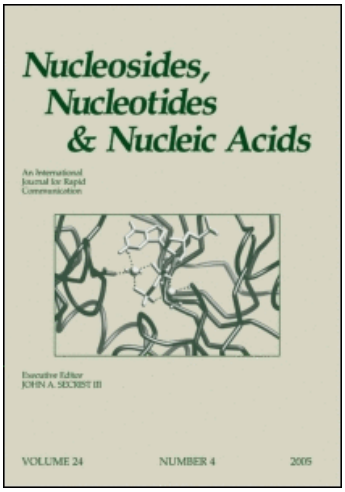

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The Novel Nucleotide 4KNTP, in High Concentrations in Erythrocytes of Renal Failure Children: a Comparison with Accumulation of Other Putative Precursors in the Plasma

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THE NOVEL NUCLEOTIDE 4KNTP, IN HIGH CONCENTRATIONS IN ERYTHROCYTES OF RENAL FAILURE CHILDREN: A COMPARISON WITH ACCUMULATION OF OTHER PUTATIVE PRECURSORS IN THE PLASMA

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□ *We have measured the concentrations of metabolites related to the turnover of NAD, which accumulate in the blood of children with renal failure. One is a novel nucleotide, identified as the N1-riboside triphosphate of 4-pyridone-3-carboxamide (4PYTP), also described as 4KNTP, which accumulates in the erythrocytes in parallel with renal failure.*

Keywords M2PY (N1-methyl-2-pyridone-5-carboxamide); 4-Pyridone-3-carboxamide N-1 riboside triphosphate (4PYTP); PCNR; Children; Renal failure; Uraemia

INTRODUCTION

The catabolite M2PY (N1-methyl-2-pyridone-5-carboxamide) is formed from nicotinamide which has been liberated by hydrolysis of the cofactor NAD: This occurs in response to DNA damage and the consequent demand for ADP-ribose-P as a substrate for PARP-1 enzyme. Thus high concentrations in the plasma of patients with liver cirrhosis have been noted,[1] and there is a natural rise in healthy subjects with age,[2] albeit at much lower levels. We have observed that M2PY is concentrated in the plasma of patients with renal failure, approximately in parallel with the accumulation of a novel nucleotide[3] in the erythrocytes. Each of the compounds is a potential

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toxin, and high concentrations may explain why patients with uraemia feel unwell.

In soluble extracts from human erythrocytes, the novel nucleotide elutes near ATP in HPLC profiles, with a characteristic UV absorption spectrum partly resembling M2PY.[4] In fact, the compound unequivocally has been identified as the N1-riboside triphosphate of 4-pyridone-3-carboxamide (4PYTP),[5] thus, its biosynthesis is unlikely to include M2PY as a precursor. The compound may also be described as 4-ketonicotinamide riboside triphosphate or 4KNTP.

MATERIALS AND METHODS

Children aged 5–17 attending regular outpatient renal clinics or the haemodialysis unit of Great Ormond Street Hospital for Children were invited to provide a small blood sample, after they or their parents had given informed consent. Heparinised blood from 34 patients (18 girls, 16 boys) was separated into plasma and washed erythrocyte fractions (from which lymphocytes and platelets had been removed): deproteinised supernatants were applied to liquid chromatography columns with UV diode array analysis (230–310 nm) as described previously.[3] The compounds M2PY and M4PY (plasma) and the riboside and ribotides (diphosphate and triphosphate) of 4-ketonicotinamide (erythrocytes) were identified, and the concentrations calculated, from the characteristic absorption spectrum and retention times compared with synthetic standard compounds.

Values for urea and creatinine in the plasma of each patient were obtained from the medical records corresponding to the same clinic visit as the blood sample.

RESULTS AND DISCUSSION

Values for the pyridones M2PY and M4PY (M4PY was usually a very small component) were combined since the two compounds arise from the same breakdown pathway.[6] Highest values were seen in the plasma of children with the most severe chronic renal failure or who were undertaking dialysis. Haemodialysis patients gave a sample just as they were about to begin mechanical dialysis, 2–3 days since the previous session, and hence these were the highest concentrations of pyridones, creatinine and urea that would be expected in the circulation of these patients. The values would be expected to fall to nearly normal ranges immediately after dialysis.

