原著 2

Urinary pyrimidine analysis in healthy newborns and newborns with urea cycle disorder

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ABSTRACT

Pyrimidine appears to be especially important in the neonatal period, but the reference ranges for urinary pyrimidines have not been reported. We measured urinary pyrimidines in 23 healthy newborns on 6 successive days after birth (from day 0 to day 6) and at 1-month of age. We also analyzed newborns with urea cycle disorders including ornithine transcarbamylase (OTC) deficiency. We measured urine samples using high-performance liquid chromatography with column switching. The reference ranges for urinary orotic acid, uracil and pseudouridine were determined. Orotic acid was greatly increased in OTC deficiency cases, but not in carbamoyl phosphate synthetase I deficiency. We speculate that urinary pyrimidine concentrations is a useful index for diagnosis in the neonatal period.

INTRODUCTION

Pyrimidine compounds are essential for synthesizing DNA and RNA in cells (Figure 1). It has been hypothesized that pyrimidines are especially important in the neonatal period due to higher cell proliferation during this period. The pyrimidine compounds present in mother's milk may also play a certain role¹⁾. However, the reference values in previous studies were below the detection limits²⁾ or they were calculated on the basis of a small number of samples³⁾.

Therefore, pyrimidine metabolism in the neonatal period requires further investigation.

Figure 1 Pyrimidine metabolism pathway. Upper; normal metabolism, lower; ornithine transcarbamylase (OTC) deficiency CP, carbamoyl phosphate: OA, orotic acid, NH₄, ammonia

Ornithine transcarbamylase (OTC) is an enzyme involved in the urea cycle. Patients with OTC deficiency exhibit increased excretion of urinary pyrimidines such as uracil and orotic acid^{4,5)}, due to accumulated carbamyl phosphate flowing into the pyrimi-

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dine metabolism pathway. Patients with OTC deficiency show wide clinical severities, from asymptomatic to lethal hyperammonemia. As the condition often occurs in the neonatal period, it is important to perform diagnoses at that time. Urinary orotic acid and uracil are a useful index for diagnosing older cases with OTC deficiency⁶⁾. Standardized data of pyrimidines in the neonatal period may be a useful index for diagnosing infant cases. We have already established a highly sensitive method for measuring urinary pyrimidines, using high-performance liquid chromatography³⁾. In this report we describe urinary pyrimidine concentrations in healthy newborns and in newborns with urea cycle disorder during the neonatal period.

EXPERIMENTAL

Controls

We collected single-voided urine samples from healthy newborns ($n=23$) for 7 successive days (from day 0 to day 6). Their gestation periods ranged from 37 weeks to 42 weeks, and their body weight at birth was 3076 ± 314 g (mean $\pm$ standard deviation). We also collected samples from 1-month-old infants ($n=54$) when they visited for a general medical check; their body weight was 4501 ± 575 g. We obtained informed consents from newborn's parents to take part in our study.

Patients with urea cycle disorders

Ornithine transcarbamylase (OTC) deficiency case 1:

The patient was born at 38 weeks of gestation with a body weight of 3560 g. As his brother was died from OTC deficiency⁷⁾, he was suspected as also having this disorder. He was admitted to NICU soon after birth, administered with water and glucose via drip infusion. As shown in Figure 2, urinary orotic acid was greatly elevated at day 0, whereas ammonia was not elevated (76 mg/dl). However, at day 1 ammonia increased to 236 mg/dl, and so treatment with benzoic acid, carnitine and arginine was started.

Ornithine transcarbamylase (OTC) deficiency case 2:

The patient was born at 38 weeks of gestation with a body weight of 3018 g. He had no asphyxia (Apgar score 9), but his activity became poor and convulsion appeared at day 2 after birth. We obtained urine samples at day 4 and ammonia was higher than 1000 mg/dl. The OTC activity in his liver tissue was less than 1% of controls, retrospectively.

