図 7

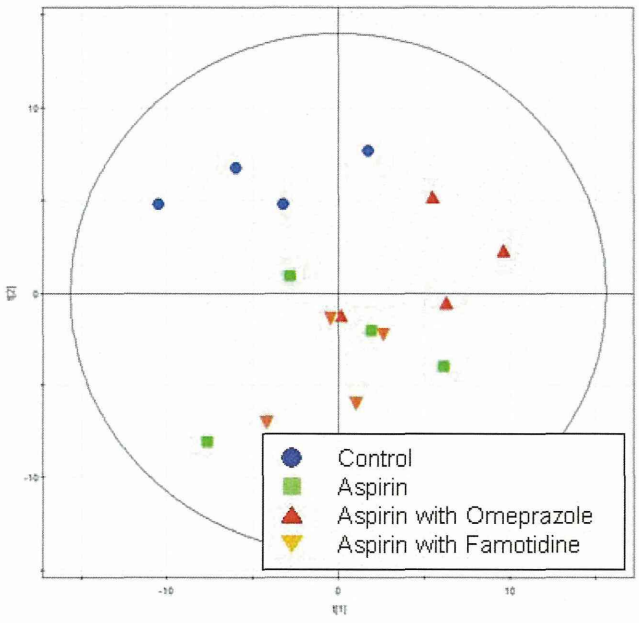
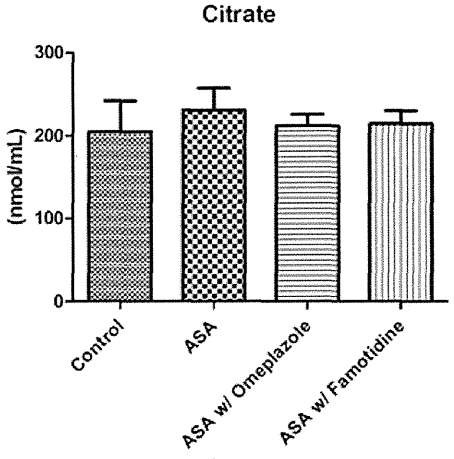
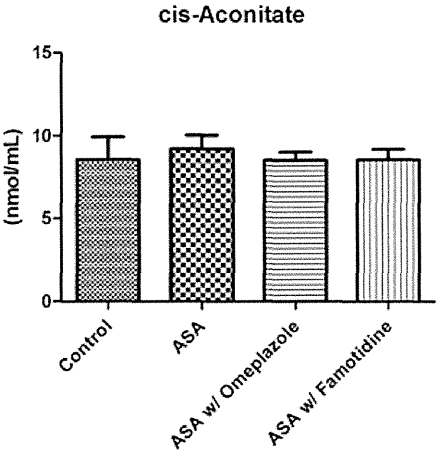