Table 1 summarizes the data for all chronic renal failure patients, stratified according to whether the child was treated by drugs/diet, haemodialysis or peritoneal dialysis. Notably, urea values were high in some of the patients

TABLE 1 Concentration of Soluble Metabolites in the Plasma of 34 Children with Chronic Renal Failure

Patients	n	M2PY + M4PY (μmol/l)	creatinine (μmol/l)	urea (μmol/l)
Nondialysis	22	8.67 ± 5.83 (0.2–21.69)	169.45 ± 92.8 (62–430)	9.9 ± 5.38 (3.2–24.8)
Haemodialysis	6	62.88 ± 26.08 (33.8–104)	1005.3 ± 258.58 (754–1457)	23.22 ± 3.04 (17.7–26.0)
Peritoneal dialysis	6	41.36 ± 9.09 (32.38–55.84)	839.17 ± 223.98 (533–1048)	13.80 ± 2.83 (9.5–18.2)
All	34	(0.2–104.0)	(62–1457)	(3.2–26.0)
Normal range		0.39 ± 0.22[a]	35–80[b]	2.5–6.0[b]

Mean ± SD (range) for 34 children, stratified according to dialysis treatment. The ranges for all the patients, and for normal/healthy subjects, are also shown.

[a]Mean ± SD for children under 16 years old.[2]

[b]Approximate reference ranges used in Great Ormond Street Hospital for Children.

who were not receiving dialysis, but all non-dialysis patients had creatinine concentrations of 500 μmol/l or less, since poor creatinine filtration was used as the criterion for progression to artificial dialysis.

In common with the adult patients studied before,[3] the concentration of 4KNTP in the erythrocytes was highest in patients with most severe renal failure. In some of the erythrocyte extracts, the diphosphate ribotide and the riboside could be recognised from their characteristic UV absorption spectrum, which was similar to that of 4KNTP. They eluted at approximately 21 minutes (4KNTP), 14 minutes (4KNDP), and 3 minutes (4KN-riboside) in the anion exchange system used here.[3] Figure 1 demonstrates that the highest mean concentrations of the riboside and 4KNDP were found in dialysis patients, who also had the highest concentrations of the triphosphate in their erythrocytes. Patients with the most severe chronic renal failure, identified by the poorest filtration of creatinine, also had measurable concentrations of riboside in their erythrocytes.

The compound 4-pyridone-3-carboxamide-1β-D ribonucleoside (4PYR, 4KN-riboside, PCNR) has been identified before in human urine[7] and in plants.[8] Analysis by mass spectrometry, NMR and chemical synthesis were consistent with our similar analysis[5] of the novel nucleotide in erythrocytes of renal failure patients as a triphosphate of this riboside.

Deficient renal filtration causes the accumulation of small molecules in the plasma, as we described in our previous studies of M2PY and M4PY in the plasma.[2,4,5] The elimination in the urine of 4PYR may be prevented during chronic renal failure, and thus it may be rapidly taken up into erythrocytes in circulation. Hence, 4PYR is seen in the erythrocytes of some patients with very poor renal function (Synesiou et al., in preparation) concomitant with an elevated concentration of 4PYTP.