Ornithine transcarbamylase (OTC) deficiency case 3:

The patient was born at 37 weeks of gestation with a body weight of 3070 g. He had no asphyxia (Apgar score 9), but he suffered from vomiting at day 4 after birth and showed convulsion at day 5. We obtained a urine sample at day 7 and ammonia was higher than 1000 mg/dl. The OTC activity in liver tissue was less than 1% of controls, retrospectively.

Carbamoyl phosphate synthetase I (CPS1) deficiency:

CPS1 deficiency is another urea cycle disorder accompanied with hyperammonemia. The patient⁸⁾ was born at 40 weeks of gestation with a body weight of 3118 g. On day 3 after birth, he showed lethargy, hypothermia, apnea and seizures. Ammonia was higher than 1000 mg/dl. CPS1 activity in liver tissue was decreased to 6% of controls.

Figure 2 Clinical and laboratory data of OTC deficiency case 1. A line; orotic acid, A dotted line; uracil.

Experimental

Urine samples were stored at -20°C . Immediately prior to analysis, each sample was passed through a $0.45\text{-}\mu\text{m}$ filter (Centricut, Kurabou, Osaka, Japan). Urinary orotic acid, uracil and pseudouridine were measured using our high-performance liquid chromatography method described previously³⁾. A reverse-phase column (Develosil ODS $150\times 6\text{mm}$ i.d.; particle size $5\text{ }\mu\text{m}$; Nomura Chemical, Seto, Japan) and an anion-exchange column (MCI GEL CK08EH, H^{+} form, $300\times 8\text{ mm}$ i.d.; particle size $9\text{ }\mu\text{m}$; Mitsubishi Kasei, Tokyo, Japan) were used. The columns were connected by a column switch, with all systems being controlled automatically by a system controller.

RESULTS

The pyrimidine concentrations in urine from healthy newborn are listed in Table 1. The orotic acid and uracil concentrations were relatively constant in the period (from day 0 to day 6), while the concentration of pseudouridine, an end product of RNA catabolism, increased gradually. As shown in Table 1, urinary levels of orotic acid and uracil in the OTC deficiency case 1 were an order of magnitude higher (178.4 and $20.1\text{ }\mu\text{mol}/\text{mmol}$ creatinine) than those in normal controls at day 0 (1.6 ± 0.7 and $1.0\pm 0.4\text{ }\mu\text{mol}/\text{mmol}$ creatinine, mean $\pm$ S.D.), while ammonia was not elevated ($76\text{ }\mu\text{g}/\text{dl}$). After treatments on this case started, orotic acid decreased but it was still higher than those in healthy newborns. The OTC deficiency cases 2 and 3 also showed increased orotic acid concentrations, but it was not elevated in the patient with CPS1 deficiency.

DISCUSSION

Orotic acid and uracil are intermediate compounds in the pyrimidine synthetic and pyrimidine catabolic pathways. It is difficult to measure the concentrations in serum because they are much lower than those in urine. The concentrations in urine may be associated with pyrimidine metabolism conditions. However, the

reference values in previous studies were below the detection limits²⁾ or they were calculated on the basis of a small number of samples³⁾. In this study we measured urinary pyrimidine such as orotic acid and uracil in a lot of urine samples from newborns. Unlike previous studies, we could measure and establish the reference ranges for these pyrimidines (Table 1). Pseudouridine, one of the pyrimidine compounds, is a modified nucleoside derived from RNA degradation, mainly tRNA. Since pseudouridine is excreted as an end product of RNA catabolism, it seems to be a faithful index of RNA turn over. It has been shown that patients with cancer or chronic viral infection excrete elevated amounts of pseudouridine in their urine. However, pseudouridine in the neonatal period has not been examined previously. Our results revealed that the pseudouridine in urine increased from day 0 to day 6 after birth. We speculate that RNA turn-over accelerates with the passage of time. Examining these pyrimidine levels in various types of neonatal patients may clarify their metabolic conditions.

