SHIMADZU
Excellence in Science

PO-CON1620E

Targeted metabonomic study of plasma from rats with acute colitis using LCMS-IT-TOF based metabonomics

Pittcon 2016 1720-2

Lingling Shen[1], Xiaojun Zhang[2], Jinting Yao[1], Qingsheng Zhong[1], Taohong Huang[1], Shin-ichi Kawano[1], Yuki Hashi[1]
[1]Shimadzu Global COE, Shimadzu (China) Co., Ltd., China, [2]Guangzhou University of Traditional Chinese Medicine, China

Targeted metabonomic study of plasma from rats with acute colitis using LCMS-IT-TOF based metabonomics

Introduction

According to published estimates, there are tens of thousands of all kinds of endogenous metabolites in the human body. Differences in the concentration range and physical & chemical properties of various metabolites in body fluids, there is no one analysis technique of all metabolites in body fluids. NMR, mass spectrometry and various separation methods became to the important analysis tools in metabonomics, such as: the liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS) etc. By using multivariable model identify methods, it is found potential markers from massive metabonomics data analysis. The diagnosis of IBD primarily depends on invasive methods, such as endoscope, however, which usually limits early diagnosis and treatments. Based on metabonomics, this paper will provide useful information for developing non-invasive and sensitive diagnosis methods for IBD. As an example of serum samples of rats with acute colitis, this paper established a method of screening potential biomarkers from the serum metabolic fingerprint by using LCMS-IT-TOF.

Methods and Materials

Sample Preparation

The rat blood was collected from the orbital venous plexus of rats, and each rat was collected 1.5 mL blood. And it transfer collected fresh blood to the EP tube of containing 100 μ L 1% heparin sodium.
Transfer 3500g of above prepared blood samples to EP tube, then centrifugate for 10 minutes to separate the plasma from the blood. Accurately measurement 200 μL plasma into 2 mL EP tube and then add 10 μL DL-2-chlorophenylalanine (0.1 mg/mL) as the internal standard and 800 μL extraction solvent (methanol: acetonitrile:acetone, 1:1:1) into it, then scroll 30s. Last centrifuge for 15 minutes in 10000 rpm, 4 °C , remove the supernatant and dried with nitrogen. Add 80 μL methanol and 20 μL purified water to dissolve, then again centrifuge for 20 minutes in 10000 rpm, 4 °C, remove the supernatant to the new bottle.

LCMS-IT-TOF analysis

Analytical Conditions

UFLC (UFLC$_{XR}$, Shimadzu Corporation, Japan)	
Column	: Shim-pack XR-ODSIII (150 mmL. x 2.0 mmi.d., 2.2 μm)
Mobile phase A	: 0.1%formic acid; Mobile phase B: Acetonitrile
Gradient program	: 5%B (0-1 min)-50%B (15 min)-90%B (18 -20min)
Flow rate	: 0.3 mL/min; Oven temperature: 40 °C; Injection volume: 5 μL
MS (LCMS-IT-TOF, Shimadzu Corporation, Japan)	
Ionization	: ESI ; Ionization voltage: +4.5 kV (positive), -3.5 kV (negative)
Nebulizing gas flow	: 1.5 L/min; Drying gas pressure: 10 L/min
CDL temperature	: 200 °C; BH temperature: 200 °c
Detector voltage	: 1.60 kV; Ion Accumulation Time: 50 ms
Tuning Method	: the automatic tuning voltage optimization and external standard calibration quality number

Results and Discussion

The precisions of the analytical method

According to the processing methods of the two groups of samples, the quality control samples (QC sample) are prepared. The stability of the data of two group sample is monitored with the stability of the data of QC sample. Each 10 needle samples into a QC sample. The precision data are given in Table 1.

Table 1 the precisions of the analytical method.

NO	Compound	Relative standard deviation RSD%		
		R.T	m/z	Area
1	DL-2-chlorophenylalanine	0.23	2.61E-04	1.77
2	phenylacetylglycine	0.20	4.04E-04	3.53
3	p-cresol glucuronide	0.19	1.03E-04	0.91
4	LysoPC (16:0)	0.07	1.76E-04	3.95

