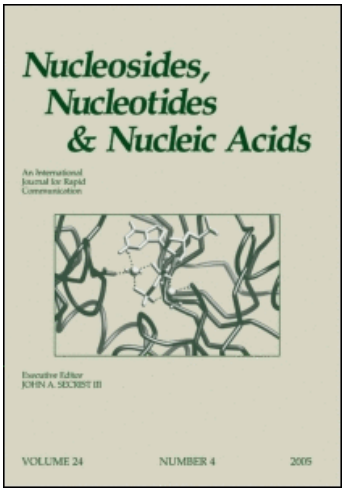

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ANALYSIS OF PYRIMIDINE SYNTHESIS DE NOVO INTERMEDIATES IN URINE DURING CRISIS OF A PATIENT WITH ORNITHINE TRANSCARBAMYLASE DEFICIENCY

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□ *Analysis of pyrimidine synthesis de novo intermediates and pyrimidine degradation products in urine samples from a decompensated patient with an ornithine transcarbamylase deficiency showed a strikingly aberrant metabolic profile. Strongly elevated levels of N-carbamyl-aspartate, orotate and uracil were present whereas the concentration of uridine was only marginally increased. The level of pyrimidine excretion appeared to be independent of the ammonia levels in blood, which were only mildly increased.*

Keywords Pyrimidine de novo; Urea cycle; Ornithine transcarbamylase deficiency

INTRODUCTION

Pyrimidine nucleotides are essential for a vast number of biological processes and they are synthesized de novo in mammalian cells through a multistep process (Figure 1). Pathological conditions, such as a deficiency of UMP synthase result in altered excretion of intermediates of the pyrimidine

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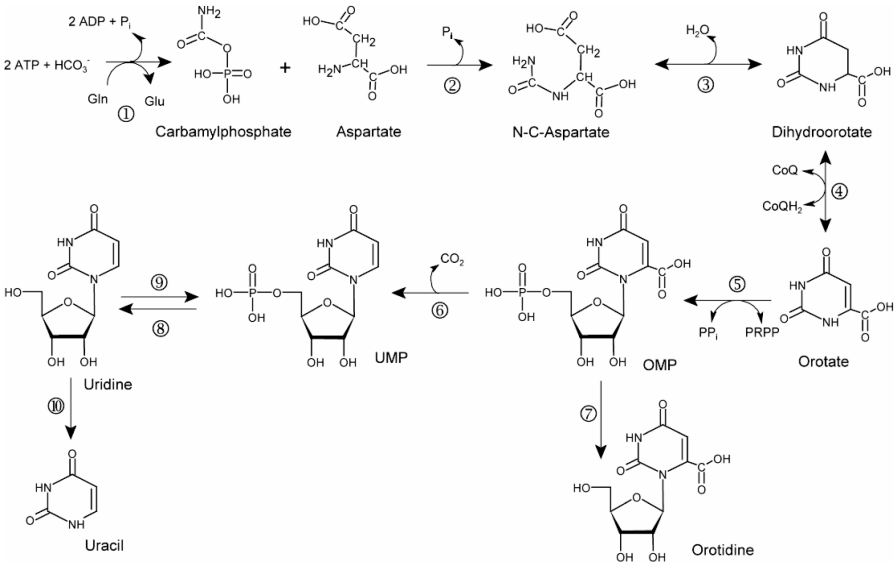

FIGURE 1 Pyrimidine de novo pathway. ①, carbamylphosphate synthetase; ②, aspartate transcarbamylase; ③, dihydroorotase; ① + ② + ③, CAD; ④, dihydroorotate dehydrogenase; ⑤, orotate phosphoribosyltransferase; ⑥, orotidine 5'-monophosphate decarboxylase; ⑤ + ⑥, UMP synthase; ⑦, orotidine 5'-monophosphate phosphohydrolase; ⑧, pyrimidine 5' nucleotidase; ⑨, uridine kinase; ⑩, uridine phosphorylase.

de novo synthesis pathway. The rate-limiting enzymes of the pyrimidine nucleotide biosynthesis and degradation pathway are OMP decarboxylase and dihydropyrimidine dehydrogenase (DPD), respectively. Patients with an inability to metabolise carbamylphosphate via the urea cycle will divert the excess carbamylphosphate to the pyrimidine biosynthesis pathway, resulting in an increased production of orotate, orotidine and uracil.[1,2] Therefore, the concentrations of the pyrimidine de novo synthesis intermediates and pyrimidine degradation products in urine are useful indicators for the diagnosis of patients suspected of an inborn error of the pyrimidine de novo synthesis pathway or a urea-cycle defect.[1,2] In this article, we present the results of the analysis of these compounds in urine samples obtained during crisis from a patient suffering from an ornithine transcarbamylase (OTC) deficiency.

