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Metabolomic study of aging in mouse plasma by gas chromatography–mass spectrometry

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ABSTRACT

Metabolomic analysis of aging was performed in plasma samples of young (8 weeks) and old (72 weeks) mice as ethoxycarbonyl/methoxime/*tert*-butyldimethylsilyl derivatives by gas chromatography–mass spectrometry (GC–MS). As new approaches, study of altered metabolism from aging was attempted by simultaneous profiling analysis of amino acids (AAs), organic acids (OAs) and fatty acids (FAs) by GC–MS in a single run combined with pattern analysis. As a result, 27 amino acids (AAs), 17 organic acids (OAs) and 24 fatty acids (FAs) were positively screened with large variations in plasma samples. Among altered metabolites, levels of six AAs (proline, methionine, 4-hydroxyproline, pipecolic acid, glutamic acid, α-aminoadipic acid) as neurotransmetters and nutrients, five OAs (2-hydroxybutyric acid, 2-hydroxyglutaric acid, *cis*-aconitic acid citric acid, isocitric acid) including intermediate metabolites in the TCA cycle, and three n-3 polyunsaturated FAs (PUFAs) of α-octadecatrienoic acid, eicosapentaenoic acid and docosahexaenoic acid as potential biomarkers were significantly different between young and old groups. Their levels were normalized to the corresponding mean values of the young group and then plotted into star symbol patterns, which were clearly distinct compared with numerical data and readily distinguishable for young and old groups. Thus, the present metabolomic screening and the star pattern recognition method might be useful for understanding the complexity of biochemical events in aging.

1. Introduction

The aging process involves genetic and environmental factors and is associated with alterations of cellular function and various biochemicals [1,2]. Generally, changes of metabolites in biological samples are related with disease states [3–6]. Recently, endogenous metabolites correlated with aging process have been reported by metabolomic studies as new approaches in biological samples [2,3,5,6]. Especially, altered amino acids (AAs), organic acids (OAs) and fatty acids (FAs) as metabolites were reported as potential biomarkers for aging in biological samples from mouse [5] and human [2,6]. These metabolites are closely interrelated in metabolic pathways. Thus, metabolic profiling analysis of AAs, OAs and FAs has become an important topic in metabolic studies. In the recent reports, metabolomic studies of aging have been attempted by gas chromatography-mass spectrometry (GC–MS), liquid chromatography-MS (LC–MS) and LC–MS/MS [2,3,5,6]. However, altered metabolism from aging was not investigated by simultaneous profiling analysis of AAs, OAs and FAs by GC–MS in single run combined with star pattern analysis in biological fluids.

In our previous reports [7,8], ethoxycarbonylation (EOC), methoximation (MO) and *tert*-butyldimethylsilyl (TBDMS) derivatives were effective for simultaneous profiling analysis of AAs, OAs and FAs from urine samples in a single run by gas chromatography (GC) and GC–mass spectrometry (MS). We also developed methods for accurate determination of AAs as EOC/TBDMS [9], OAs as MO/TBDMS [10,11] and FAs as TBDMS derivatives [12,13] by GC–MS in the selected ion monitoring (SIM) mode. Then these were applied to biological samples from patients with bladder [14], gastric cancer [15], Alzheimer's disease [16], Parkinson's disease [17], and stroke [12] for biochemical monitoring and detection of new biomarkers.

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Table 1
Altered amino acid levels in mouse plasma samples of young and old groups.