Control
Aspirin
Aspirin with Omeprazole
Aspirin with Famotidine

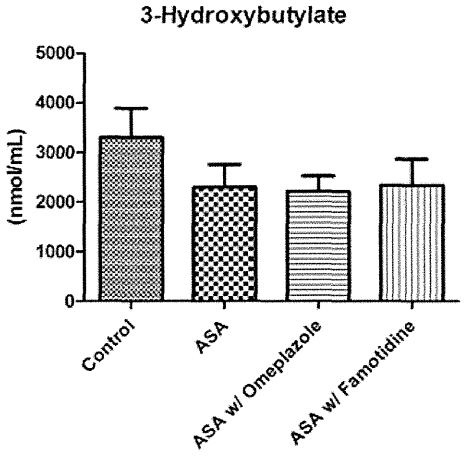
図 8

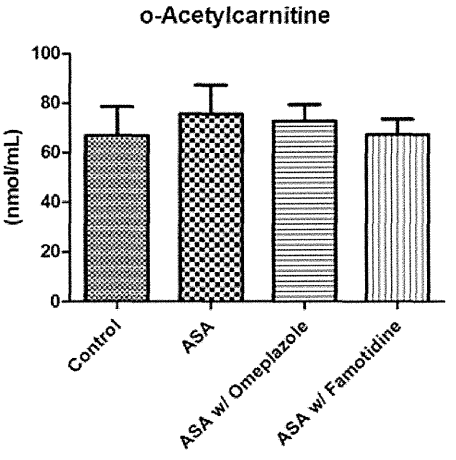
TCA Cycle



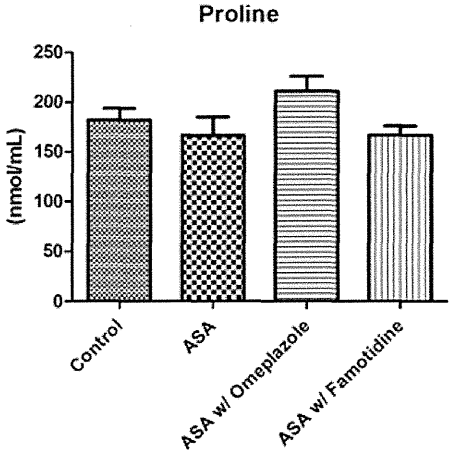


beta-Oxidization





Collagen metabolism



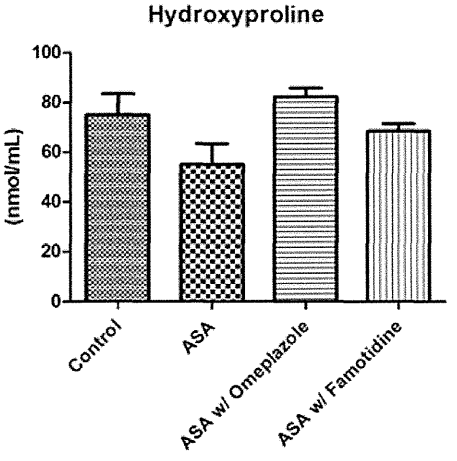


図 9

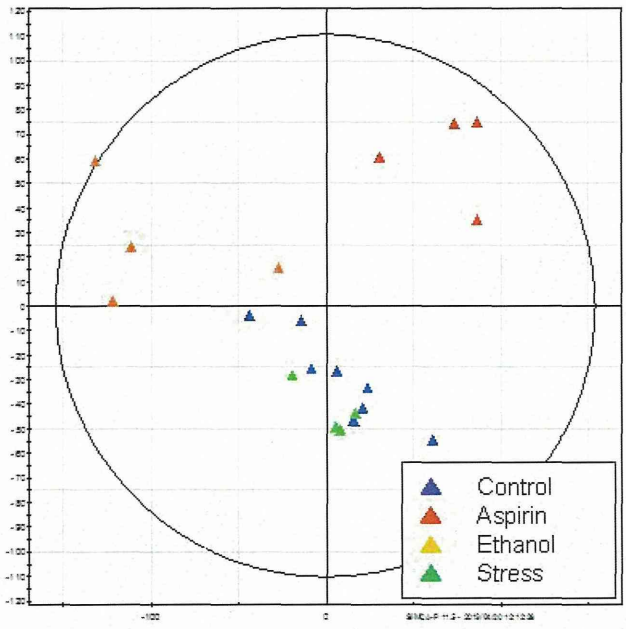


図 10

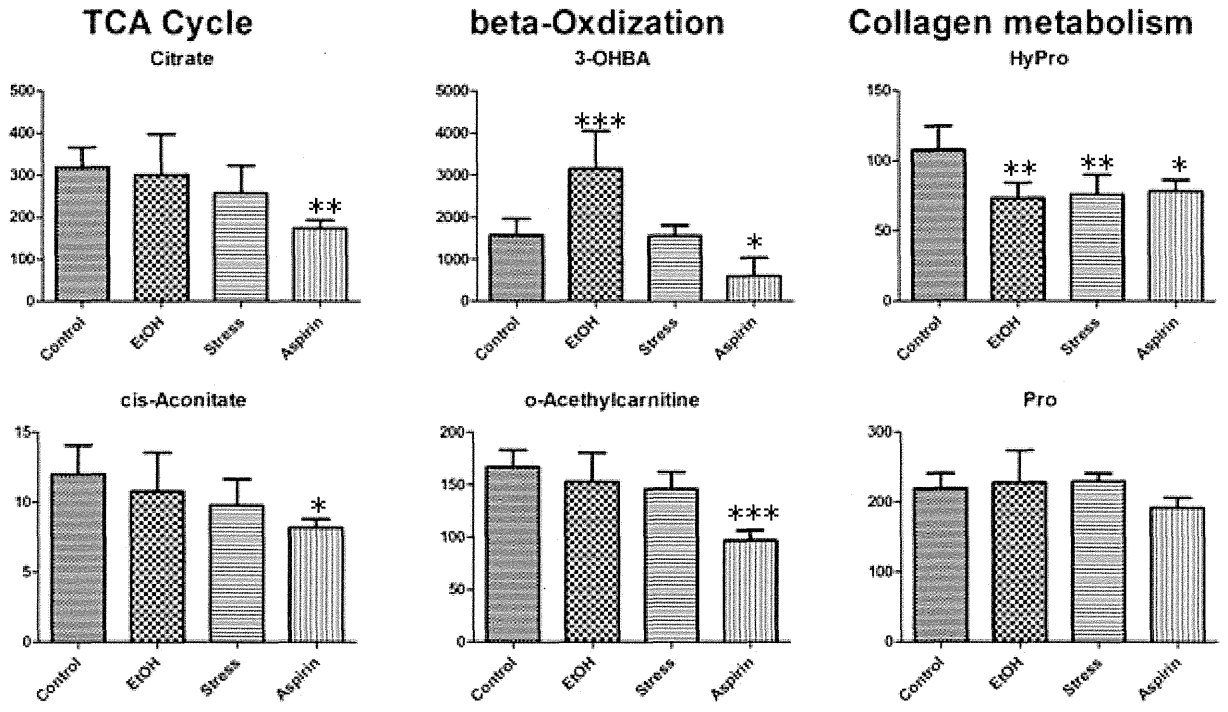
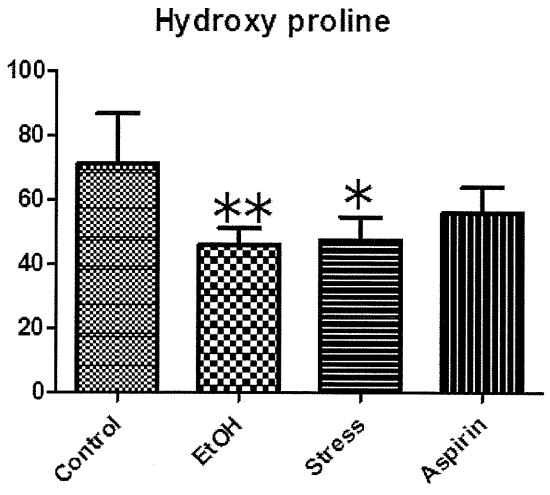

TCA Cycle
Citrate
500
400
300
200
100
0
**
Control
EtOH
Stress
Aspirin
beta-Oxdization
3-OHBA
5000
4000
3000
2000
1000
0
***
*
Collagen metabolism
HyPro
150
100
50
0
**
**
*
cis-Aconitate
15
10
5
0
*
o-Acethylcarnitine
200
150
100
50
0
***
Pro
300
200
100
0

