• Basic Research •

Metabolic profile analysis of free amino acids in experimental autoimmune uveoretinitis rat plasma

Jun-Guo Guo[1,2,3], Xin-Miao Guo[4], Xing-Rong Wang[2,3,5], Jing-Zhen Tian[1], Hong-Sheng Bi[2,3,5]

[1]College of Pharmacy, Shandong University of Traditional Chinese Medicine, Jinan 250355, Shandong Province, China
[2]Shandong Provincial Key Laboratory of Integrated Traditional Chinese and Western Medicine for Prevention and Therapy of Ocular Diseases, Jinan 250002, Shandong Province, China
[3]Eye Institute, Shandong University of Traditional Chinese Medicine, Jinan 250002, Shandong Province, China
[4]School of Management, Shandong University of Traditional Chinese Medicine, Jinan 250355, Shandong Province, China
[5]Affiliated Eye Hospital, Shandong University of Traditional Chinese Medicine, Jinan 250002, Shandong Province, China
Correspondence to: Jing-Zhen Tian. No.4655, Daxue Road, Jinan 250355, Shandong Province, China. tianjingzhen@163.com; Hong-Sheng Bi. No.48, Yingxiongshan Road, Jinan 250002, Shandong Province, China. hongshengbi1@163.com
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Abstract

• AIM: To determine the differences of amino acid (AA) levels in experimental autoimmune uveoretinitis (EAU).

• METHODS: AA analysis of the plasma samples in EAU rats induced by interphotoreceptor retinoid-binding protein emulsion were performed with high performance liquid chromatography (HPLC) and phenylisothiocyanate (PITC) pre-column derivation methods were performed. Using partial least squares discriminant analysis (PLS-DA), the potential biomarkers were identified in EAU rat plasma, and the metabolic pathways related to EAU were further analyzed.

• RESULTS: The method results showed that linear (r≥0.9957), intra-day reproducible [relative standard deviation (RSD)=0.04%-1.33%], inter-day reproducible (RSD=0.06%-2.07%), repeatability (RSD=0.03%-0.89%), stability (RSD=0.05%-2.48%) and recovery (RSD=1.98%-4.39%), with detection limits of 0.853-11.4 ng/mL. The metabolic profile in EAU rats was different from that in the control groups five AAs concentrations were increased and nine AAs were reduced. Moreover, five metabolic pathways were related to the development of EAU.

• CONCLUSION: The developed method is a simple, rapid and convenient for determination of AAs in EAU rat plasma, and these findings will provide a comprehensive insight on the metabolic profiling of the pathological changes in EAU.

• KEYWORDS: experimental autoimmune uveoretinitis; phenylisothiocyanate; high performance liquid chromatography; amino acid; metabolic profile; rat

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INTRODUCTION

Uveitis is an inflammatory and autoimmune eye disease including Vogt-Koyanagi-Harada syndrome, Behcet's disease, acute retinal necrosis, Fuchs syndrome and birdshot retinochoroidopathy. Uveitis is the frequent cause of visual impairment worldwide mostly affecting individuals of young adults and children[1-3]. Experimental autoimmune uveoretinitis (EAU) has been extensively used as an ideal animal model to research the pathophysiology of uveitis and to develop new effective therapeutic strategies over the last decade[4-6]. EAU can be induced in rats and mice by using pathogenic epitopes, such as interphotoreceptor retinoid-binding protein (IRBP), S-Ag, and rhodopsin. The most popular rat for EAU studies is Lewis rat, which typically takes an acute, monophasic course and develops severe uveitis, and it can be observed by external examination of the eyes with a flashlight[7]. Recently, expression changes in miRNAs, immunohistochemical analysis and *in vivo* optical coherence tomography assessment of EAU have facilitated the understanding its mechanisms[8-9]. However, the metabolism changes in rat plasma still limits in the light of our understanding.

The metabolism of amino acids (AAs) can adjust the total amount of nitrogen balance, and can promote the synthesis of enzymes, hormones and some vitamin levels[10-12]. The metabolism of AAs was closely related to disease. The type and concentration of AAs in the blood were dynamic equilibrium state in physiological conditions. However, when the body anomalies, the dynamic balance will be out of control, and the AAs concentration will be change[13]. The analysis of metabolites is mainly involved in metabolic fingerprinting and metabolic profiling[14]. The detection techniques of metabolites

were mainly gas chromatography mass spectrometry[15], nuclear magnetic resonance[16] and liquid chromatography cass spectrometry[17]. Beyond the analysis of free AAs in plasma could hold great promise to enhance our understanding of the complex relationship between AAs and EAU.

