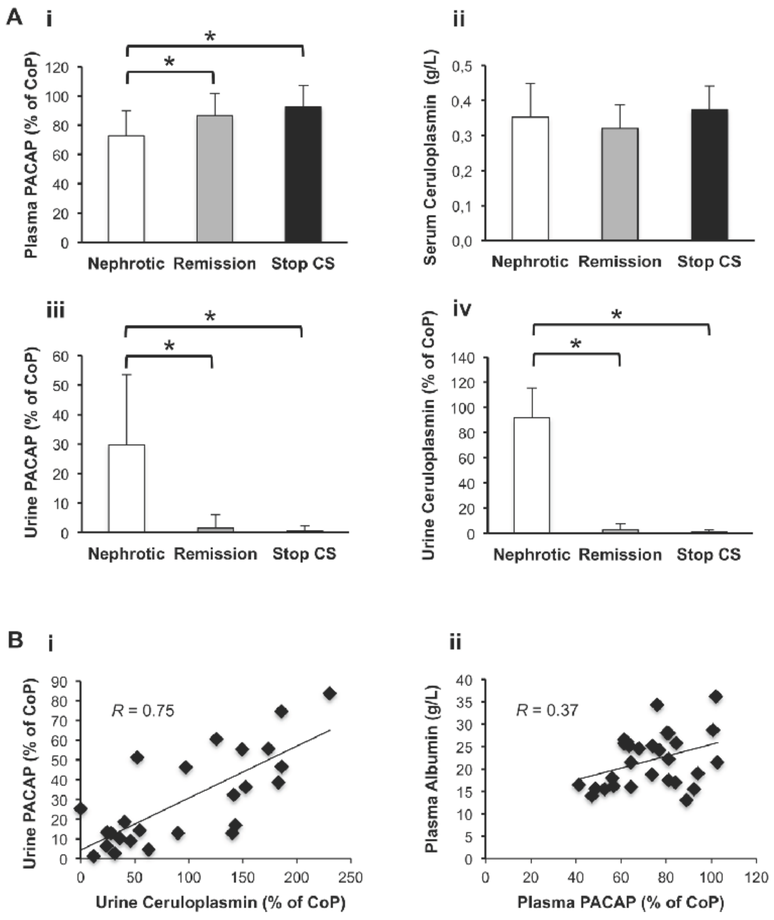
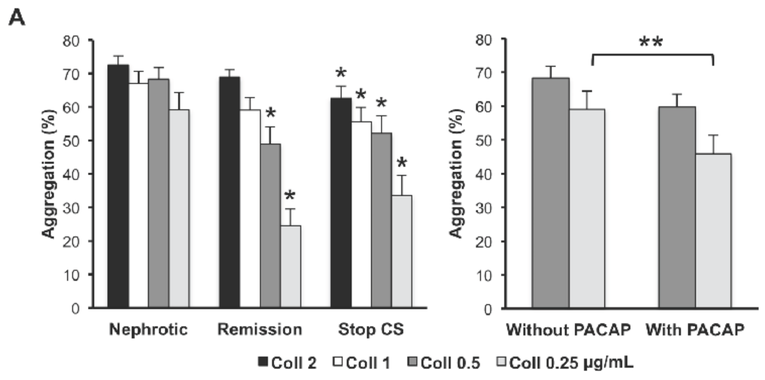


Figure 3: Urinary loss of PACAP and ceruloplasmin during nephrotic state in INS patients
(A) Plasma PACAP (i), serum ceruloplasmin (ii), urine PACAP (iii), and urine ceruloplasmin (iv) levels during nephrotic state, 4 weeks after start of remission and 4 weeks after stop of corticosteroid treatment. Significant lower values of plasma PACAP were found during nephrotic state in comparison to remission and after stop of corticosteroids. Serum ceruloplasmin levels were not changed. PACAP and ceruloplasmin were present in the urine during nephrotic state, while almost no presence of these proteins was found during remission and after stop of corticosteroids. Bars represent means + SD. (*) $P < 0.05$.
CoP = control plasma pool, CS = corticosteroids.
(B) Correlations between urine PACAP and ceruloplasmin (i) and between plasma PACAP and plasma albumin (ii) during nephrotic state. *R* values represent Pearson correlation coefficients, with significance $P < 0.05$.



B

	Platelet aggregation	
	Slope (95% confidence interval)	*P* value
Plasma PACAP	-0.57 (-0.76 – -0.38)	<0.001*
Plasma PACAP, corrected for serum albumin	-0.56 (-0.78 – -0.35)	<0.001*
Serum albumin	-0.37 (-1.16 – 0.41)	0.33
Serum albumin, corrected for plasma PACAP	-0.09 (-0.64 – 0.46)	0.72

Figure 4: Platelet aggregation responses to collagen
(A) Left: Platelet aggregation responses to collagen for different concentrations of collagen during nephrotic state, during remission and after stop of treatment. A significant higher aggregation response was seen during nephrotic state than during remission. (*) $P < 0.05$ compared to the aggregation in response to the same collagen concentration in de nephrotic state. Bars represent means + SEM.
Right: Platelet aggregation responses to collagen for 2 concentrations of collagen with and without addition of recombinant PACAP during nephrotic state. A decreased aggregation response was seen after addition of recombinant PACAP. (**) $P < 0.05$ for the collagen concentration of 0.25 µg/mL. Bars represent means + SEM.
(B) Correlations between platelet aggregation and plasma PACAP levels or serum albumin levels, during nephrotic state. Slopes with 95% confidence intervals and *P* values are shown. A significant correlation was observed between platelet aggregation responses to collagen and plasma PACAP levels. This result persisted after correction for plasma albumin levels. There was no correlation between platelet aggregation responses to collagen and plasma albumin levels, also not after correction for PACAP plasma levels. Slopes were calculated with a linear model for repeated measures with unstructered residual covariance matrix, at significance level (*) $P < 0.05$.

Conclusion

We demonstrate plasma PACAP deficiency in children with INS, playing a role in the platelet hyperaggregability and probably in the increased risk for arterial thrombosis in NS.

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