図 11


Hydroxy proline
100
80
60
40
20
0
**
*
Control
EtOH
Stress
Aspirin

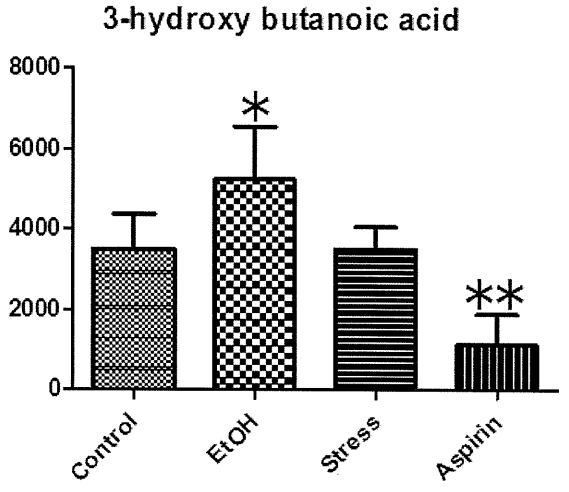

3-hydroxy butanoic acid
8000
6000
4000
2000
0
*
**
Control
EtOH
Stress
Aspirin

表 1　代謝物質標準液濃度

	各代謝物質濃度（μM）	内部標準物質(IS)濃度（μM）
陽イオン ALLSTD	20	200
陽イオン 112STD	20	200
陰イオン ALLSTD	20	200
陰イオン 112STD	50	200
陰イオン 追加依頼 3 物質 (アスピリン、サリチル酸、イブプロフェン)	50	200

表 2 胃潰瘍の面積（実験-1）

	Control	Aspirin		Ibuprofen	
		3 mg/kg	300 mg/kg	8 mg/kg	800 mg/kg
1 hr	ND (0/4)	ND (0/4)	ND (0/4)	ND (0/4)	ND (0/4)
5 hr	ND (0/4)	ND (0/4)	2.48 ± 1.14 (4/4)	ND (0/4)	18.08 ± 18.72 (4/4)
24 hr	ND (0/4)	ND (0/4)	0.08 ± 0.15 (1/4)	ND (0/4)	3.76 ± 4.33 (4/4)

Data are expressed as mean + standard deviation of the area of ulceration (mm2)

Values in parentheses are the incidence rate of animals with gastric ulceration.

ND: not detected

表 3　抽出された低分子代謝物の臓器内濃度の群平均及び有意差検定の結果

			Aspirin		Ibuprofen	
Metabolite	Time point	Control	3 mg/kg	300 mg/kg	8 mg/kg	800 mg/kg
Citrate	1 hr	194±21	202±23	120±25 (**)	202±26	135±39 (*)
	5 hr	190±18	187±33	120±8 (**)	172±23	124±19 (**)
	24 hr	269±75	203±42	213±50	175±60	169±52
Cis-aconitate	1 hr	4.7±0.3	4.4±0.5	2.8±0.7	4.6±0.6	3.6±1.0
	5 hr	5.4±0.6	4.6±1.0	3.1±0.4 (**)	4.1±1.0 (*)	3.1±0.9 (**)
	24 hr	7.7±2.0	5.6±0.7	5.7±1.0	4.9±1.6 (*)	3.7±1.1 (**)
Succinate	1 hr	235±34	252±30	227±28	229±29	139±27 (**)
	5 hr	220±7	231±14	195±8 (*)	205±11	155±11 (**)
	24 hr	349±60	323±44	295±80	270±110	278±87
o-Acetyl carnitine	1 hr	201±14	196±9	150±20 (*)	206±36	149±29 (*)
	5 hr	205±9	198±39	128±13 (**)	164±12	131±25 (**)
	24 hr	237±41	237±33	193±41	195±75	145±39
3-Hydroxybutanoic acid	1 hr	565±141	575±110	285±67 (*)	445±234	188±60 (**)
	5 hr	690±326	610±211	206±40 (*)	394±130	404±134
	24 hr	596±298	397±217	317±227	326±206	175±70 (*)
Proline	1 hr	339±23	325±28	274±24 (**)	329±25	317±18
	5 hr	274±23	303±24	247±10	292±22	233±17 (*)
	24 hr	434±78	433±35	428±131	367±134	385±98
Hydroxyproline	1 hr	158±14	130±10 (*)	113±8 (**)	128±19 (*)	130±10 (*)
	5 hr	118±23	128±13	65±11 (**)	118±24	89±21 (*)
	24 hr	171±46	154±28	145±36	122±37	100±26

Mean concentration (nmol/g tissue) and standard deviation.
Asterisks indicate statistically significant differences. **, $p<0.01$ *, $p<0.05$.