AAs were organic substances containing both amino and acid groups, which were traditionally classified as essential what mammalian cannot synthesis and non-essential what the mammalian can synthesis for humans and animals. Due to variation of side chains, AAs have their own unique catabolic pathway and have remarkably different biochemical properties and functions[18]. In recent studies, it has been showed that AAs directly participate in cell signaling, cell specific metabolism of nutrients, oxidative stress and efficiency of utilization of dietary proteins[19-21]. Abnormal metabolism of AAs impairs growth and development of body homeostasis, and even death[22]. In addition, AAs are pivotal syntheses precursors for hormones and nitrogenous substances, have enormous biological importance. The balance among AAs is crucial for the whole body. AAs exist in organisms as free or bound forms. High performance liquid chromatography (HPLC) combined with chemical derivatization is the most widely used analytical technique in AA determination in plasma, skeletal muscle, urine ciliary epithelium, aqueous and lens[23-26].

In the present study, we performed the plasma AA analysis using HPLC with phenylisothiocyanate (PITC) pre-column derivation methods in EAU rats, and mainly focused on the alterations of AAs concentration profile and the metabolism of AAs alterations of the plasma between EAU rats and control samples.

MATERIALS AND METHODS

Ethical Approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. All animals were fed and maintained according to the guidelines of Care and Use of Laboratory Animals published by the China National Institute of Health.

Animals and Chemicals In the study, we used female Lewis rats (SPF grade, 160-180 g, 6-8 weeks old) to induce EAU. Lewis rats purchased from Peking Vital River Laboratory Animal Ltd., Beijing, China.

$IRBP_{1177-1191}$ was synthesized by Sangon Biotech (Shanghai, China) Co., Ltd. Freund's complete adjuvant and PITC were purchased from Sigma-Aldrich, St. Louis, MO, USA. Eighteen kinds of AAs and standard references were provided by National Institutes for Food and Drug Control of China (Beijing, China). Acetonitrile, methanol and acetic acid were HPLC grade were purchased from Tedia (Product of Tedia, USA). Other analytical grade reagents were obtained from Shanghai Sinopharm Chemical Reagent Co., Ltd.

Apparatus and Software HPLC system includes UltiMate 3000 Pump, 3000 Autosampler, 3000 RS Column Compartment, 3000 Diaode Array Detector (DIONEX USA), Chromeleon software (version 6.80) for data acquisition, signal processing and calculation were used for all separation. The electronic balance (MS205DU, Mettler-Toledo, Germany) was checked with certified weight. SIMCA-P software (version 11.5) was used for data analysis.

Induction and Assessment of Experimental Autoimmune Uveoretinitis To induce EAU, the rats were immunized by a single injection 0.1 mL prepared peptide antigen in one footpad described in our previous study[27] and 14 unimmunized rats were set as control group. All animals were monitored by slit-lamp biomicroscopy for ocular inflammation, and scored[28]. The eye inflammation was also confirmed by histopathological analysis. After day 12 immunization, and the plasma samples and eyes tissue were collected. The plasma were anticoagulated with heparin, centrifuged at 2700 g for 10min, supernatant stored at -80℃ prior to analysis. The eyes tissues were fixed for histological examination[29].

Preparation of Sample A volume of 300 μL PITC was transferred to a brown volumetric flask (25 mL), diluted to the mark with acetonitrile and then stored at 4℃ as the solution of PITC (0.1 mol/L). Of 1.3 mL triethylamine was transferred to a brown volumetric flask (10 mL), diluted with acetonitrile and then stored at 4℃ as solution of triethylamine solution (THS, 0.1 mol/L). AAs standard solutions were made by precisely weighing each reagent about 10 mg, transferred to a brown volumetric flask (10 mL), and then diluted with 0.1 mol/L hydrochloric acid. Further, 1 mL of each AAs standard solution was transferred to a 25 mL volumetric flask, diluted to the volume as mixed stock solution.

Before analysis, the samples supernatant were thawed at room temperature. For reducing the effect of solvent to obtain a good peak shape, a 300 μL of sample supernatant was diluted with 600 μL of acetonitrile, centrifuged at 13 000 g at 4℃ for 10min, 200 μL supernatants were transferred to an Eppendorf tube. Of 100 μL PITC and 100 μL THS were added and mixed, after 60min at room temperature, 600 μL of n-hexane was added and mixed thoroughly. After standing for 10min, the mixed solutions were divided into two, and the lower solution was obtained and filtered by 0.22 μm strainer before injecting into an HPLC system for analysis.