The chromatograms of samples

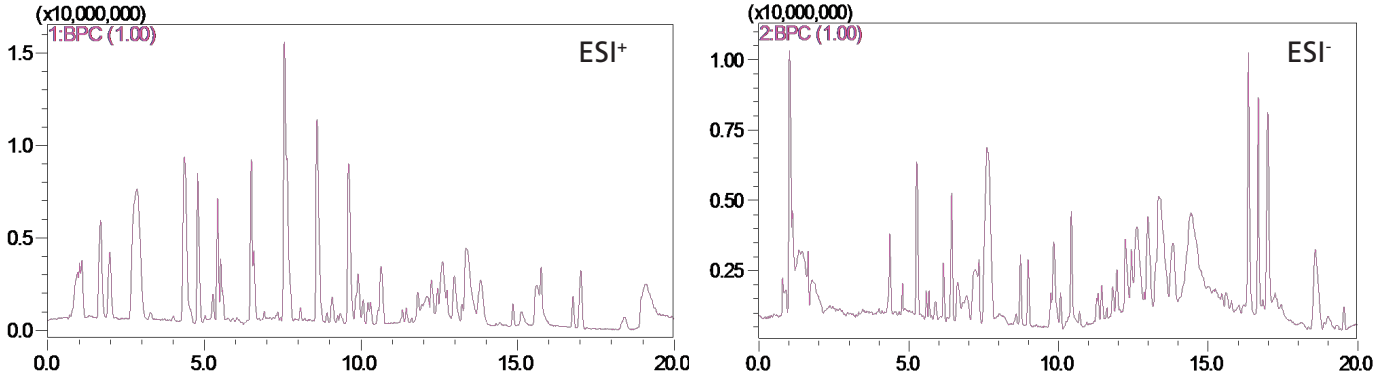

Figure 1. BPC chromatograms of normal group

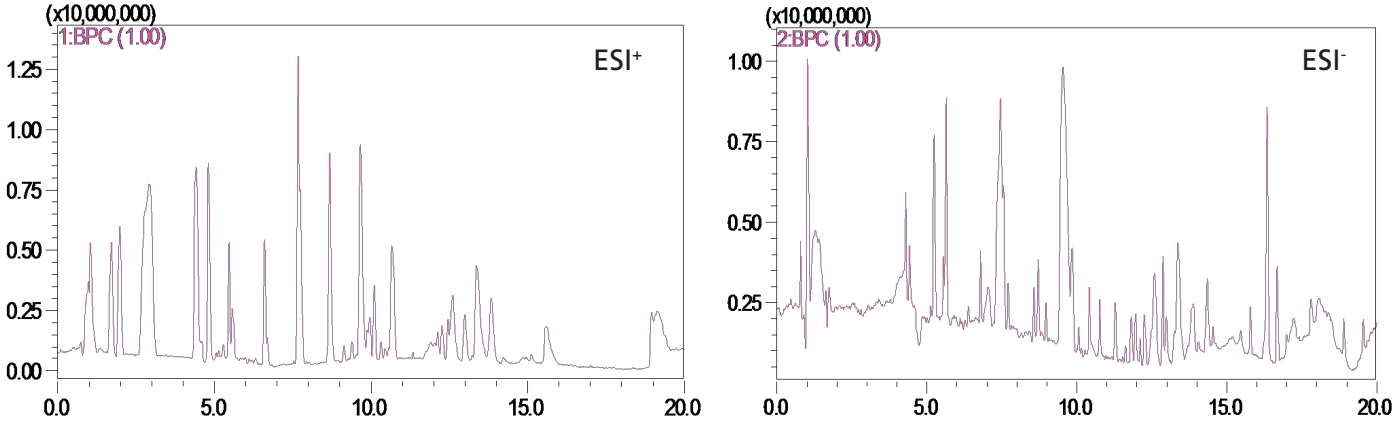

Figure 2. BPC chromatograms of TNBS mode group

Targeted metabonomic study of plasma from rats with acute colitis using LCMS-IT-TOF based metabonomics

The results of statistical analysis

The dates of two groups are analyzed by using the Profiling solution metabonomics software, and then the analysis results are imported by the Simca-P statistical analysis software to establish OPLS model. This OPLS model would distinguish the TNBS group and normal group, and screened for potential markers. The results are given in Table 2.