Table 1 Urinary pyrimidine in healthy newborns and in urea cycle disorders

	Urinary pyrimidine ($\mu\text{mol}/\text{mmol}$ creatinine, mean $\pm$ S.D.)		
	Orotic acid	Uracil	Pseudouridine
<i>Healthy newborn</i>			
day 0	1.6 ± 0.7	1.0 ± 0.4	58.6 ± 19.6
1	1.3 ± 0.5	0.9 ± 0.4	88.7 ± 22.9
2	1.3 ± 0.9	1.3 ± 0.6	93.6 ± 26.0
3	1.4 ± 0.6	1.8 ± 0.8	99.6 ± 29.6
4	1.4 ± 0.6	2.1 ± 0.8	100.5 ± 29.6
5	1.6 ± 0.6	2.1 ± 1.0	101.8 ± 22.1
6	1.7 ± 0.5	1.8 ± 0.8	100.9 ± 27.7
1 month	2.1 ± 1.2	12.8 ± 7.8	138.5 ± 23.9
<i>Urea cycle disorder</i>			
OTC deficiency			
Case 1 day 0	178.4	20.1	91.6
OTC deficiency			
Case 2 day 4	797.7	646.0	135.8
OTC deficiency 3			
Case 3 day 7	1982.1	51.0	273.5
CPS deficiency			
day 3	n.d.	0.9	125.5

It is well known that urinary orotic acid and uracil are a useful index for diagnosing older cases with OTC deficiency. In this study we found that orotic acid and uracil were elevated in neonatal cases with OTC deficiency. As far as we are aware, the OTC deficiency case 1 is the first reported case in which orotic acid was measured before the onset of hyperammonemia. Interestingly, we found that orotic acid began to increase before ammonia increased. We speculate that the measurement of orotic acid predicts a risk of hyperammonemia, allowing early and better treatments thereof. On the other hand, neither orotic acid nor uracil were elevated in the case with CPS I deficiency. They may be a useful index for the diagnosis.

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健康新生児および尿素サイクル異常症における尿中ピリミジン分析

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ピリミジンは新生児期の代謝にとって重要な役割を担っていると考えられているが、この時期の尿中ピリミジンの基準値は確立されていない。今回我々は、健康な新生児23名について、生後0日から6日、および生後1ヶ月時に、随時尿を採取した。そして、尿中オロット酸、ウラシル、シュードウリジンを高速液体クロマトグラフィー／カラムスイッチング法を用いて測定して基準値を算出した。日齢0, 1, 2, 3, 4, 5, 6, 生後1ヶ月時の尿中オロット酸の濃度 ($\mu\text{mol}/\text{mmolcreatinine}$, $\text{mean} \pm \text{S.D.}$) は、 1.6 ± 0.7 , 1.3 ± 0.5 , 1.3 ± 0.9 , 1.4 ± 0.6 , 1.4 ± 0.6 , 1.6 ± 0.6 , 1.7 ± 0.5 , 2.1 ± 1.2 であった。尿中ウラシルの濃度は、 1.0 ± 0.4 , 0.9 ± 0.4 , 1.3 ± 0.6 , 1.8 ± 0.8 , 2.1 ± 0.8 , 2.1 ± 1.0 , 1.8 ± 0.8 , 12.8 ± 7.8 で

あった。尿中シュードウリジンの濃度は、 58.6 ± 19.6 , 88.7 ± 22.9 , 93.6 ± 26.0 , 99.6 ± 29.6 , 100.5 ± 29.6 , 101.8 ± 22.1 , 100.9 ± 27.7 , 138.5 ± 23.9 であった。

さらに、高アンモニア血症などの症状をきたす尿素サイクル異常症の新生児についても、尿中ピリミジンの分析を行った。オルニチントランスカルバミラーゼ欠損症では、尿中オロット酸、ウラシルとも異常高値を示し、特に、オロット酸はアンモニアの上昇する前から増加していた。一方、カルバミルリン酸合成酵素欠損症ではオロット酸などの増加は認められなかった。以上の結果より、尿中ピリミジンの測定は新生児期の尿素サイクル異常症の診断に有用であることを確認することができた。

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