Chromatographic Conditions Isolation of rat plasma AAs was carried out with chromatography by using gradient elution on an Atlantis dC_{18} (3 μm, 4.6×150 mm, Waters USA) column coupled with diode array detector (DAD). Mobile A was acetonitrile, methanol and water (3:1:1, v/v). Mobile B

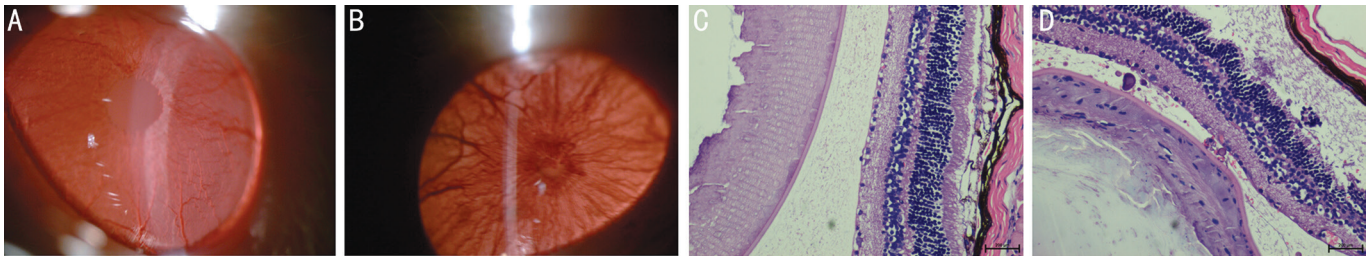

Figure 1 Examination of EAU on day 12 after immunization A: Control group monitored by a slit lamp; B: EAU group monitored by a slit lamp. Irregular iris hyperemia, damaged iris with flare and cells, and cloudy aqueous humor; C: Histopathology examination of control group; D: Histopathology examination of EAU group, iris, ciliary body and anterior chamber neutrophil, monocytes and T lymphocytes infiltration. Scale bar=200 μm.

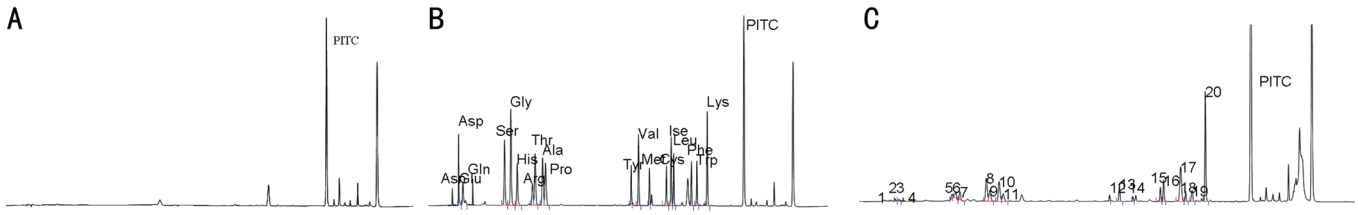

Figure 2 The chromatogram of HPLC A: Blank chromatogram of AAs. B: Chromatogram of standard AAs. 1) Asp: 3.127min; 2) Glu: 3.643min; 3) Ser: 8.707min; 4) Gly: 9.470min; 5) His: 10.243min; 6) Arg: 12.103min; 7) Thr: 12.427min; 8) Ala: 13.333min; 9) Pro: 13.693min; 10) Tyr: 24.097min; 11) Val: 24.997min; 12) Met: 26.327min; 13) Cys: 28.393min; 14) Iso: 28.963min; 15) Leu: 29.282min; 16) Phe: 31.440min; 17) Try: 32.107min; 18) Lys: 33.367min and PITC 37.907min. C: Plasma sample.

was 925 mL water contained 12.6 g sodium acetate plus 70 mL acetonitrile, and the pH value was 6.5 after adjustment with glacial acetic acid. The above mobile were filtered by 0.22 μm and degassed ultrasonically. Gradient elution was set as 0 A to 96% A from 0 to 6min, 6% A to 9% A from 6 to 17min, 9% A to 23% A from 17 to 20min, 23% A to 45% A from 20 to 32min, 45% A to 55% A from 32 to 34min, 55% A to 100% A from 34 to 40min, 100% A to 0 A from 40 to 45min. The column temperature was 30℃, flow rate was 0.8 mL/min, and the wavelength was 254 nm. A volume of 5 μL of the plasma samples from both EAU and the control group were injected at a random order.