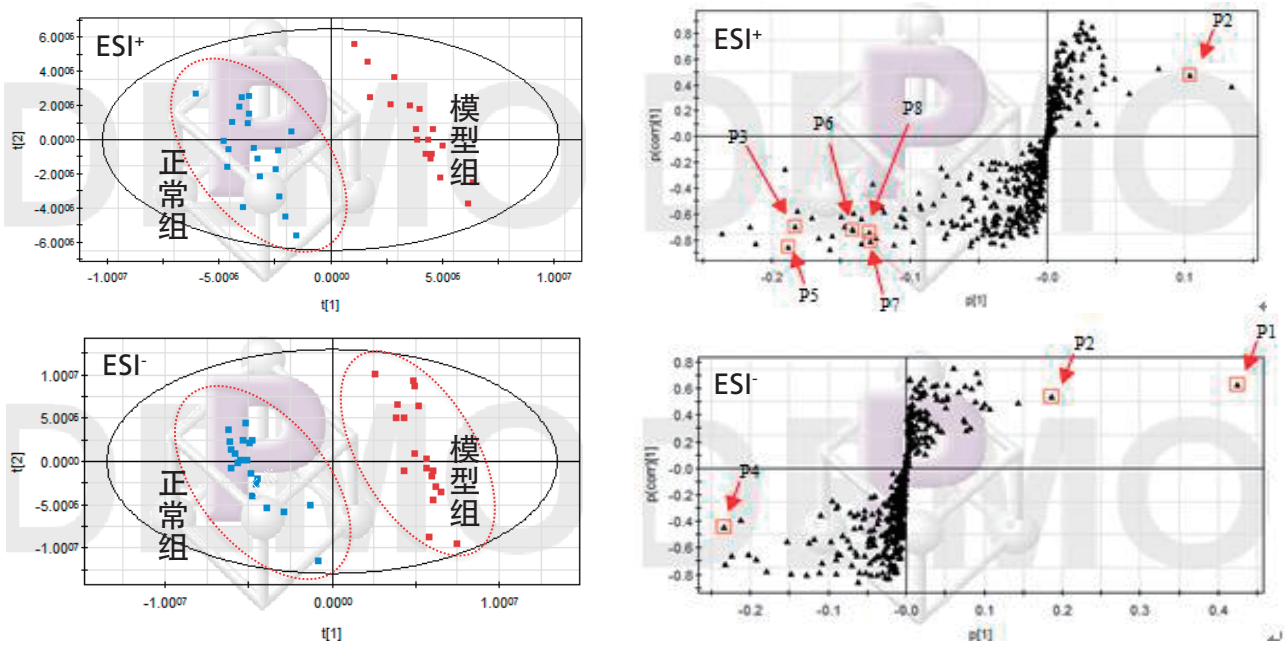

Figure 3. OPLS and S-plot of sample

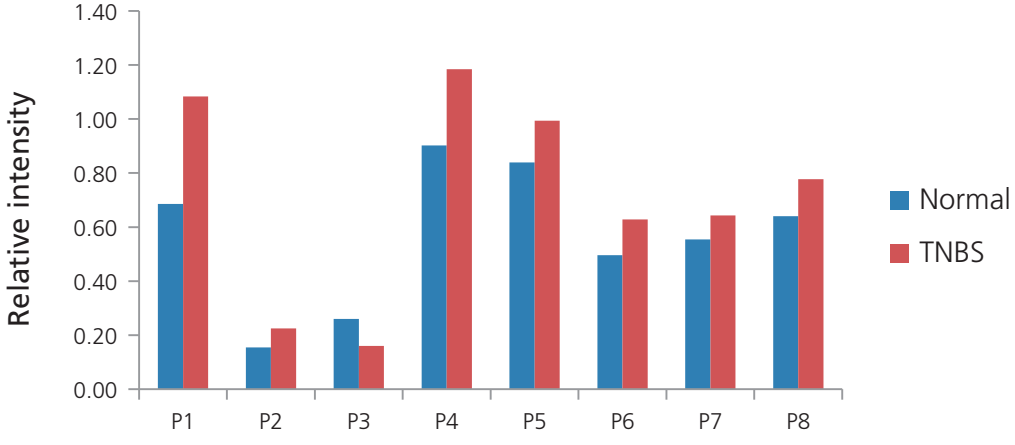

Figure 4. Differences between the two groups in the potential markers

Targeted metabonomic study of plasma from rats with acute colitis using LCMS-IT-TOF based metabonomics

Identification of potential markers

The m/z of the possible compounds screened out by Simca-P software import into the Metlin metabolism of drugs database to search its structure, and then according to the MSn mass spectra, the structural information would be further inferred. At the same time, this possible compounds also should meet with pharmacological and metabolic rule. The potential markers will be finally found from this possible compounds.

Table 2 The potential markers

No.	Ion mode	R.T (min)	m/z	VIP	P Value	Intensity change	Mol. For	Compound
P1	ESI^-	5.645	283.0823	8.03	4.25E-04	↑	$C_{13}H_{16}O_7$	p-cresol glucuronide
P2	ESI^-	5.562	192.0681	3.37	4.49E-03	↑	$C_{10}H_{11}NO_3$	Phenylacetylglycine[a]
	ESI^+	5.567	194.0802	2.22	5.27E-03	↑		
P3	ESI^+	3.291	232.1514	3.57	1.21E-03	↓	$C_{11}H_{21}NO_4$	Butyryl carnitine
P4	ESI^-	16.67	303.2321	6.82	2.05E-02	↑	$C_{20}H_{32}O_2$	Arachidonic acid
P5	ESI^+	12.979	496.3377	3.97	1.78E-05	↑	$C_{24}H_{50}O_7NP$	LysoPC(16:0)
P6	ESI^+	15.615	524.3690	3.23	4.86E-04	↑	$C_{26}H_{54}O_7NP$	LysoPC(18:0)
P7	ESI^+	12.246	520.3371	2.66	2.34E-05	↑	$C_{26}H_{50}O_7NP$	LysoPC(18:2)
P8	ESI^+	13.832	522.3536	2.55	1.05E-03	↑	$C_{26}H_{52}O_7NP$	LysoPC(18:1)