No.	Amino acid	μg/100 μL of plasma (mean ± SD)		P value[a]	Normalized mean values[b]	
		8 weeks (n = 4)	72 weeks (n = 3)		8 weeks (n = 4)	72 weeks (n = 3)
1	Alanine	4.84 ± 0.44	4.09 ± 0.70	0.071	1.0	0.85 ± 0.14
2	Glycine	4.45 ± 0.71	4.24 ± 0.96	0.376	1.0	0.95 ± 0.22
3	α-Aminobutyric acid	0.06 ± 0.02	0.09 ± 0.02	0.090	1.0	1.39 ± 0.34
4	Valine	2.72 ± 0.60	2.26 ± 0.28	0.138	1.0	0.83 ± 0.10
5	Leucine	3.80 ± 2.13	5.62 ± 1.07	.0.120	1.0	1.48 ± 0.28
6	Isoleucine	0.82 ± 0.21	0.72 ± 0.11	0.243	1.0	0.88 ± 0.13
7	Proline	1.79 ± 0.38	1.11 ± 0.26	0.024	1.0	0.62 ± 0.15
8	γ-Aminobutyric acid	0.005 ± 0.001	0.011 ± 0.010	0.146	1.0	2.10 ± 1.91
9	Pipecolic acid	0.025 ± 0.002	0.039 ± 0.008	0.008	1.0	1.55 ± 0.30
10	Pyroglutamic acid	3.46 ± 1.52	2.55 ± 0.51	0.187	1.0	0.74 ± 0.15
11	Methionine	0.67 ± 0.11	0.52 ± 0.05	0.041	1.0	0.78 ± 0.08
12	Serine	20.2 ± 8.3	16.7 ± 13.5	0.343	1.0	0.83 ± 0.67
13	Phenylalanine	0.23 ± 0.07	0.29 ± 0.19	0.284	1.0	1.28 ± 0.85
14	Cysteine	0.57 ± 0.42	0.73 ± 0.74	0.363	1.0	1.28 ± 1.31
15	Aspartic acid	0.07 ± 0.01	0.06 ± 0.02	0.126	1.0	0.81 ± 0.22
16	N-Methyl-DL-aspartic acid	0.01 ± 0.00	0.01 ± 0.00	0.208	1.0	0.93 ± 0.15
17	4-Hydroxyproline	3.59 ± 0.76	1.45 ± 0.23	0.003	1.0	0.40 ± 0.06
18	Homocysteine	0.16 ± 0.08	0.19 ± 0.10	0.379	1.0	1.14 ± 0.60
19	Glutamic acid	0.77 ± 0.09	1.11 ± 0.13	0.005	1.0	1.44 ± 0.17
20	Asparagine	0.21 ± 0.11	0.12 ± 0.06	0.133	1.0	0.57 ± 0.28
21	Ornithine	1.05 ± 0.45	1.51 ± 0.15	0.079	1.0	1.44 ± 0.14
22	α-Aminoadipic acid	0.05 ± 0.01	0.08 ± 0.02	0.018	1.0	1.68 ± 0.38
23	Glutamine	7.43 ± 2.17	8.00 ± 1.67	0.361	1.0	1.08 ± 0.22
24	Lysine	4.85 ± 1.72	4.59 ± 1.32	0.417	1.0	0.95 ± 0.27
25	Histidine	5.15 ± 1.12	5.00 ± 1.24	0.436	1.0	0.97 ± 0.24
26	Tyrosine	0.10 ± 0.05	0.17 ± 0.22	0.286	1.0	1.63 ± 2.09
27	Tryptophan	0.06 ± 0.02	0.08 ± 0.02	0.106	1.0	1.36 ± 0.36

[a] Student's *t*-test at 95% confidence level of young and old groups.
[b] Values normalized to the corresponding mean values of young group.

In addition, a pattern recognition method is needed to accurately discriminate between normal and disease states in a complex metabolic profile. In our previous reports, star symbol plotting was useful for visual monitoring and discrimination of normal and disease states [12,14–16].

Thus, in this work, GC–MS-SIM combined with star symbol plotting method as new approaches was applied to the monitoring of altered AAs, OAs, and FAs in plasma samples of young (8 weeks) and old (72 weeks) mice.

2. Materials and methods

2.1. Chemicals and reagents

AAs, OAs, FAs, methoxyamine hydrochloride, ethyl chloroformate (ECF) and triethylamine (TEA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). *N*-Methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) was obtained from Pierce (Rockford, IL, USA). Toluene, diethyl ether, ethyl acetate, dichloromethane and sodium chloride of pesticide grade obtained from Kanto Chemical (Tokyo, Japan). All other chemicals of analytical grade used as received.

2.2. Preparation of standard solutions

Standard stock solutions were individually prepared at 10 μg/μL of AAs including norvaline as internal standard (IS) in 0.1 M HCl, OAs including 3,4-dimethoxybenzoic acid as IS, and FAs including pentadecanoic acid as IS in methanol. Working solutions of 0.1 μg/μL were then prepared by diluting each stock solution with 0.1 M HCl for AAs and with methanol for OAs and FAs, respectively. All standard solutions were stored at 4 °C.

2.3. Plasma samples from young and old mice

Young (8 weeks, n = 4) and old (72 weeks, n = 3) female C57BL/6 N mice (Koatech, Pyeongtaek, Republic of Korea) were used for metabolomics study. Blood obtained after all mice sacrificed, then plasma was isolated and immediately frozen at −70 °C until analyzed. This study was carried out with the permission of the ethics committee on animal experiments of the Sunchon National University (Suncheon, Republic of Korea).

2.4. Gas chromatography-mass spectrometry

GC–MS analysis SIM mode was performed using an Agilent 7890 N gas chromatograph, interfaced with an 5975C mass-selective detector (70 eV, electron impact mode) and installed with an Ultra-2 (5% phenyl-95% methylpolysiloxane bonded phase; 25 m × 0.20 mm i.d., 0.11 μm film thickness) cross-linked capillary column (Agilent Technologies, Atlanta, GA, USA). The temperatures of injector, interface and ion source were 260, 300, and 230 °C, respectively. Helium used as the carrier gas at a flow rate of 0.5 mL/min in constant flow mode. Samples were introduced in split-injection mode (10:1), and the oven temperature was initially maintained at 100 °C for 2 min and then programmed to 300 °C (10 min) at a rate of 5 °C/min. In scanning mode, the mass range was 50–750 u at a rate of 0.43 scans/s.

2.5. Sample preparation for simultaneous profiling analysis of AAs, OAs and FAs in plasma

AAs, OAs and FAs in mouse plasma samples were determined as their EOC/MO/TBDMS derivatives according to our previously described methods [7,8]. Briefly, proteins were removed by adding 1 mL of acetonitrile to 0.1 mL of plasma containing 0.1 μg of norvaline, 3,4-dimethoxybenzoic acid and pentadecanoic acid as ISs. Following centrifugation, 1 mL of distilled water added to the