Series of standard AA solutions were prepared and analyzed by HPLC to establish the linear relationship. The precision was calculated by the intra-day and inter-day reproducibility of the same sample. The stability of sample was detected for 0, 8, 16, 24, 48 and 72h. The repeatability was tested by five continuous injection of the same sample. The sample recovery was also detected by divided mixed AA standard solutions in different concentrations. The detection limits for each AA was estimated by the lowest concentration accorded to three times the standard deviation of the blank signal.

Statistical Analysis The relative standard deviations (RSD) of samples peak were calculated to evaluate the precision, stability and repeatability. The raw data were exported for SIMCA-P analysis. Parameters of partial least squares discriminant analysis (PLS-DA) were used to evaluate the models indicating and the ability of prediction. Variable importance in the projection (VIP) value choice the potential biomarkers[30].

Metabolic Biomarkers and Pathways The potential biomarkers were selected according to the VIP values greater than 1. The metabolic pathways were interpreted with KEGG (http://www.kegg.com) and HMDB (http://www.hmdb.ca) databases.

RESULTS

Experimental Autoimmune Uveoretinitis Clinical and Histopathology Assessment The Lewis rats' eyes were observed every-day after immunization with IRBP emulsion. Compared with control group, EAU rats were detected every day and were found that the inflammation appeared on day 8 after immunization, and the intraocular inflammation, such as opaque anterior chamber and obscured pupil, was the most severe on day 12 (Figure 1A, 1B). The results were also similar to our previous study[28]. Histopathologic examination of the obtained eyes on day 12 after immunization demonstrated apparent changes for the iris, ciliary body and anterior chamber (Figure 1C, 1D).

High Performance Liquid Chromatography Result We selected the PITC as derivatizing regent for plasma AA determination by HPLC method. This method produces stable derivatives and the reaction kinetic was fast to all AAs. The blank chromatogram of plasma AAs was shown in Figure 2A. The chromatogram of the standard reference containing 18 kinds of AAs was shown in Figure 2B, and the chromatogram of plasma AAs sample was shown in Figure 2C. It was found

that the standard reference contained 18 kinds of AAs were separated well.

The linear equation, range and correlation coefficient for the standard reference containing 18 kinds of AAs were shown in Table 1. RSD of intra-day and inter-day within five days were less than 2% and 3%, respectively. The RSD of sample stability was less than 3% for 0, 8, 16, 24, 48 and 72h. The average recoveries of AAs RSD were between 1.98% and 4.39%, and the RSD were less than 5% (Table 2). The detection limits varied from 0.853 to 11.4 ng/mL.

In EAU group, we found that the concentrations of five AAs (*i.e.* Thr, Val, Cys, Iso, Leu) were increased in plasma compared to those in control group. Meanwhile, the concentrations of another eight AAs (*i.e.* Asp, Glu, Gly, His, Ala, Pro, Tyr, Met, Trp) were decreased compared to those in control group. The rest of AAs hardly changed (Figure 3, Table 3).

Multi-component Statistical Analysis Principal component analysis (PCA) score plot of EAU and control groups shown in Figure 4A. Separation between EAU and controls was clear. This results show that the metabolic profiling of AAs could be used for understanding EAU. In order to select the potential biomarkers, PLS-DA analysis was performed according to variable importance. A clear separation between EAU and controls could be seen in Figure 4B. The PLS-DA model had a good capability for the classification of EAU from control group.

Potential Metabolic Biomarkers and Pathways In order insight into the metabolic changes among EAU, the PLS-DA analysis was used to choice potential biomarkers. The VIP of PLS-DA of Tyr, Gly, Met, His, Ala, Leu and Thr was more than 1.00, VIP of Ser and Phe was no more than 0.50, and the others between 1.00 and 0.05 (Figure 4C). The value of VIP more than 1.00 was used in the selection, the potential biomarkers were confirmed by standard samples. Final biomarkers were Tyr, Gly, Met, His, Ala, Leu and Thr. The biomarkers related to glycine, serine and threonine metabolism, phenylalanine, tyrosine and tryptophan biosynthesis, valine, leucine and isoleucine degradation and biosynthesis, alanine metabolism, phenylalanine metabolism, alanine, aspartate and glutamate metabolism, cysteine and methionine metabolism and tyrosine metabolism, which may play important roles in the metabolic changes of EAU.

