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Disturbances of Purine Nucleotide Metabolism in Uremia

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The increased concentration of adenosine triphosphate (ATP) in erythrocytes from patients with chronic renal failure (CRF) has been observed in many studies but the mechanism leading to these abnormalities still is controversial. It is believed that hyperphosphatemia and metabolic acidosis triggering enhanced reutilization of purine bases are the factors responsible for changes in erythrocyte nucleotide concentration. During the past decade we have performed several studies. A summary of the obtained results is presented. A high-performance liquid chromatography technique was used for the determination of plasma and intraerythrocyte nucleotide concentrations. Labeled adenine and adenosine were used for measuring adenine incorporation. In CRF patients treated conservatively increased concentrations of ATP levels and other nucleotides such as adenosine diphosphate were found. Adenosine monophosphate and hypoxanthine levels were lower than in controls. In hemodialyzed patients both ATP and adenosine monophosphate intraerythrocyte concentrations were higher than in controls. At the same time, adenosine monophosphate and hypoxantine level were comparable with levels in healthy people. The main pattern of nucleotides during hemodialysis remained unchanged, independent from the mode of therapy. The only exception was a decreased level of hypoxantine. Results of a consecutive study have suggested that the increased rate of adenine incorporation into the adenine nucleotide pool could be partially responsible for the increased ATP concentration in uremic erythrocytes. Last but not least, trying to elucidate the pathomechanism of adenine nucleotide disturbances in uremia, we have found that the concentration of N-methyl-2-pyridone-5-carboxamide (2PY), one of the end products of nicotinamide-adenine dinucleotide degradation, were enhanced in CRF patients to values that are potentially toxic. Our findings suggest that 2PY could be a novel uremic toxin. Disturbances of nucleotide metabolism are one of the important components of uremic syndrome.
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Different metabolic disturbances are associated with uremic syndrome. One of the components of these abnormalities are changes in purine nucleotide metabolism. Increased adenosine triphosphate (ATP) concentrations in erythrocytes of patients with chronic renal failure (CRF) were found in 1964 by Hurt and Chanutin.[1] On the other hand, we found in our studies, performed in the early 1970s, that glucose use by uremic erythrocytes is increased significantly in comparison with healthy red blood cells.[2,3] It is possible that both glucose metabolism disturbances and adenine nucleotide disturbances may partially participate in the pathomechanism of anemia observed in patients with CRF. The increased concentration of ATP in erythrocytes of patients with this syndrome was confirmed in several studies performed in very reliable research centers.[4-8] Nevertheless, mechanisms leading to these abnormalities still are obscure and controversial. Some studies showed that hyperphosphatemia and metabolic acidosis observed in uremic patients most likely is responsible in part for the appearance of these disturbances.[9,10] Although definitive confirmation of this still is lacking.

Because human erythrocytes are not able to synthesize

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Table 1 Adenine Nucleotide Concentration in Blood of Patients With CRF Treated Conservatively and Undergoing Hemodialysis

Group	ATP nmol/mL	Adenosine Diphosphate nmol/mL	Adenosine Monophosphate nmol/mL	ATP/Adenosine Diphosphate	ADP/Adenosine Monophosphate
Control (n = 13)	1,273 ± 198	171.8 ± 44.9	21.7 ± 13	7.74 ± 1.7	10.5 ± 5.2
Predialysis (n = 25)	1,757* ± 282	122.7* ± 19.4	8.13* ± 4.9	14.6* ± 2.6	18.5* ± 7
Hemodialysis (n = 30)					
Before	2,029* ± 467	133.3 ± 31	19.4 ± 11	11.1 ± 5	12.9 ± 5
After	2,076* ± 211	138 ± 23	12.5 ± 4	11.05 ± 6	14.0 ± 5
24 hours after HD	2,064* ± 431	114* ± 28	11.0* ± 4	18.1* ± 7	10.4 ± 5

NOTE. Mean ± SD shown.
*$P < .05$ versus control values.