表 4　抽出された低分子代謝物の血中濃度の群平均及び有意差検定の結果

Metabolite	Time point	Control	Aspirin		Ibuprofen		Correlation
			3 mg/kg	300 mg/kg	8 mg/kg	800 mg/kg	
Citrate	1 hr	112±5	99±10	99±14	112±13	113±8	p<0.01
	5 hr	116±9	106±10	97±13	103±6	100±10	
	24 hr	106±7	96±6	94±18	96±5	84±3	
Cis-aconitate	1 hr	5.3±0.2	5.0±0.8	4.7±0.5	5.4±0.5	5.7±0.6	p<0.01
	5 hr	5.8±0.6	5.6±0.6	4.2±0.3 (**)	5.3±0.5	5.1±0.4	
	24 hr	5.8±0.9	5.4±0.1	5.0±0.8	5.5±0.1	5.2±0.3	
Succinate	1 hr	20±2	19±3	18±1	18±1	18±2	NS
	5 hr	22±2	20±1 (*)	24±1	20±1	19±0 (**)	
	24 hr	19±1	19±2	17±1	18±2	21±3	
o-Acetyl carnitine	1 hr	11.4±1.5	11.6±1.6	11.8±4.4	12.4±2.1	8.8±1.4	p<0.01
	5 hr	15.6±3.9	14.3±1.6	8.7±1.0 (**)	9.8±1.3 (*)	7.3±2.6 (**)	
	24 hr	15.1±3.6	17.0±2.6	15.1±0.7	20.6±8.9	8.0±2.4	
3-Hydroxy butanoic acid	1 hr	1299±241	1253±130	579±101 (**)	983±343	466±152 (*)	p<0.01
	5 hr	1515±731	1260±261	320±102 (**)	880±203 (*)	660±213 (**)	
	24 hr	971±477	725±235	501±299	699±293	311±128 (*)	
Proline	1 hr	160±18	157±19	116±18 (**)	137±13	135±14	p<0.01
	5 hr	150±14	141±10	118±14 (*)	149±16	104±16 (**)	
	24 hr	138±5	153±13	139±26	140±10	127±20	
Hydroxyproline	1 hr	57±5	51±5	40±8 (**)	50±3	51±3	p<0.01
	5 hr	52±6	53±4	28±4 (**)	52±6	41±5 (*)	
	24 hr	47±5	46±10	38±10	43±5	30±8 (*)	

Mean concentration (nmol/mL) and standard deviation.

Asterisks indicate statistically significant differences. **, p<0.01 *, p<0.05.

NS: not significant

表 5　胃潰瘍の面積（実験-2）

Group	Control	Aspirin	Aspirin with Omeprazole	Aspirin with Famotidine
Test article	Vehicle	Aspirin 100mg/kg	Aspirin 100mg/kg	Aspirin 100mg/kg
Co-administration (administrated 30 min. before test article administration)	Vehicle	Vehicle	Omeprazole 60 mg/kg	Famotidine 5mg/kg
Number of animals	4	4	4	4
Severity of gastric ulceration	ND (0/4)	0.863 ± 0.426 (4/4)	ND (0/4)	ND (0/4)

Data are expressed as mean + standard deviation of the area of ulceration (mm2)
Values in parentheses are the incidence rate of animals with gastric ulceration.
ND: not detected

表 5　胃潰瘍の発現例数(実験-2)

Group / Model	Control	Aspirin	Ethanol	Stress
Test article	Vehicle	Aspirin 100mg/kg	Ethanol 5mL/kg	Vehicle
Experimental room temperature	Room temperature	Room temperature	Room temperature	Cold room (4℃)
Number of animals	8	4	4	4
Gastric ulceration	ND	4/4	4/4	4/4

Values are the incidence rate of animals with gastric ulceration.
ND: not detected

参考文献

CHOI, E. Y., HWANG, H. J., KIM, I. H. & NAM, T. J. 2009. Protective effects of a polysaccharide from Hizikia fusiformis against ethanol toxicity in rats. *Food Chem Toxicol,* 47, 134-9.

GABRIEL, S. E. & FEHRING, R. A. 1992. Trends in the utilization of non-steroidal anti-inflammatory drugs in the United States, 1986-1990. *J Clin Epidemiol,* 45, 1041-4.

GABRIEL, S. E., JAAKKIMAINEN, L. & BOMBARDIER, C. 1991. Risk for serious gastrointestinal complications related to use of nonsteroidal anti-inflammatory drugs. A meta-analysis. *Ann Intern Med,* 115, 787-96.

HASEBE, T. 1987. Collagen and collagenase in ulcer tissue--2. Restraint and water immersion induced gastric lesions and effects of cimetidine and misoprostol. *Tokai J Exp Clin Med,* 12, 181-90.

HASEBE, T., HARASAWA, S., MIWA, T., SHIBATA, T. & INAYAMA, S. 1987. Collagen and collagenase in ulcer tissue--1. The healing process of acetic acid ulcers in rats. *Tokai J Exp Clin Med,* 12, 147-58.

HAWKEY, C. J. 2000. Nonsteroidal anti-inflammatory drug gastropathy. *Gastroenterology,* 119, 521-35.

HONDA, K., FUKUDA, S., ISHIKAWA, S. E., KUZUYA, T. & SAITO, T. 1994. Role of endogenous vasopressin in development of gastric ulcer induced by restraint and water immersion. *Am J Physiol,* 266, R1448-53.

SHAKER, E., MAHMOUD, H. & MNAA, S. 2010. Anti-inflammatory and anti-ulcer activity of the extract from Alhagi maurorum (camelthorn). *Food Chem Toxicol,* 48, 2785-90.

SOMASUNDARAM, S., RAFI, S., HAYLLAR, J., SIGTHORSSON, G., JACOB, M., PRICE, A. B., MACPHERSON, A., MAHMOD, T., SCOTT, D., WRIGGLESWORTH, J. M. & BJARNASON, I. 1997. Mitochondrial damage: a possible mechanism of the "topical" phase of NSAID induced injury to the rat intestine. *Gut,* 41, 344-53.

STEINMEYER, J. 2000. Pharmacological basis for the therapy of pain and inflammation with nonsteroidal anti-inflammatory drugs. *Arthritis Res,* 2, 379-85.

SZEWCZYK, A. & WOJTCZAK, L. 2002. Mitochondria as a pharmacological target. *Pharmacol Rev,* 54, 101-27.

TAKEDA, M., TAKAGI, T., YASHIMA, Y. & MAENO, H. 1982. Effect of a new potent H2-blocker, 3-[[[2-(diaminomethylene)amino]-4-thiazolyl]methyl]thio]-N2-sulfamoyl propionamidine (YM-11170), on gastric secretion, ulcer formation and weight of male accessory sex organs in rats. *Arzneimittelforschung,* 32, 734-7.