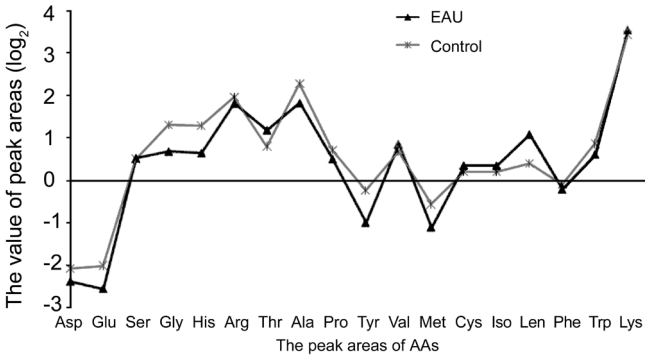

Figure 3 The changes of plasma AAs in EAU and control groups.

Table 1 The linear equation, range and correlation coefficient of 18 kinds of AAs

Amino acids	Linear equation	Linear range (μmol/mL)	Correlation coefficient
Asp	y=9.434x-0.141	0.0862-0.862	0.9987
Glu	y=9.033x-0.117	0.0850-0.850	0.9978
Ser	y=13.713x+0.114	0.1152-1.152	0.9992
Gly	y=12.015x-0.099	0.2073-2.073	0.9995
His	y=11.969x+0.094	0.0900-0.900	0.9979
Arg	y=12.432x-0.246	0.1078-1.078	0.9957
Thr	y=12.769x-1.037	0.1576-1.576	0.9968
Ala	y=11.152x-0.106	0.1138-1.138	0.9990
Pro	y=11.476x+0.176	0.1158-1.158	0.9993
Tyr	y=11.180x+0.008	0.0628-0.628	0.9968
Val	y=17.585x+0.013	0.0736-0.736	0.9995
Met	y=11.251x-0.002	0.0625-0.625	0.9997
Cys	y=7.0880x-0.001	0.1169-1.169	0.9999
Iso	y=11.853x+0.014	0.1008-1.008	0.9992
Leu	y=11.639x+0.003	0.0825-0.825	0.9991
Phe	y=17.342x-0.236	0.0642-0.642	0.9999
Trp	y=14.392x-0.072	0.0562-0.562	0.9999
Lys	y=22.350x+0.027	0.0741-0.741	0.9996

DISCUSSION

EAU model was first established in 1963, subsequently induced with S-Ag, IRBP, rhodopsin, recoverin, or phosducin. Further, it was found that the development of diverse retinal proteins which induced was mediated by T cells[7]. EAU are widely used to imitate human diseases of autoimmune nature, although the animal models were not reproduced the full spectrum of the disease. Clinically, the EAU has served as an ideal animal model to develop new effective therapeutic strategies. EAU in Lewis rat was typically takes an acute and anterior uveitis, a good clinical predictor. AAs represent certain nutritional conditions, and their disorders reflect various forms of aggression. However, the content of plasma AAs in EAU remain obscure. In the present study, a PITC pre-column derivation and HPLC method for the simultaneous determination of plasma AA in EAU and control Lewis rat were established. Furthermore, the concentration of AAs in EAU was compared to this in control group; PCA and PLS-DA were built in order to find correlations of AAs in plasma between EAU rats and control group.

There are several deproteination methods of AAs, such as organic solvents precipitation, ultrafiltration, dialysis and strong acids[24,31]. The advantage of ultrafiltration deproteination method was simple without the other reagents, but may decrease recovery rate because of the interactions and

Table 2 The precision, repeatability, stability and recovery of established AA analysis methods RSD, %

AAs	Reproducible				Repeatability (*n*=5)		Stability (*n*=6)		Recovery (*n*=9)
	Intra-day (*n*=6)		Inter-day (*n*=5)						
	RT	PA	RT	PA	RT	PA	RT	PA	
Asp	0.05	0.58	0.06	0.84	0.03	0.38	0.05	1.25	3.58
Glu	0.04	0.82	0.06	1.03	0.04	0.56	0.06	1.64	4.39
Ser	0.09	0.15	0.13	0.56	0.04	0.26	0.08	2.03	3.65
Gly	0.05	0.33	0.08	0.95	0.05	0.31	0.08	1.89	2.87
His	0.06	0.21	0.06	0.82	0.05	0.15	0.12	1.57	3.78
Arg	0.04	0.68	0.07	1.23	0.06	0.43	0.11	2.36	4.03
Thr	0.04	1.33	0.06	2.07	0.06	0.89	0.08	2.48	3.72
Ala	0.05	0.79	0.08	1.59	0.05	0.65	0.07	1.56	2.91
Pro	0.05	0.43	0.08	1.02	0.05	0.38	0.08	1.82	2.53
Tyr	0.06	0.25	0.08	1.18	0.03	0.24	0.09	1.75	3.07
Val	0.05	0.54	0.09	0.95	0.03	0.48	0.07	1.95	1.98
Met	0.04	0.36	0.05	1.05	0.04	0.32	0.06	2.08	2.69
Cys	0.08	0.57	0.1	1.31	0.04	0.51	0.07	1.91	3.04
Iso	0.11	0.83	0.12	1.58	0.05	0.14	0.08	2.51	2.59
Leu	0.12	0.76	0.12	1.61	0.05	0.12	0.09	2.37	2.82
Phe	0.12	0.61	0.13	1.39	0.06	0.34	0.11	1.38	2.38
Trp	0.13	0.29	0.15	1.24	0.06	0.31	0.11	1.65	2.55
Lys	0.15	0.34	0.18	1.37	0.07	0.28	0.13	1.73	2.94

