Increased platelet count and aggregability due to urinary loss of PACAP in congenital nephrotic syndrome

B. Eneman1,2, K. Freson3, B. Van den Heuvel4, C. Van Geet5, E. Levchenco1
1Department of Growth and Regeneration, KU Leuven; 2FWO Vlaanderen; 3Department of Molecular and Vascular Biology, KU Leuven

Introduction
Thrombotic complications occurring in up to 15% of patients represent a severe burden in congenital nephrotic syndrome (CNS). (1) The underlying mechanisms are multifactorial and are mainly unravelled in regard to the venous thrombosis, while elevated blood platelet counts and platelet hyperaggregability increase the risk of arterial thrombosis. The pituitary adenylate cyclase-activating polypeptide (PACAP) is a highly conserved neuropeptide. (2) The role of PACAP as an inhibitor of megakaryocyte maturation and platelet function has recently been established. (3) PACAP interferes with the regulation of apoptosis in megakaryocytes, via stimulation of NFκB signaling 4 (transcaudal PACAP in plasma is bound to ceruloplasmin (5), we assumed that urinary loss of ceruloplasmin in CNS might lead to PACAP deficiency, leading to thrombocytosis and increased platelet reactivity.

The aim of this study was to investigate plasma PACAP levels in relation to blood platelet counts and aggregability in patients with CNS and to examine if addition of recombinant PACAP changes growth of hematopoietic stemcells and differentation into megakaryocytes in CNS. 


Results

1. All patients in nephrotic stage had plasma PACAP deficiency (14-40%, p<0.001) and excessive urinary PACAP excretion (figure 1,2).

2. In patient A, B and C a bilateral nephrectomy was performed because of ongoing very severe nephrosis despite full treatment. In these three patients, we saw a significant increase in plasma albumin, plasma PACAP and serum ceruloplasmin and a significant decrease in platelet count after nephrectomy (figure 3). A strong correlation (R2 > 0.95) was found between platelet count and plasma PACAP levels (figure 3).

3. In patient A, plasma PACAP levels and platelet counts were measured the day before and the days after bilateral nephrectomy. Plasma PACAP levels progressively rose within days after nephrectomy (figure 4) and blood platelet counts normalized (figure 5).

4. Hematopoietic stemcells were isolated from peripheral blood in patient B and C and in vitro differentiated into colony forming unit (CFU) megakaryocytes. In one condition PACAP (1μM) was added to the culture dish on day 0, 4 and 8, while in the other condition no PACAP was added. There was a significantly lower amount of colonies after addition of PACAP in nephrotic stage (figure 6).

5. In patient B and C platelet aggregation was tested before and after bilateral nephrectomy. In analogy to PACAP deficient mice, an increased platelet aggregation response to collagen was found during nephrotic stage, while platelets after bilateral nephrectomy showed normal reactivity towards collagen (figure 7).

Conclusion
Our observations provide new insights on the mechanisms of arterial thrombosis in NS and is a proof-of-principle that PACAP deficiency exists in CNS. In analogy to mice, PACAP deficiency in CNS seems to play an important role in the thrombocytosis, by stimulating megakaryopoiesis, and in platelet hyperaggregability. When confirmed in larger studies, PACAP replacement or stimulation of PACAP receptors might become a valuable therapeutic option for prevention of arterial thrombosis in NS.

Table 1: Clinical characteristics of patients

Figure 1: Immunoblot analysis of PACAP in plasma samples. A-D: CNS patients, CoP: control plasma post.

Figure 2: Immunoblot analysis of PACAP in urine samples. A-D: CNS patients, CoU: control urine sample, CoP: control plasma post.

Figure 3: Plasma albumin, PACAP and ceruloplasmin levels and platelet counts before and after bilateral nephrectomy and correlation between platelet count and plasma PACAP.

Figure 4: Immunoblot analysis of PACAP in plasma of patient A, daily during 5 days after bilateral nephrectomy and after 417 days.

Figure 5: Bilet platelet counts in plasma of patient A, at different time points from 1 month before to 1 month after bilateral nephrectomy.

Figure 6: Amount of colonies of CFU megakaryocytes after 11 days of culture, with and without addition of PACAP.

Figure 7: Platelet aggregation responses after addition of different collagen concentrations.

Patient B

Patient C