UM, S. Y., PARK, J. H., CHUNG, M. W., KIM, K. B., KIM, S. H., CHOI, K. H. & LEE, H. J. 2012. Nuclear magnetic resonance-based metabolomics for prediction of gastric damage induced by indomethacin in rats. *Anal Chim Acta,* 722, 87-94.

WALLACE, J. L. & GRANGER, D. N. 1996. The cellular and molecular basis of gastric mucosal defense. *FASEB J,* 10, 731-40.

研究成果の刊行に関する一覧表

雑誌　　2009年度

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Soga, T., Igarashi, K., Itoh, C., Mizobuchi, K., Zimmermann, H., Tomita, M.	"Metabolomic Profiling of Anionic Metabolites by Capillary Electrophoresis Mass Spectrometry"	Anal. Chem.	81(15)	6165-6174	2009
曽我朋義	メタボロミクス－網羅的代謝物質解析の医学・医療へのあらたな応用	医学のあゆみ	231(12,13)	1143	2009
平山明由、曽我朋義	ヒト癌組織のメタボローム解析	医学のあゆみ	231(12,13)	1145-1149	2009
曽我朋義	メタボローム測定装置の発明	発明 The Invention	106(11)	29-31	2009

研究成果の刊行に関する一覧表

雑誌　　2010年度

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Husain, A., Sato, D., Jeelani, G., Suematsu, M., **Soga, T.**, Nozaki, T.	"Metabolome Analysis Revealed Increase in S-methylcysteine and Phosphatidylisopropanolamine Synthesis upon L-cysteine Deprivation in The Anaerobic Protozoan Parasite *Entamoeba Histolytica*"	J. Biol. Chem.	285(50)	39160-39170	2010
Sugimoto, M., Hirayama, A., Robert, M., Abe, S., **Soga, T.**, Tomita, M.	"Prediction of metabolite identity from accurate mass, migration time prediction and isotopic pattern information in CE-TOFMS data"	Electrophoresis	31(14)	2311-2318	2010
Sugimoto, M., Hirayama, A., Ihiskawa, T., Baran, R., Robert, M., Uehara, K., **Soga, T.**, Tomita, M.	"Differential Metabolomics Software for Capillary Electrophoresis-Mass Spectrometry Data Analysis"	Metabolomics	6(1)	27-41	2010
Horai, H., Arita, M., Kanaya, S., Nihei, Y., Ikeda, T., Suwa, K., Ojima, Y., Tanaka, K., Tanaka, S., Aoshima, K., Oda, Y., Kakazu, Y., Kusano, M., Tohge, T., Matsuda, F., Sawada, Y., Horai, Y. M., Nakanishi, H., Ikeda, K., Akimoto, N., Maoka, T., Takahashi, H., Ara, T., Sakurai, N., Suzuki, H., Shibata, D., Neumann, S., Iida, T., Tanaka, K., Funatsu, K., Matsuura, F., **Soga, T.**, Taguchi, R., Saito, K., Nishioka, T.	"MassBank: A Public Repository for Sharing Mass Spectral Data for Life Sciences"	J. Mass Spectro.	45(7)	703-714	2010
曽我朋義	CE-MS法によるメタボローム解析と医薬への応用	遺伝子医学MOOK	19号	66-70	2011
曽我朋義	CE-MSメタボロミクスの最新動向	ぶんせき	461(11)	585-590,	2010

研究成果の刊行に関する一覧表

雑誌　　2011年度

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Toyoda, Y., Tamai, M., Kashikura, K., Kobayashi, S., Fujiyama, Y., **Soga, T.,** Tagawa, Y.	"Acetaminophen-induced Hepatotoxicity in a Liver Tissue Model Consisting of Primary Hepatocytes Assembling Around an Endothelial Cell Network"	Drug Metab. Dispos.	40(1)	169-177	2012
Sugimoto, M., Kawakami, M., Robert, M., **Soga, T.,** Tomita, M.	"Bioinfomatics Tools for Mass Spectroscopy-Based Metabolomic Data Processing and Analysis"	Curr. Bioinf.	7(1)	96-108	2012
Sugimoto, M., Ikeda, S., Niigata, K., Tomita, M., Sato, H., **Soga, T.**	"MMMDB: Mouse Multiple Tissue Metabolome Database"	Nucl. Acids Res.	40 (D1)	D809-D814	2012
Iino, K., Sugimoto, M., **Soga, T.,** Tomita, M.	"Profiling of the Charged Metabolites of Traditional Herbal Medicines Using Capillary Electrophoresis time-of-flight Mass Spectrometry"	Metabolomics	8(1)	99-108	2012
Soga, T., Sugimoto, M., Honma, M., Mori, M., Igarashi, K., Kashikura, K., Ikeda, S., Hirayama, A., Yamamoto, T., Yoshida, H., Otsuka, M., Tsuji, S., Yatomi, Y., Sakuragawa, T., Watanabe, H., Nihei, K., Saito, T., Kawata, S., Suzuki, H., Tomita, M., Suematsu, M.	"Serum Metabolomics Reveals γ-glutamyl Dipeptides as Biomarkers for discrimination among Different Forms of Liver Disease"	J. Hepatol.	55(4)	896-905	2011
Deng, L., Shoji, I., Ogawa, W., Kaneda, S., **Soga, T.,** Jiang, D., Ide, Y., Hotta, H.	"Hepatitis C Virus Infection Promotes Hepatic Gluconeogenesis through an NS5A-Mediated, FoxO1-Dependent Pathway"	J. Virol.	85(17)	8556-8568	2011
Hayashi, K., Sasamura, H., Hishiki, T., Suematsu, M., Ikeda, S., **Soga, T.,** Itoh, H.	"Use of Serum and Urine Metabolome Analysis for the Detection of Metabolic Changes in Patients with Stage 1-2 Chronic Kidney Disease"	Nephro-Urol. Mon.	3(3)	164-171	2011