AA: Amino acid; RT: Retention time; PA: Peak areas; AR: Average recoveries.

Table 3 The concentration of plasma AAs of EAU and control group μmol/mL, *n*=14

AAs	TAU group	Control group	Change	AAs	TAU group	Control group	Change
Asp	0.0353[a]	0.0401	↓	Tyr	0.0442[a]	0.0755	↓
Glu	0.0318[a]	0.0405	↓	Val	0.1031	0.0905	↑
Ser	0.0969	0.0956	-	Met	0.0432[a]	0.0622	↓
Gly	0.1423[a]	0.2149	↓	Cys	0.1809	0.1627	↑
His	0.1238[a]	0.1978	↓	Iso	0.1069	0.0960	↑
Arg	0.3044	0.3351	-	Leu	0.1831	0.1131	↑
Thr	0.2603	0.2184	↑	Phe	0.0638	0.0677	-
Ala	0.3296[a]	0.4480	↓	Trp	0.1121[a]	0.1321	↓
Pro	0.1087[a]	0.1278	↓	Lys	0.5281	0.4836	-

AA: Amino acid; EAU: Experimental autoimmune uveoretinitis. ↓: AAs descended more than 10% compared to control group; ↑: AAs increased more than 10%; -: The change of AAs no more than 10%. Compared with control group, [a]P<0.05.

filter, and experimental costs are higher. The deproteination methods of high speed centrifugation and dialysis need special equipments and the experimental costs also higher. Pretreatment need strong acid, and plasma is not evidently diluted. However, strong acids can affect the life of column, the separation of samples, and not remove all plasma protein either. Organic solvents can remove most plasma protein, although requires larger volumes, cause the sample to be diluted. In the present study, the bio-sample was plasma, and the content of plasma AAs in EAU rats and control group was higher. So the plasma sample was not concentrated after the pretreatment with organic solvents for injection.

The simpler and greater sensitivity method of simultaneous determination of AAs in the body-fluids is HPLC, which usually applies reversed-phase C_8 or C_{18} silica-based column with pre-column derivization. Gas chromatography, capillary electrophoresis, ion exchange chromatography and post-column derivization are also used[32]. PITC, benzylisothiocyanate, benzenedialdehyde, 5-dimethlyamino-1-naphthalene sulfonyl chloformate, 9-fluorenylmethyl chloroformate, and 6-aminoquinoyl-N-hydroxysuccinimidyl carbamate are used for pre-column derivatization of AAs. PITC can react quantitatively with free and hydrolysis AAs to form stable phenylthiocarbamyl AA derivatives and only one product for each AA. The excess reagents and byproducts could interfere by adding organic solvents and then remove