nucleotides de novo, only salvage of adenine and adenosine could be a source of purine moiety in the nucleotide molecule. Also, increased plasma concentrations of adenine together with increased levels of 5-phospho-rybose-1-pyrophosphate (PRPR) seem to play an important role in this phenomena. Renal replacement therapy, without any doubt, positively influences these disturbances. Nevertheless, the real effect of different methods of this therapy is not described very precisely. In our center, thanks to close collaboration of nephrologists and biochemists, several studies were performed during the past decade that tried to elucidate these issues.[11-20] In this article, we summarize the results obtained in these studies and try to reach common conclusions. All nucleotide estimations were made using a reversed-phase high-performance liquid chromatography technique.[21-23] A detailed description of patient characteristics and the methodology used in our studies has been described in our previous publications.[11-22]

Adenine Nucleotides in Erythrocytes of Uremic Patients: Influence of Renal Replacement Therapy

Four of our independent studies confirmed that both blood and intraerythrocyte concentrations of ATP are increased significantly in CRF patients. The exceptions to these results are presented in Table 1 and Fig. 1. It was shown in predialysis patients (mean creatinine concentration, 8 mg/dL) and in patients treated with maintenance hemodialysis as well. During hemodialysis sessions, ATP concentrations did not change independently from the type of concentrate (acetate or bicarbonate) used for this procedure.

In predialysis patients, ADP, adenosine monophosphate, and hypoxanthine (not shown) concentrations were significantly lower than in control subjects. In hemodialyzed patients the concentrations of both nucleotides were on the same level before and after the dialysis session. Only the hypoxanthine concentration decreased 2-fold after this procedure, however, it did not reach normal values. All of these results were obtained using cuprophane dialysers.[12,13] On the other hand, during more recent studies when polysulphone dialysers were used, a significant reduction in both erythrocyte ATP and plasma adenine levels were observed immediately after hemodialysis, but 48 hours later high erythrocyte ATP and plasma adenine concentrations were restored (Fig. 1). Additionally, in this study we have observed patients after successful kidney transplantation. In this group both intraerythrocyte ATP and plasma adenine concentrations reached control values. In both series of studies, in predialysis patients, a significant positive correlation was found between erythrocyte ATP and creatinine concentrations. In the first group, the ATP concentration correlated with the phosphate level as well.[12]

The results of our studies summarized earlier provide evidence that adenine nucleotide turnover is increased in patients with CRF and this process is related to the severity of the disease. It also was shown that renal replacement therapy has a positive impact on these abnormalities.

Mechanisms Leading to ATP Increase in Uremic Erythrocytes

The increase of the purine nucleotide pool, mainly ATP, in erythrocytes of CRF patients has been well described. However, the mechanism responsible for this abnormality is far from being clear. Our observations lead to the hypothesis that the increased rate of adenine incorporation into the adenine nucleotide pool is responsible for the increased level of ATP in uremic erythrocytes and that the increase of adenine concentration was dependent on the severity of CRF in the plasma of patients with this syndrome made this thesis even more attractive. To check this possibility in one of our studies we evaluated the rate of adenine nucleotide synthesis (from adenine) in uremic erythrocytes and the effect of high concentrations of phosphates and a low pH level (similar to that observed in patients with CRF) on adenine incorporation into the adenine nucleotide pool in erythrocytes from healthy subjects and uremic predialysis patients. Labeled [8^{14} C] adenine (25 μCi/mmol) and adenosine (20 μCi/mmol) were used for the studies and the detailed methodology is described elsewhere.[14] It was shown that in isolated erythrocytes of uremic patients adenine was incorporated into the adenine nucleotide pool 2-fold faster than in healthy erythrocytes (Table 2). Under this same condition the incorporation of adenosine was comparable in erythrocytes from both