For example, it can be speculated that P2 compound could be Phenylacetylglycine from Figure 5, and the compound is confirmed by using its standard sample. Phenylacetylglycine is a gut microbial co-metabolite, and the increased level of phenylacetylglycine may play a role in disturbing gut microbiota homeostasis. Therefore, Phenylacetglycine may be a potential marker for intestinal diseases.

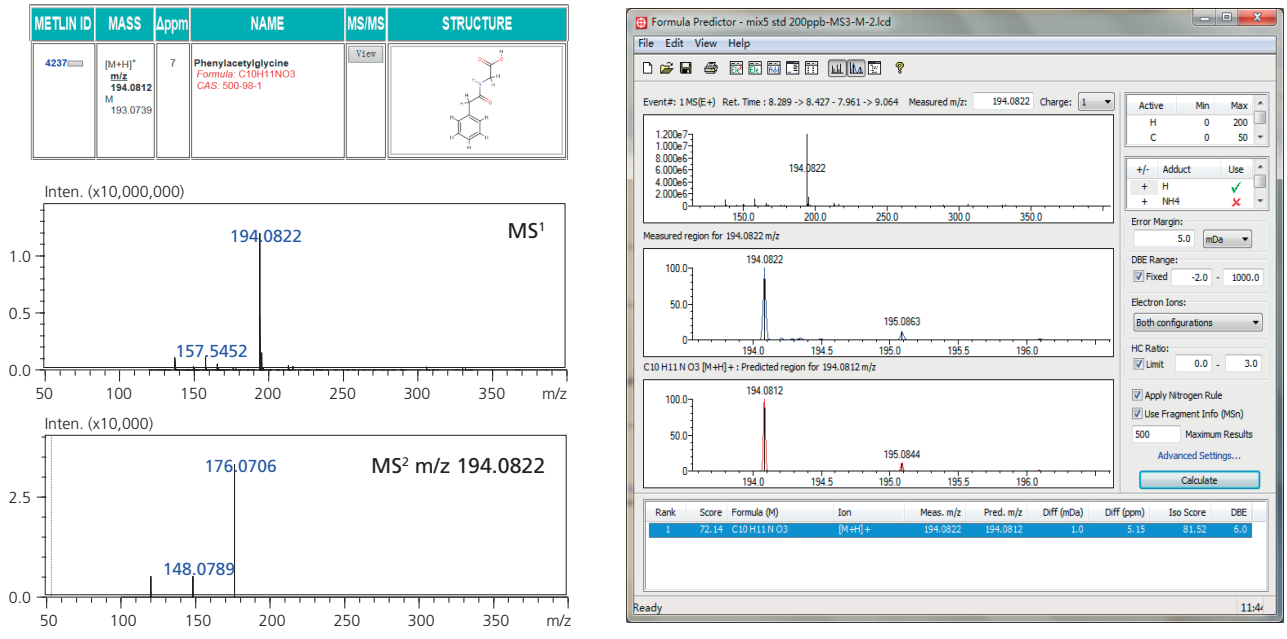

Figure 5. MS^n and Prediction results of P2 (m/z 194.0822)

Targeted metabonomic study of plasma from rats with acute colitis using LCMS-IT-TOF based metabonomics

The same time, it can be speculated that P1 compound could be p-cresol glucuronide. This compound is similar with the P1, may be a potential marker for intestinal diseases.
P5-P8 compounds in table 2 are lysophosphatidylcholine species, and this kinds of compounds have proinflammatory properties. The increased level of LysoPCs reflects the existence of some inflammation in acute colitis, and this is consistent with the actual.

Conclusions

This application news established a method of screening potential biomarkers from the serum metabolic fingerprint by using SHIMADZU LCMS-IT-TOF. This method described systematically the process from data collection, data preprocessing, pattern recognition and finally screening potential biomarker. The PLS model showed that there were significant differences in the metabolites of TNBS rats and healthy rats, and 8 compounds screened were identified as potential biomarkers, such as henylacetylglycine, p-cresol glucuronide, Butyryl-L-carnitine, 8Z, 11Z, 14Z, 18Z-eicosatetraenoic acid and LysoPCs (PC16:0, PC18:0, PC18:2, PC18:1).

First Edition: March, 2016

SHIMADZU

Shimadzu Corporation
www.shimadzu.com/an/