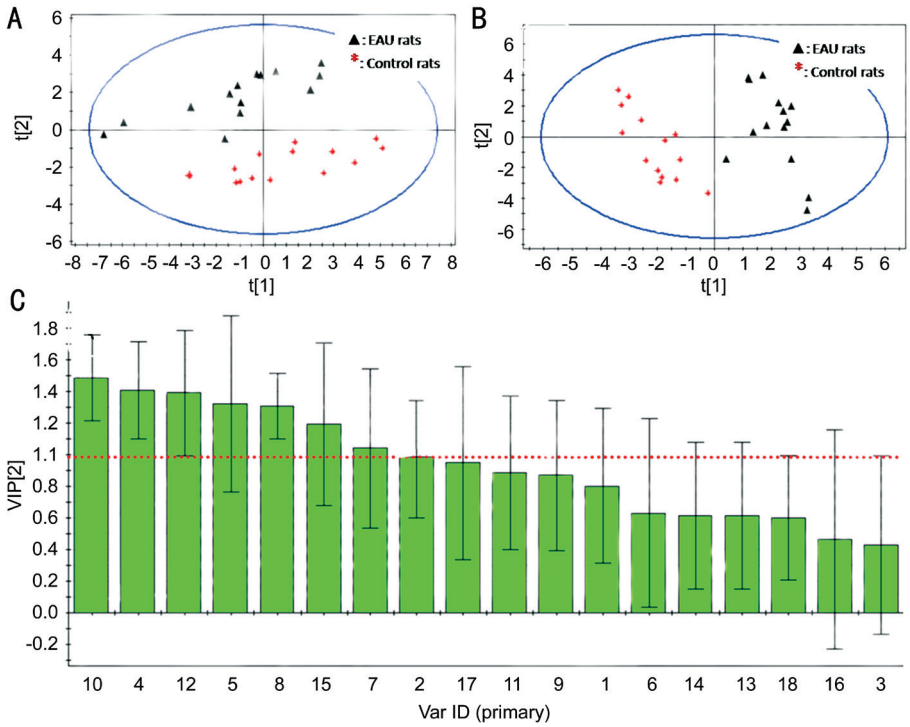

Figure 4 Score plots between EAU group and control group A: PCA score plots; B: PLS-DA score plots; C: VIP of PLS-DA.

them. HPLC with pre-column derivatization was used to analysis the levels of AAs in rat plasma, urine, spinal fluid, tissue samples of mammals[20,23-24]. Based on the method of PITC derivatization, we used HPLC with DAD to separate and analyze the free AAs levels in rat's plasma samples.

In this study, we established a method for detecting AAs contents in plasma. A single, reliable analytical method was required. We finally selected a 3 μm column (4.6×150 mm) from several different lengths, internal diameter and filler particle, such as Venusil MP C_{18} 3 μm 2.1×100 mm, Acclaim C_{18} 5 μm 4.6×250 mm and Atlantis dC_{18} 3 μm 4.6×150 mm. Furthermore, we optimized the separation procedure of different gradients to obtain an appropriate resolution value. We tested different gradients from conditions according to previously method[33]. The response variable used in this way was the chromatographic resolution of the AA standard solutions. The retention time reproducibility of 18 kinds of AAs was studied, finally gradient elution was selected.

In this study, we found that plasma AAs was significant difference between EAU and control group. There were 9 kinds of AAs in EAU rats whose concentrations were lower than in control group, 5 kinds of AAs were higher in EAU (Figure 3, Table 3). Notably among these AAs, Tyr, Gly, Met, His, Ala, Glu, Try, Pro and Asp were decereased, while the levels of Leu, Thr, Val, Iso and Cys were increased. The most decreased AA was Tyr, which is the essential AA, and related to Tyr with dopa, dopamine, norepinephrine and epinephrine, and also the product of metabolism of phenylalanine. In this study, the content of Phe was hardly changed. In contrast, the most increased AA was Leu, demonstrating that signal transduction pathways related to Leu may contribute to interpretation of cellular signals[34]. Moreover, AAs can also influence protein by regulating protein synthesis and degradation. Because of Tyr, Leu, Gly and Met represent a biologic molecules exerting dynamic, highly disparate physiologic processes in EAU. Thus, we have applied an analytical tools to understand of AA in EAU.

Metabolic profiling can provide a window to study of systematic and functional living organisms[35]. The role of AAs in ophthalmology is growing, which lots of studies has been demonstrated[20-21]. Plasma metabolites was source of information about metabolic events in the organism[36]. AAs play important roles in retinal function and neurotransmission[37]. Many ocular diseases related changes to AA levels[38]. Each AA is the result of a balance between input and removal. The change of AAs in the plasma of EAU may represent markers of changed catabolism in these rats and play a role.

In the study, we found that tyrosine and histidine were significantly lower in plasma in EAU rats than in control samples. Tyr is a semi-essential AA that is only synthesized by the hydroxylation of phenylalanine and by the enzyme phenylalanine hydroxylase[39]. Histidine is an alpha-AA, which precursor for histamine and carnosine biosynthesis. Histidine metabolism are marked by increased histidine. Elevated level of histidine is accompanied by symptoms from mental to physical retardation. Histidine have anti-oxidant, anti-inflammatory and anti-secretory properties[40]. Histidine has been shown to reduce insulin resistance, suppress inflammation

