

Rapid analysis of orotic acid in dried blood spots using liquid chromatography-tandem mass spectrometry (MS/MS)

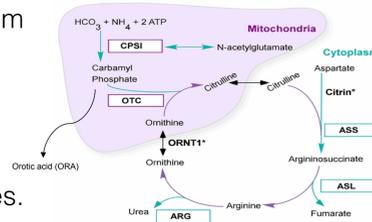
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Introduction

- The urea cycle disorders (UCDs) result from inherited molecular defects in metabolism of waste nitrogen produced by protein turnover and other nitrogen containing molecules. The waste nitrogen is converted to ammonia and transported to the liver where it is processed via urea cycle. Defect of enzyme in the urea cycle results in accumulation of ammonia by which the patient can rapidly develop cerebral edema and related signs of hypothermia, seizures, and death. UCDs include seven diseases. The incidence of UCDs is estimated at least 1 in 25,000 birth.
- Since late 1990's, analysis of acylcarnitines (AC) and amino acids (AA) using ESI-MS/MS has become popular in newborn screening (NBS). This screening is able to detect more than 20 types of inborn errors of metabolism, including two UCDs of argininosuccinic acid synthase deficiency (CIT1) and argininosuccinic aciduria (ASLD).
- We developed a simple analytical method of orotic acid (ORA), a diagnostic marker in ornithine transcarbamylase deficiency (OTCD) and also CIT1 or ASLD, on conventional method of NBS.



Results

Fig.1 Calibration curve of ORA

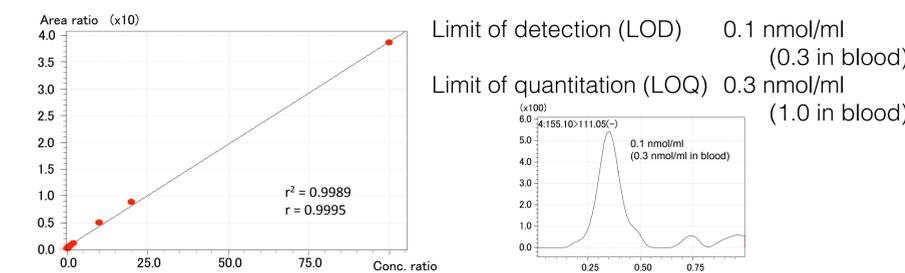
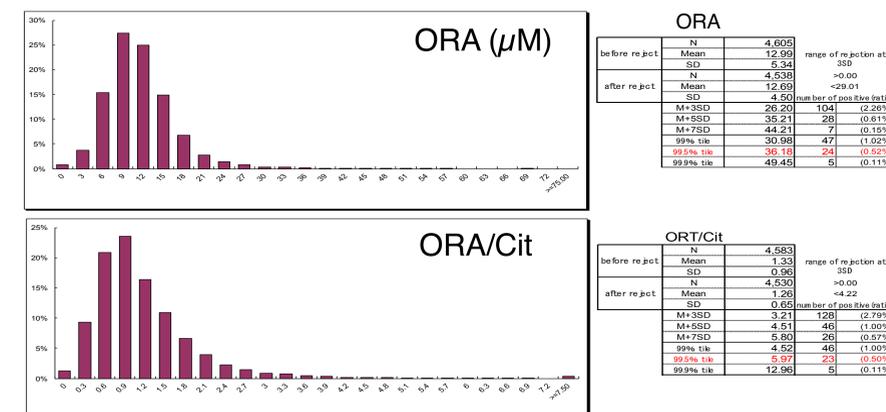


Table.1 Profiles of amino acids in pts with OTCD and CPS1D

	ORA	ORA/Cit	Arg	Cit	Met	Orn
1 OTCD (symptomatic)	400.68	71.7	17.25	5.59	55.06	80.32
2 OTCD (symptomatic)	185.72	17.9	24.21	10.38	11.77	225.64
3 OTCD (symptomatic)	201.34	78.3	92.85	2.57	33.36	109.45
4 OTCD (symptomatic, newborn)	674.14	>1000	5.00	0.00	26.15	124.45
5 OTCD (symptomatic, newborn)	2806.74	>1000	11.31	0.00	31.99	81.09
6 CPS1D	7.83	19.58	13.09	0.40	19.71	27.82
7 OTCD (pre-symptomatic, newborn)	8.83	1.60	4.82	5.51	7.81	79.21
8 OTCD (pre-symptomatic, newborn)	14.73	1.70	17.72	8.67	11.11	134.59
Mean+3SD	26.2	3.21	44.51	23.43	24.08	271.4

unit: nmol/ml

Fig2. Histogram of OA and OA/Cit ratio in NBS samples (n=4,605)