MATERIALS AND METHODS

The pyrimidine de novo synthesis intermediates and pyrimidine degradation products were measured in urine samples using HPLC-tandem mass spectrometry, as described before.[2]

RESULTS

Case Report

The male patient was diagnosed with OTC deficiency at the age of 5 months. At the age of 8 years, he presented with fever and lowered consciousness. According to the parents, he had been suffering from a mild upper respiratory infection since 3 days. The day before admission, a protein-free, high-caloric feeding had been started. Blood ammonia was only mildly increased (138 μM, controls <50 μM) and not corresponding to his decreased consciousness. An emergency treatment was started with intravenously administered glucose (10 mg/kg/min) and high-dosed sodium benzoate, arginine and carnitine to induce anabolism and optimize ammonia excretion. Despite this treatment, his clinical condition deteriorated resulting in complete coma, 6 hours after admission. On the suspicion of convulsions, treatment with diazepam, midazolam, and fenytoine was started and he was intubated and ventilated. The ammonia level was only 98 μM. After 24 hours, maintenance therapy with sodium phenylbutyrate and citrulline was restarted. The treatment with sodium benzoate was stopped for reasons of putative neurotoxicity, which did not result in a clinical improvement. In addition hemodialysis was started. The next day, an electrocephalogram revealed a strongly decreased basal pattern corresponding to the encephalopathy. Ammonia levels remained surprisingly low, with levels around 100 μM. Two days later clinical signs of cerebral oedema were observed and a brain CT revealed hypodensity, corresponding to encephalopathy. Despite mannitol treatment, brain death was established 4 days after admission and the patient died.

Metabolic Investigations

Metabolic studies showed normal plasma levels of glutamine (605 μmol/l) and decreased levels of arginine (15 μmol/l) upon admission. The next day, glutamine appeared to be strongly elevated in cerebrospinal fluid (2,400 μmol/l). However, a virtually normal glutamine concentration was observed in plasma (717 μmol/l) and the level of arginine remained low, despite high dose intravenous administration. Urine analysis revealed slightly increased levels of N-C-aspartate, orotate, and uridine in addition to strongly increased concentrations of uracil (Table 1, Urine I). The levels of N-C-aspartate, orotate, and uracil were strongly increased 2 days later and a progressive increase in these metabolites was observed during the subsequent hours Table 1, Urine II).

TABLE 1 Pyrimidine de novo Metabolites in Urine (μmol/mmol Creatinine) of a Patient with Ornithine Transcarbamylase Deficiency During Crisis

Compound	Urine I	Urine II (t = 0 h)	Urine II (t = 5 h)	Urine II (t = 8 h)	Controls (n = 155) Mean ± SD
N-C-Aspartate	5.3	83	99	127	0.8 ± 0.7
Dihydroorotate	<0.1	<0.5	<0.5	<0.3	0.01 ± 0.07
Orotate	11	215	311	777	1.2 ± 0.9
Orotidine	2.2	2.9	3	0.7	1.4 ± 1.0
Uridine	2.9	5	5.5	6.3	0.4 ± 0.7
Uracil	206	461	542	657	7.9 ± 6.0

n.d., not detectable.
N-C-Aspartate, N-carbamyl-aspartate.

DISCUSSION

The accumulation of carbamylphosphate, which is associated with inherited defects of the urea cycle, stimulates the pyrimidine de novo synthesis pathway, resulting in an increased production of orotate. In addition, some patients also showed strongly elevated concentrations of orotidine, uridine, and uracil.[2] In our study, a patient suffering from an OTC deficiency presented with strongly elevated urinary levels of N-C-aspartate, orotate, and uracil, which is in line with results obtained for other OTC deficient patients.[2] Remarkably, the concentration of uracil in urine of our patient is even comparable to that observed for patients with a complete deficiency of DPD.[2] Despite intensive therapy, the accumulation of pyrimidine de novo synthesis metabolites increased after admission, indicating progressive metabolic decompensation.

In patients with a defect in one of the enzymes of the urea cycle, the level of pyrimidine excretion usually correlates with the blood ammonia levels.[1] However, in our patient only moderately increased levels of ammonia and normal levels of glutamine were present in blood whereas massive amounts of pyrimidine de novo synthesis intermediates appeared in urine. In addition, the apparent discrepancy between the normal concentration of glutamine in plasma versus the highly elevated level in cerebrospinal fluid remains enigmatic. Our results show that the concentrations of the pyrimidine de novo synthesis intermediates and pyrimidine degradation products in urine are useful indicators, and possibly even more sensitive parameters than ammonia or glutamine levels for monitoring the metabolic condition of patients with an inborn error of the urea cycle.

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