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Suzuki, T., Toyohara, T., Akiyama, Y., Takeuchi, Y., Mishima, E., Suzuki, C., Ito, S., **Soga, T.,** Abe, T.	"Transcriptional Regulation of Organic Anion Transporting Polypeptide SLCO4C1 as a New Therapeutic Modality to Prevent Chronic Kidney Disease"	J. Pharm. Sci.	100(9)	3696-3707	2011
平山明由、曽我朋義	CE-MSメタボローム測定法	Diabetes Frontier	22(6)	pp673-678	2011
曽我朋義	新規酸化ストレスマーカーγ-グルタミルジペプチドによる肝疾患スクリーニング	酸化ストレスと肝疾患	第7巻	pp115-123	2011

研究成果の刊行に関する一覧表

雑誌　　2012年度

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Takeuchi, K., Ohishi, M., Ota, S., Suzumura, K., Naraoka, H., Ohata, T., Seki, J., Miyamae, Y., Honma, M., **Soga, T.**	"Metabolic Profiling to Identify Potential Serum Biomarkers for gastric ulceration induced by nonsteroid anti-inflammatory drugs"	J. Proteome Res.	12(3)	1399-1407	2013
Tanaka, S., Taga, H., Maehara, K., Kaneshima, A., Machino, M., Onuma, H., Kaneko, M., Sakagami, H., Sugimoto, M., **Soga, T.** and Tomita, M.	"Pilot Study of Changes in Salivary Metabolic Profiles Induced by Template Therapy"	In Vivo.	26(6)	1015-1020	2012
Hirayama, A., Tomita, M., and **Soga, T.**	"Sheathless capillary electrophoresis-mass spectrometry with a high-sensitivity porous sprayer for cationic metabolome analysis"	Analyst	137(21)	5026–5033	2012
Kato, T., Niizuma, S., Inuzuka, Y., Kawashima, T., Okuda, J., Kawamoto, A., Tamai, Y., Iwanaga, Y., **Soga, T.,** Kita, T., Kimura, T., Shioi, T.	"Analysis of Liver Metabolism in a Rat Model of Heart Failure"	Int. J. Cardiol.	161(3)	130-136	2012
Sugimoto, M., Sakagami, H., Yokote, Y., Onuma, H., Kaneko, M., Mori, M., Sakaguchi, Y., **Soga, T.**, Tomita, M.	"Non-targeted metabolite profiling in activated macrophage secretion "	Metabolomics	8(4)	624-633	2012

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
曽我朋義	メタボロミクスによるがんの診断マーカー探索	現代化学	3(504)	pp52-56	2013

Anal. Chem. **2009**, *81*, 6165–6174

Metabolomic Profiling of Anionic Metabolites by Capillary Electrophoresis Mass Spectrometry

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We describe a sheath flow capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) method in the negative mode using a platinum electrospray ionization (ESI) spray needle, which allows the comprehensive analysis of anionic metabolites. The material of the spray needle had significant effect on the measurement of anions. A stainless steel spray needle was oxidized and corroded at the anodic electrode due to electrolysis. The precipitation of iron oxides (rust) plugged the capillary outlet, resulting in shortened capillary lifetime. Many anionic metabolites also formed complexes with the iron oxides or migrating nickel ion, which was also generated by electrolysis and moved toward the cathode (the capillary inlet). The metal−anion complex formation significantly reduced detection sensitivity of the anionic compounds. The use of a platinum ESI needle prevented both oxidation of the metals and needle corrosion. Sensitivity using the platinum needle increased from several- to 63-fold, with the largest improvements for anions exhibiting high metal chelating properties such as carboxylic acids, nucleotides, and coenzyme A compounds. The detection limits for most anions were between 0.03 and 0.87 μmol/L (0.8 and 24 fmol) at a signal-to-noise ratio of 3. This method is quantitative, sensitive, and robust, and its utility was demonstrated by the analysis of the metabolites in the central metabolic pathways extracted from mouse liver.

Metabolism is the entire network of chemical reactions that occur in a cell in order to maintain life, in which one metabolite is transformed into another by a sequence of enzymes. Among the whole cellular metabolic network, central carbon metabolism, composed of glycolysis, the pentose phosphate pathway, and the tricarboxylic acid (TCA) cycle, plays key functions in substrate degradation, energy and cofactor regeneration, and biosynthetical precursor supply (DNA, RNA, proteins, peptideglycan, and lipid bilayers).[1,2] Interestingly, all the components involved in the central carbon and energy metabolism are negatively charged: phosphorylated saccharides, phosphorylated carboxylic acids, carboxylic acids, coenzyme A (CoA) compounds, nucleotides, and nicotinamide adenine dinucleotides.

As the importance of metabolomics is recognized, several large-scale metabolite analysis methods using GC/MS,[3] LC/MS,[2,4,5] NMR[6−8] or Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS)[9,10] have been developed. However, only a limited number of methodologies enable the simultaneous analysis of the anionic metabolites due to their extremely large physicochemical diversity.