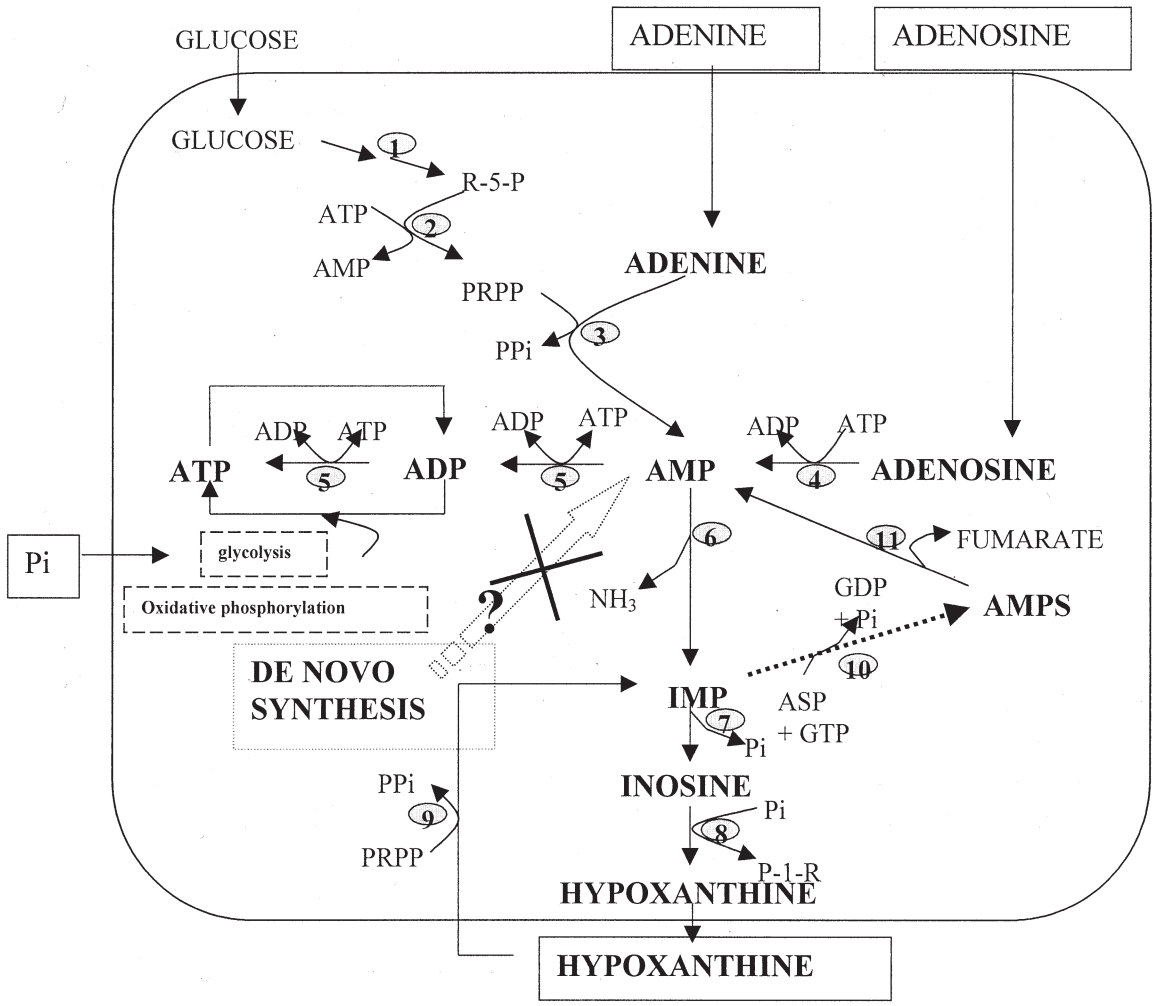

Figure 1 Erythrocyte purine nucleotide metabolism. Salvage pathways: (1) pentosomonophosphate cycle, (2) phosphoribosylpyrophosphate synthetase, (3) adenine phosphoribosyltransperase, (4) adenosine kinase, (5) adenylate kinase, (6) adenosine monophosphate deaminase, (7) 5′ nucleotidase, (8) purine nucleoside phosphorylase, (9) hypoxanthine-quanine phosphoribosyl-transperase (HGPRT), (10) adenylocuccinate (AMPS) synthetase (lack of that enzyme activity in red blood cells), (11) adenylosuccinate lyase.

