Anomalous Neutrophil Inositol Triphosphate Production in Idiopathic Calcium-Oxalate Nephrolithiasis

S.		Cantaro ^a
L.		Calò ^a
A.		Piccoli ^a
F.		Marchini ^a
G.		Gambaro ^a
S.		Favaro ^a
H.E.		Williams ^b
A.		Borsatti ^b

^aDepartment of Internal Medicine, First Division of Nephrology, University of Padova, Italy; ^bUniversity of California, Davis School of Medicine, Davis, Calif., USA

Dr. Lorenzo Calò, Istituto di Medicina Interna, Divisione di Nefrologia, Università di Padova, Via Giustiniani, 2, I-35128 Padova (Italy)

Sir,

We have previously reported that in red blood cells (RBC) of renal stone formers, the rate of oxalate self-exchange is faster than normal [1] and that it can be normalized by disulfonic stilbenes such as DIDS and SITS [2] which are known inhibitors of the anion carrier band 3. These cells also exhibited increased phosphorylation of the RBC anion exchanger (band 3) [3] which, together with the demonstration of a reduction in oxalate self-exchange after depletion of RBC adenosine 5'-triphosphate [3], suggests that this anion carrier, like other ion exchangers [4], requires phosphorylation to function normally, raising, in this way, the possibility of an increased protein kinase activity as a basis for this abnormality [5].

Although the kinase(s) involved in the phosphorylation of band 3 is not known, we have evidence that the protein kinase A activation with forskolin and the protein kinase C (PK-C) activation by diacyl glycerol (DAG) increases the transmembrane flux of oxalate in normal controls up to the level characteristic of stone formers, while, on the contrary, the inhibition of intracellular Ca²⁺-mediated events by trifluoperazine, reverted to normal the high oxalate transmembrane flux of stone formers [6].

Following the hypothesis of an abnormality of the phospholipid sensitive calcium-dependent protein kinase activity in idiopathic calcium oxalate nephrolithiasis, we investigated whether some defect along the intracellular calcium-dependent pathway leading to PK-C activation is present in stone formers,

Table 1. Basal and stimulated IP₃ (pmol/ml) production in neutrophils of healthy controls and stone formers

and we focused on phospholipase C (PLC) activity in terms of inositol triphosphate (IP₃) production. PLC activity, in fact, gives rise to IP₃, that mobilizes intracellular calcium, and to DAG, a known activator of PK-C [7].

To this end, we determined the basal and formyl-methionyl-leucyl-phenylalanine (FMLP)-stimulated levels of IP₃ in neutrophils of 6 patients affected by calcium oxalate nephrolithiasis whose RBC showed a high rate of oxalate self-exchange, in comparison with 6 age- and sex-matched healthy controls.

Neutrophils of patients and controls were prepared from fresh blood samples as previously reported [8], resuspended in the assay medium and adjusted at a concentration of 20×10^6 cells/ml for resting and FMLP-stimulated (3×10^{-7} M for 10 s) IP₃ determinations. IP₃ was determined by RIA after diethyl ether extraction by a commercially available kit (Amersham). The results are shown in table 1. Basal IP₃ levels were significantly higher in patients in comparison with controls

and, while IP₃ release significantly increases after FMLP stimulation in controls, FMLP was not able to increase IP₃ in stone formers. The higher basal IP₃ levels found in stone formers are in keeping with an anomaly, in these patients, along the intracellular pathway leading to protein kinase activation and in which Ca²⁺ plays a pivotal role. Moreover, the higher basal levels of IP₃, together with a lack of increment after FMLP found in stone formers, suggest that IP₃ production is already maximally stimulated in the basal condition, meaning that PLC in these patients is, in the basal condition, already working at its zenith. A higher PLC activity could give rise also to an increased DAG intracellular level making likely an increased PK-C activity.

In conclusion, these preliminary data support the hypothesis of an increased protein kinase activity as a basis for the suggested defect in anion carrier in 'primary' nephrolithiasis and point to PK-C as the kinase involved in its phosphorylation.

© 1992 S. Karger AG, Basel

0028-2766/92/

0612-0241\$2.75/0

References

1 Baggio B, Gambaro G, Marchini F, Cicerello E, Borsatti A: Raised transmembrane oxalate flux

in red blood cells in idiopathic calcium oxalate nephrolithiasis. *Lancet* 1984;ii: 12-13.

2 Baggio B, Gambaro G, Borsatti A, Clari G, Moret V: Relation between band 3 red blood

cell protein and transmembrane oxalate flux in stone formers. *Lancet* 1984;ii:223-224.

3 Baggio B, Clari G, Marzaro G, Gambaro G, Borsatti A, Moret V: Altered red blood cell membrane protein phosphorylation in idiopathic calcium oxalate nephrolithiasis. *IRCS Med*

Sci 1986;14:368-369.

Bursaux E, Hilly M, Bluze A, Poyart C: Organic phosphates modulate self-exchange across the human erythrocyte membrane. *Biochim Bio-phys Acta* 1984;777:253-260. Baggio B, Clari G, Marzaro G, Gambaro G, Borsatti A, Moret V: Erythrocyte membrane protein phosphorylation in urolithiasis. *Contrib Nephrol* 1987;58:156-159. Cantaro S, Marchini F, Calò L, Gambaro G, Piccoli A, Williams HE, Baggio B, Borsatti A: Oxalate exchange in red blood cells of calcium oxalate stone formers: A pharmacologic study; in Walker VR, Sutton RAL, Cameron ECB, Pak VC, Robertson GW (eds): *Urolithiasis*. New York, Plenum Press, 1989, pp 443-445.

Berridge JM, Irvine RF: Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature* 1984;312:315-321.

Di Virgilio F, Calò L, Cantaro S, Favaro S, Piccoli A, Borsatti A: Resting and stimulated free calcium levels in neutrophils from patients with Bartter's syndrome. *Clin Sci* 1987;72:483-488.

242

Cantaro/Calò/Piccoli/Marchini/Gambaro/ Leucocyte IP₃ Production in Nephrolithiasis
Favaro/Williams/Borsatti