Recently, approaches based on capillary electrophoresis mass spectrometry (CE-MS)[11,12] and CE time-of-flight mass spectrometry (CE-TOFMS)[13] have emerged as powerful tools for the comprehensive analysis of charged metabolites and have played a critical role in understanding intricate biochemical and biological systems.[13−19]

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(1) Sonenshein, A. L. *Nat. Rev. Microbiol.* **2007**, *5*, 917–927.
(2) Yang, W. C.; Sedlak, M.; Regnier, F. E.; Mosier, N.; Ho, N.; Adamec, *J. Anal. Chem.* **2008**, *80*, 9508–9516.
(3) Fiehn, O.; Kopka, J.; Dormann, P.; Altmann, T.; Trethewey, R. N.; Willmitzer, L. *Nat. Biotechnol.* **2000**, *18*, 1157–1161.
(4) Plumb, R.; Granger, J.; Stumpf, C.; Wilson, I. D.; Evans, J. A.; Lenz, E. M. *Analyst* **2003**, *128*, 819–823.
(5) Yoshida, H.; Mizukoshi, T.; Hirayama, K.; Miyano, H. *J. Agric. Food Chem.* **2007**, *55*, 551–560.
(6) Reo, N. V. *Drug Chem. Toxicol.* **2002**, *25*, 375–382.
(7) Coen, M.; Lenz, E. M.; Nicholson, J. K.; Wilson, I. D.; Pognan, F.; Lindon, J. C. *Chem. Res. Toxicol.* **2003**, *16*, 295–303.
(8) Coen, M.; Ruepp, S. U.; Lindon, J. C.; Nicholson, J. K.; Pognan, F.; Lenz, E. M.; Wilson, I. D. *J. Pharm. Biomed. Anal.* **2004**, *35*, 93–105.
(9) Aharoni, A.; Ric de Vos, C. H.; Verhoeven, H. A.; Maliepaard, C. A.; Kruppa, G.; Bino, R.; Goodenowe, D. B. *Omics* **2002**, *6*, 217–234.
(10) Hirai, M. Y.; Yano, M.; Goodenowe, D. B.; Kanaya, S.; Kimura, T.; Awazuhara, M.; Arita, M.; Fujiwara, T.; Saito, K. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 10205–10210.
(11) Soga, T.; Ohashi, Y.; Ueno, Y.; Naraoka, H.; Tomita, M.; Nishioka, T. *J. Proteome Res.* **2003**, *2*, 488–494.
(12) Edwards, J. L.; Chisolm, C. N.; Shackman, J. G.; Kennedy, R. T. *J. Chromatogr., A* **2006**, *1106*, 80–88.
(13) Soga, T.; Baran, R.; Suematsu, M.; Ueno, Y.; Ikeda, S.; Sakurakawa, T.; Kakazu, Y.; Ishikawa, T.; Robert, M.; Nishioka, T.; Tomita, M. *J. Biol. Chem.* **2006**, *281*, 16768–16776.
(14) Ishii, N.; Nakahigashi, K.; Baba, T.; Robert, M.; Soga, T.; Kanai, A.; Hirasawa, T.; Naba, M.; Hirai, K.; Hoque, A.; Ho, P. Y.; Kakazu, Y.; Sugawara, K.; Igarashi, S.; Harada, S.; Masuda, T.; Sugiyama, N.; Togashi, T.; Hasegawa, M.; Takai, Y.; Yugi, K.; Arakawa, K.; Iwata, N.; Toya, Y.; Nakayama, Y.; Nishioka, T.; Shimizu, K.; Mori, H.; Tomita, M. *Science* **2007**, *316*, 593–597.
(15) Ohashi, Y.; Hirayama, A.; Ishikawa, T.; Nakamura, S.; Shimizu, K.; Ueno, Y.; Tomita, M.; Soga, T. *Mol. Biosyst.* **2008**, *4*, 135–147.
(16) Yoshida, S.; Imoto, J.; Minato, T.; Oouchi, R.; Sugihara, M.; Imai, T.; Ishiguro, T.; Mizutani, S.; Tomita, M.; Soga, T.; Yoshimoto, H. *Appl. Environ. Microbiol.* **2008**, *74*, 2787–2796.

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Application of the sheath flow CE-MS to the analysis of anionic metabolites has been successfully performed using the "negative mode" and a cationic polymer coated capillary.[20] This methodology reverses electroosmotic flow (EOF)[21] toward the anode (the MS direction) to prevent a deleterious current drop. The method enabled the large-scale determination of anionic metabolites, however, with limitations. Several anionic metabolites such as citrate, nucleotides, and CoA compounds were not detected or were detected as poorly shaped peaks.[20,22,23] To overcome the problem, a pressure-assisted CE-MS method[24] was used for these analytes.[22,23] While successful, two separate CE-MS methods for anions were necessary,[11] and moreover, these approaches were not ideal. Additionally, the capillaries frequently clogged.

Recently, it was found that these problems were caused by metal ions generated from the stainless steel needle of the electrospray ionization (ESI) sprayer. Iron oxides formed at the capillary outlet, while nickel ions migrated into the separation capillary and formed complexes with many anions. Here, we propose an improved sheath flow CE-MS method for the analysis of anionic metabolites using a platinum ESI spray needle. The platinum material has a low ionization tendency and does not generate metal ions through electrolysis. This approach overcame the problems and provided robust and sensitive analysis of most of the anionic metabolites and was successfully applied to the quantitative analysis of mouse hepatic metabolites in the central carbon and energy metabolic pathways.

EXPERIMENTAL SECTION

Chemicals. Glycerophosphate was purchased from Nacalai Tesque (Kyoto, Japan), 2-morpholinoethanesulfonate (MES, internal standard) from Dojindo (Kumamoto, Japan), and hexakis-(2,2-difluorothoxy)-phosphazene (Hexakis) from SynQuest Laboratories (Alachua, FL). All other reagents were obtained from Sigma-Aldrich (St. Louis, MO) or Wako (Osaka, Japan). Individual stock solutions of carboxylic acids, phosphorylated saccharides, and phosphorylated carboxylic acids were prepared at a concentration of 100 mM, and stock solutions for other compounds of 10 mM were prepared in Milli-Q water, except for fumarate and trimesate (reference peak) which were prepared in 0.1 M NaOH and succinyl CoA which was prepared in 0.1 M HCl. The working mixture standard was prepared by diluting these stock solutions with Milli-Q water just before injection. All chemicals were of analytical or reagent grade. Water was purified with a Milli-Q purification system (Millipore, Bedford, MA).