groups of subject. It also must be noted that adenine incorporation into the adenine nucleotide pool was observed when erythrocytes from healthy subjects were incubated in the medium imitating conditions expected in severe renal failure (pH 7.1, inorganic phosphate, 2.4 mmol/L).[14] These data confirmed the theory that high extracellular inorganic phosphate concentration alone, or especially in combination with low extracellular pH level, have a stimulatory effect on the adenine incorporation into the adenine nucleotide pool. These experiments were extended in the more recent study in which erythrocytes from patients with different severities of CRF were studied.[18] We found that in erythrocytes from patients with the highest severity of this syndrome adenine was incorporated into the adenine nucleotide pool at a rate

Table 2 Incorporation of [8^{14}C] Adenine Into the Adenine Nucleotide Pool in the Erythrocytes of CRF Patients and of Healthy Subjects (Control)

	Incubation Time (min)			
	0	10	20	40
Control (n = 6)	0.0	4.2 ± 0.8	6.1 ± 0.6	11.6 ± 2.4
CRF (n = 6)	0.0	6.6 ± 1.7	11.8 ± 1.8	22.4 ± 4.4
Statistics	NS	NS	$P < .002$	$P < .002$

NOTE. Incubation was performed under physiologic concentration of inorganic phosphate (1.2 mmol/L) and at pH 7.4. Incorporation rate is expressed in nmol/mL red blood cells.

Abbreviation: NS, not specified

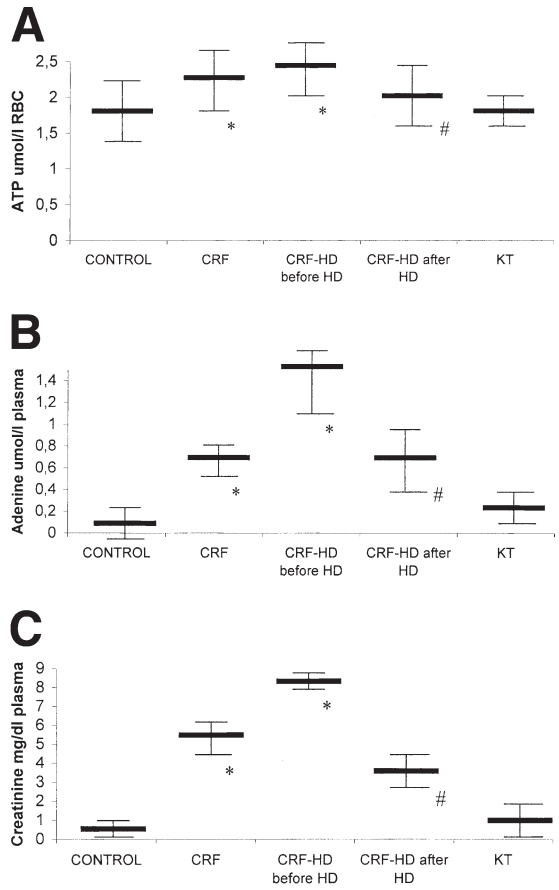

Figure 2 (A) Erythrocyte ATP, (B) plasma adenine, and (C) creatinine concentrations in healthy subjects and patients with CRF. *$P <$ .001 versus healthy subjects, #$P <$ .001 versus before hemodialysis. Healthy subjects: n = 26; predialyzed patients: CRF, n = 30; hemodialyzed patients: CRF-HD, n = 11; CRF-HD before HD, immediately before hemodialysis; CRF-HD after HD, immediately after hemodialysis; KT, patients after successful kidney transplantation (n = 12).