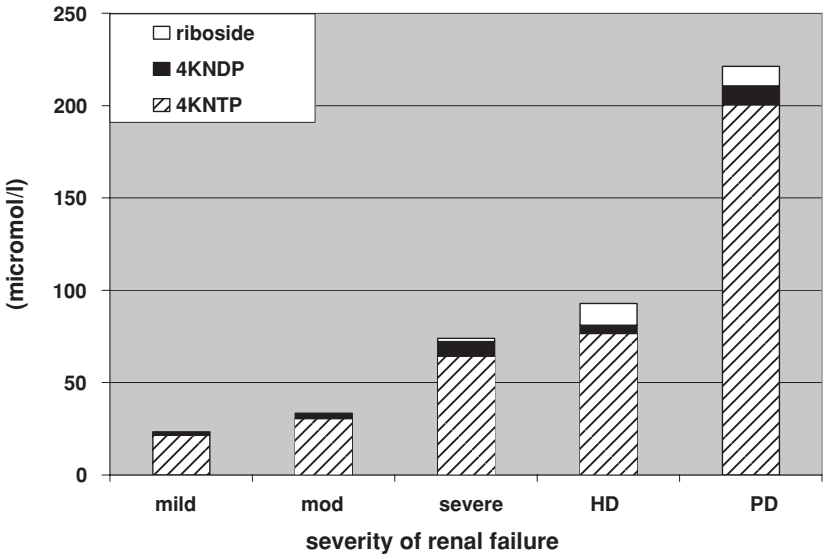

FIGURE 1 Relationship of 4KNTP concentration with degree of renal failure. The mean concentrations of the riboside and ribotides of 4-pyridone-3-carboxamide are shown for erythrocyte extracts of children with chronic renal failure: mild (creatinine $\leq$125 μmol/l, n = 7); moderate (creatinine 126–190 μmol/l, n = 9); severe (creatinine $\geq$190 μmol/l, n = 6): HD, treated with 3 times weekly haemodialysis, n = 6; PD, treated with continuous ambulatory peritoneal dialysis, n = 6.

REFERENCES

1. Pumpo, R.; Sarnelli, G.; Spinella, A.; Budillon, G.; Cuomo, R. The metabolism of nicotinamide in human liver cirrhosis: a study on N-methylnicotinamide and 2-pyridone-5-carboxamide production. *Am. J. Gastroenterol.* **2001**, 96, 1183–1187.
2. Slominska, E.M.; Rutkowski, P.; Smolenski, R.T.; Szutowicz, A.; Rutkowski, B.; Swierczynski, J. The age-related increase in N-methyl-2-pyridone-5-carboxamide (NAD catabolite) in human plasma. *Mol. Cell. Biochem.* **2004**, 267, 25–30.
3. Carrey, E.A.; Smolenski, R.T.; Edbury, S.M.; Laurence, A.; Marinaki, A.M.; Duley, J.A.; Zhu, L.; Goldsmith, D.J.A.; Simmonds, H.A. Origin and characteristics of an unusual pyridine nucleotide accumulating in erythrocytes: positive correlation with degree of renal failure. *Clinica Chimica Acta* **2003**, 335, 117–129.
4. Carrey, E.A.; Smolenski, R.T.; Edbury, S.M.; Laurence, A.; Marinaki, A.M.; Duley, J.A.; Zhu, L.M.; Goldsmith, D.J.A.; Simmonds, H.A. An unusual pyridine nucleotide accumulating in erythrocytes: its identity and positive correlation with renal failure. *Nucleosides, Nucleotides and Nucleic Acids* **2004**, 23, 1135–1139.
5. Slominska, E.M.; Carrey, E.A.; Foks, H.; Orlewska, C.; Wieczerzak, E.; Sowinski, P.; Yacoub, M.H.; Marinaki, A.M.; Simmonds, H.A.; Smolenski, R.T. Identification of a novel naturally occurring nucleotide: 4-pyridone-3-carboxamide-1β-D-ribonucleoside triphosphate in the human erythrocytes. *J. Biol. Chem.* **2006** (in press).
6. Mrochek, J.E.; Jolley, R.L.; Young, D.S.; Turner, W.J. Metabolic response of humans to ingestion of nicotinic acid and nicotinamide. *Clin. Chem.* **1979**, 22, 1821–1827.
7. Dutta, S.P.; Crain, P.F.; McCloskey, J.A.; Chheda, G.B. Isolation and characterization of 1-β-D-ribofuranosylpyridin-4-one-3-carboxamide from human urine. *Life Sci.* **1979**, 24, 1381–1388.
8. Bringmann, G.; Ochse, M.; Wolf, K.; Kraus, J.; Peters, K.; Peters, E.-M.; Herderich, M.; Assi, L.A.; Tayman, F.S.K. 4-oxonicotinamide-1-(1′-β-D-ribofuranoside) from *Rothmannia longiflora* Salisb. (Rubiaceae). *Phytochemistry* **1999**, 51, 271–276.