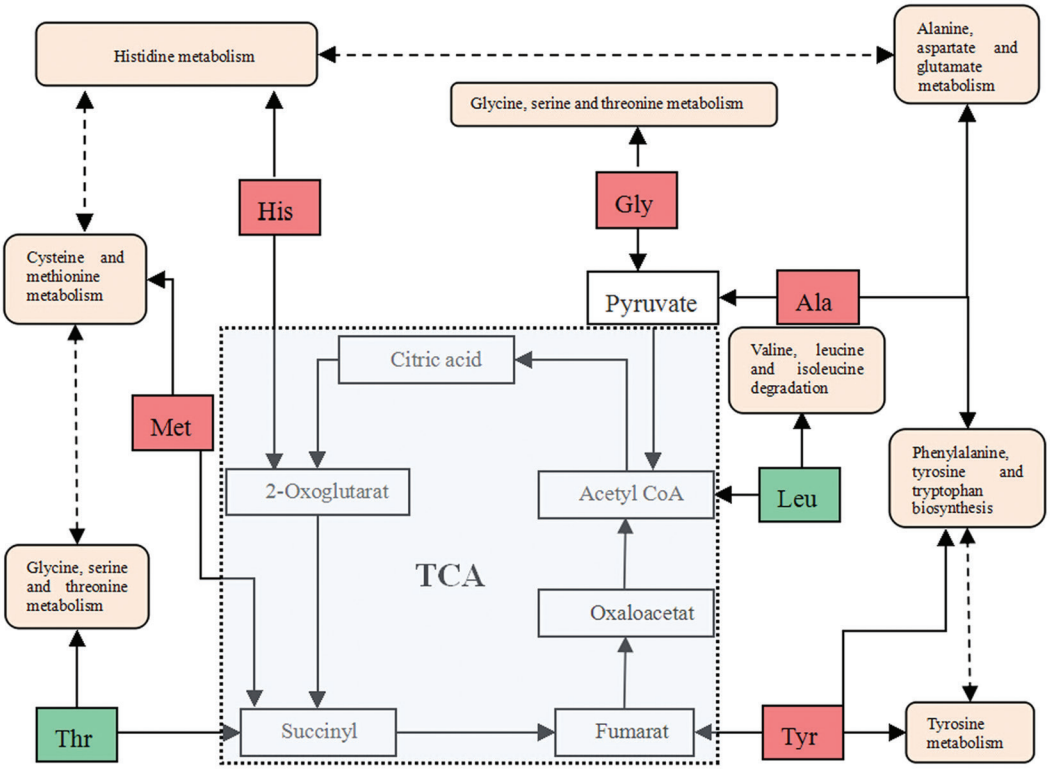

Figure 5 Schematic network of the metabolic pathways related to EAU Red-labeled metabolites descended, Green-labeled metabolites increased. Light brown-labeled refers to the metabolic pathways.

and oxidative stress in metabolic syndrome obese women. Furthermore, histidine appears to suppress pro-inflammatory expression *via* NF-κB pathway by increasing oxidative stress within adipocytes[41]. Low histidine are associated with inflammation, protein-energy wasting, and greater mortality in patients with chronic kidney disease[42]. Low His maybe caused by switch from oxidative phosphorylation to glycolysis. Therefore, we speculate that observed decreases in plasma Tyr and His concentration may be attributable to the cause of EAU pathological changes. Nevertheless, further studies are needed to investigate these possibilities in uveitis.

The biomarkers and their metabolic pathways merely show the isolated change of plasma AAs in EAU. However, the metabolic network built combined with biomarkers and metabolic pathways can display the holistic changes. These AAs metabolites correlated with each other affected six metabolic pathways (Figure 5). Tricarboxylic acid (TCA) cycle is a major source of adenosine triphosphate production, correlated with pathways for AA, fat, and glucose metabolisms[43]. The TCA cycle bypassed the switch from oxidative phosphorylation to glycolysis is a core pathway for the metabolism of AAs[44-45]. Therefore, we hypothesize that the imbalance of AAs in the plasma in EAU rats will influence the TCA cycle by switch from oxidative phosphorylation to glycolysis. The decreased and increased levels of AAs indicate the disturbance of TCA cycle. We herein consider that the metabolic biomarkers and pathways are impacted by the conditions of internal environment based on the results obtained from plasma metabolomic profiling.

In this study, we reported the development of the model AAs analysis of the plasma samples in EAU and determination the differences of AA levels in experimental group and control. The results show that the metabolic profile in EAU rats was different from control groups five AAs concentrations were increased and nine AAs were reduced. The content of Tyr, Gly, Met, His, Ala, Leu and Thr was differences of AA in experimental group and control. It was also found that EAU might be related to five metabolic pathways, such as glycine, serine and threonine metabolism, *etc.* These results suggest that plasma metabolic profiling of AAs could be a platform for understanding EAU. The developed method is simple, rapid and convenient for AAs in EAU rat plasma, and these findings will provide a comprehensive insight on the metabolic profiling of the pathological changes in EAU.

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