Methods

[Analytical system]

- MS/MS: LCMS-8040 triple quadrupole mass spectrometer (Shimadzu, Kyoto) equipped with an ESI positive and negative source
- LC: Nexera MP system with SIL-30ACMP multiplate autosampler (Shimadzu, Kyoto)

[Materials and reagents]

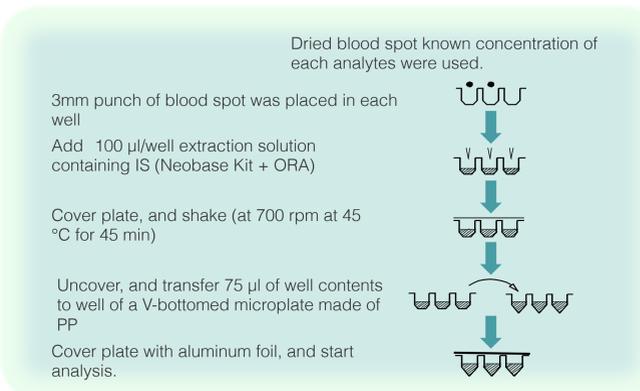
- Orotic acid (ORA) standard was purchased from Sigma-Aldrich (St. Louis, MO), and [1,3-15N₂]OA of internal standard (IS) was obtained from Cambridge Isotope Laboratories (Andover, MA, USA).
- NeoBase Non-derivatized MSMS Kit (PerkinElmer) was used for analysis of other amino acid and acylcarnitine.
- Other chemicals were obtained from Wako (Japan).

[Analytical methods]

- multiple reaction monitoring (MRM) mode
- ESI : positive mode: Neobase Kit, **negative mode: ORA**
- Flow rate of mobile phase:

Time	Flow
0 min	0.1 mL/min
0.1 min	0.05 mL/min
0.65 min	0.1 mL/min
0.66 min	1 mL/min
1 min	1 mL/min
- MS Interface Parameter
 - DL temp. : 250 °C
 - Nebulizing Gas Flow : 3 L/min
 - Heat Block Temp. : 400 °C
 - Drying Gas Flow : 12 L/min
- Sample injection volume: 1 μl
- Software : Lab Solutions Neonatal Solution (Shimadzu, Kyoto)
- MRM settings:

ORA m/z	155.10>111.05
ORA IS m/z	157.10>113.05



SUMMARY

- Quantification of orotic acid (ORA) can be added on newborn screening method using combination of negative mode without extending analytical time.
- In patients with OTCD, the values of ORA were significantly increased at symptomatic status, although the difference between pre-symptomatic patients with OTCD and normal controls in ORA level was not seen.
- Quantification of ORA should be useful for distinguished OTCD from CPS1D, when value of citrulline is relatively low. Further analysis of samples from patients with UCDs is required.

Summary of Results

- ORA in dried blood spot could be measured in negative mode, and the quantity range was about 0.1 to 100 nmol/ml.
- The value of ORA has significant difference between controls and symptomatic pts with OTCD.
- The value of ORA was not increased in asymptomatic pts with OTCD.

Discussion & Conclusions

- Why ORA was not elevated significantly in two asymptomatic neonatal patients with OTCD?
 - ❖ Low protein load? residual enzyme activity?
 - ❖ Further analysis in asymptomatic pts with OTCD is expected.
- In symptomatic situation, analysis of ORA was useful to distinguish OTCD from CPS1D.
- Analysis of ORA requires negative mode, while conventional amino acids and acylcarnitines were analyzed in positive mode. The LC-8040, a popular MS/MS in NBS field, has polarity switching rate of 15 msec. This rapid switching rate made it possible to analyze ORA without extending analytical time (about 14 point/analysis).