Metabolite Extraction. Liver tissue (approximately 300 mg) was immediately plunged into methanol (1 mL) containing 300 μM each of L-methionine sulfone and 2-morpholinoethanesulfonate (MES) (internal standards) and homogenized for 2 min to inactivate enzymes. Then Milli-Q water (500 μL) was added, 300 μL of the solution was transferred, and 200 μL of chloroform was added and mixed well. The solution was centrifuged at 15 000 rpm for 15 min at 4 °C, and the separated 200 μL aqueous layer was centrifugally filtered through a Millipore 5 kDa cutoff filter to remove proteins. The filtrate (100 μL) was lyophilized and dissolved in 50 μL of Milli-Q water containing reference compounds (200 μM each of trimesate and 3-aminopyrrolidine). The solution (2 μM) was diluted with 18 μL of Milli-Q water and then injected into the CE-TOFMS system.[13]

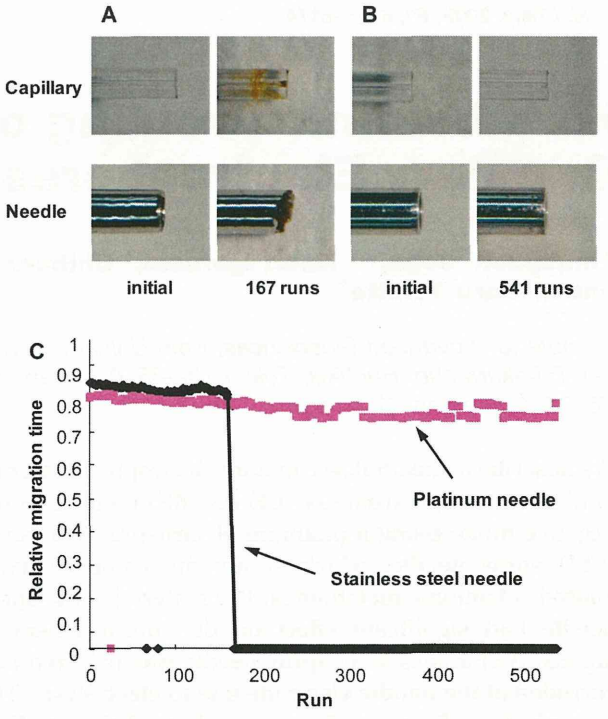

Figure 1. Robustness of the CE-TOFMS system in the anion standard analysis. Photograph of the capillary outlet and the tip of the needle of the COSMO(+) capillary obtained by the CE-ESI-MS sprayer with (A) the SST316Ti stainless steel needle and (B) the platinum needle. (C) Endurance of the system between the SST316Ti stainless steel needle (black) and platinum needle (magenta). The endurance was expressed using the relative migration time of lactate, which was calculated by normalization with the migration time of MES (internal standard). Experimental conditions are described in the Experimental Section.

Instrumentation. All CE-ESI-MS experiments were performed using an Agilent CE capillary electrophoresis system, an Agilent G3250AA LC/MSD TOF system, an Agilent1100 series isocratic HPLC pump, a G1603A Agilent CE-MS adapter kit, and a G1607A Agilent CE-ESI-MS sprayer kit (Agilent Technologies, Waldbronn, Germany). The CE-MS adapter kit includes a capillary cassette which facilitates thermostating of the capillary, and the CE-ESI-MS sprayer kit which simplifies coupling the CE system with MS systems was equipped with an electrospray source. For system control and data acquisition, we used G2201AA Agilent ChemStation software for CE and Agilent TOF (Analyst QS) software for TOFMS.

In addition, the original Agilent SST316Ti stainless steel (Fe/Cr/Ni/Mo/Ti; 68:18:11:2:1) ESI needle was replaced with passivated SST316Ti stainless steel (with 1% formic acid and 20%

(17) Sato, S.; Soga, T.; Nishioka, T.; Tomita, M. *Plant J.* **2004**, *40*, 151–163.
(18) Kinoshita, A.; Tsukada, K.; Soga, T.; Hishiki, T.; Ueno, Y.; Nakayama, Y.; Tomita, M.; Suematsu, M. *J. Biol. Chem.* **2007**, *282*, 10731–10741.
(19) Williams, B. J.; Cameron, C. J.; Workman, R.; Broeckling, C. D.; Sumner, L. W.; Smith, J. T. *Electrophoresis* **2007**, *28*, 1371–1379.
(20) Soga, T.; Ueno, Y.; Naraoka, H.; Ohashi, Y.; Tomita, M.; Nishioka, T. *Anal. Chem.* **2002**, *74*, 2233–2239.
(21) Lukacs, K. D.; Jorgenson, J. W. *J. High Resolut. Chromatogr. Chromatogr. Commun.* **1985**, *8*, 407–411.
(22) Soga, T.; Ueno, Y.; Naraoka, H.; Matsuda, K.; Tomita, M.; Nishioka, T. *Anal. Chem.* **2002**, *74*, 6224–6229.
(23) Soga, T.; Ishikawa, T.; Igarashi, S.; Sugawara, K.; Kakazu, Y.; Tomita, M. *J. Chromatogr., A* **2007**, *1159*, 125–133.
(24) Cao, P.; Moini, M. *Electrophoresis* **1998**, *19*, 2200–2206.