approximately 10-fold higher than in red blood cells from healthy patients. Nevertheless direct correlation between the rate of adenine incorporation and the different degree of CRF, reflected by serum creatinine concentration, was not found.[18] It is important to remember that it is well established that human erythrocytes cannot synthesize adenine nucleotide molecules (Fig. 1).[24,25] Summarizing this part of experiments is necessary to emphasize that in CRF patients a high concentration of plasma adenine was observed (Fig 2), correlating with the severity of kidney failure. Furthermore, the plasma adenine concentration in CRF patients directly correlated with the erythrocyte adenine nucleotide concentration and the adenine incorporation pool. This suggests that the ATP increase in uremic erythrocytes is the result of metabolic changes shown earlier. Combined with results presented in other articles dealing mainly with degradation of adenine nucleotides in CRF erythrocytes, one can assume that rather the accelerated nucleotide synthesis and not nucleotide degradation is the primary cause of the adenine nucleotide pool increase in uremic erythrocytes.[15]

Nicotinamide-Adenine Dinucleotide Degradation Product: The Family of Novel Uremic Toxins

During the earlier-described studies in high-performance liquid chromatograms of uremic blood extracts, an unknown peak was noted.[12] After further identification using techniques with better separation ability the compound responsible for the appearance of this peak was identified as metabolic nicotinamide-adenine dinucleotide-N-methyl-2 pyridone-5-cosboxamide (2PY).[19] Table 3 shows that the 2PY concentration is increased many times in predialysis CRF patients. The direct correlation was found between serum 2PY concentration and serum creatinine concentration and a negative correlation was found between the concentration of this compound and creatinine clearance.[20] The data presented in Table 3 show that 2PY concentration decreases considerably after hemodialysis and increases 48 hours later. In patients after successful kidney transplantation, the concentration of this compound decreases permanently. We suggested in our previous report that an increase of 2PY concentration caused by impairment of its excretion in urine by altered kidneys may play an important role in the development of uremic toxemia, especially as an inhibitor of poly (adenosine diphosphate-ribose) polymerase (PARP;19). To confirm this hypothesis further experiments were performed checking the inhibitory effect of 2PY on PARP-1 activity in vitro. It was shown that 2PY inhibits activity of this important enzyme in a dose-dependent manner.[20] The considerably enhanced concentration of 2PY–PARP-1 inhibitor in CRF patients might restrict the DNA repair events and promote cell, tissue, and organ dysfunction present in uremic patients. It seem possible that this phenomenon impairing poly (adenosine diphosphate-ribosylation)

Table 3 The Serum 2PY Concentration in Patients With Different Severities of Renal Insufficiency

					Hemodialysis (n = 11)			
	Control (n = 19)	CRF (n = 10)			Before HD (HD1)	After HD (HD2)	48 hours After HD (HD3)	KT (n = 12)
		CRF1	CRF2	CRF3				
Creatinine (μmol/L)	78 ± 16	210 ± 98	540 ± 68	827 ± 145	733 ± 128	324 ± 73	707 ± 112	111 ± 20
2PY (μmol/L)	0.83 ± 0.18	4.1 ± 2.8	9.2 ± 4.4	15.5 ± 5.8	19.3 ± 6.4	10.2 ± 3.4	17.3 ± 4.2	1.64 ± 0.16

NOTE. The effect of hemodialysis (4-h session; polysulphone membrane) and kidney transplantation. Control, control group; CRF, groups of predialysis patients with CRF; HD, group of patients on hemodialysis treatment; KT, group of patients after kidney transplantation. Statistical analysis: CRF1 versus control: $P <$ .001; CRF2 versus CRF1: $P <$ .005; CRF3 versus CRF2: $P <$ .05; HD1 versus CRF3: n.s; HD2 versus HD1 and HD3 versus HD2: $P <$ .001; KT versus control: $P <$ .01.

process is partly responsible for the increased incidence of neoplasms in CRF patients.[26] 2PY fulfils all the necessary criteria for uremic toxins[27] and this nicotinamide-adenine dinucleotide degradation product could be one of them.

Generally one can conclude that disturbances of purine nucleotide metabolism are one of the important components of uremic syndrome.

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