Serum Pharmacochemical Analysis of Mongolian Medicine Agar-8 for Combined Allergic Rhinitis and Asthma Syndrome

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Abstract: Combined Allergic Rhinitis and Asthma Syndrome (CARAS) refers to a respiratory allergic reaction disease that occurs concurrently in both the upper respiratory tract (allergic rhinitis) and the lower respiratory tract (asthma). Mongolian medicine has shown significant efficacy in treating CARAS, particularly Mongolian Medicine Agar-8, which has garnered widespread attention from patients and medical professionals during CARAS treatment. To date, the material basis of Agar-8 in the treatment of CARAS remains unclear. In this study, a serum drug fingerprint of Agar-8 for CARAS was established. The results identified 67 differential metabolized oxidative products in the blood, with seven small molecules being primarily screened. Among these, apigenin, fumaric acid, L-phenylalanine, and deoxycholic acid are suggested as the potential pharmacodynamic substances of Agar-8 in treating CARAS. Therefore, this study lays the foundation for future CARAS research.

Keywords: Mongolian Medicine; Combined Allergic Rhinitis and Asthma Syndrome; Serum Pharmacochemistry; Agar-8; Pharmacodynamic Substances

1. Introduction

Combined Allergic Rhinitis and Asthma Syndrome (CARAS) refers to a respiratory allergic reaction disease that occurs concurrently in both the upper respiratory tract (allergic rhinitis) and the lower respiratory tract (asthma) [1-3]. The principle of treating allergic diseases is to avoid or eliminate allergens. However, the allergens of CARAS are often substances in the natural environment, such as mugwort and pollen, which cannot be easily isolated or avoided. Clinically, the treatment of allergic rhinitis often shows significant short-term effects but tends to relapse, and there is no cure currently available [4-6]. Mongolian medicine considers CARAS to fall within the category of "Yama disease" in Mongolian medical theory. It is believed that the primary internal causes of this disease are Yama evil, Chusi, and Shira [7,8]. The external causes are weak physical condition, excessive fatigue, exposure to wind and cold leading to Yama insect invasion, which ascends through the qi and blood to invade the nasal cavity; or prolonged use of tobacco and alcohol, excessive consumption of rich foods, leading to an imbalance of Chusi and Shira, resulting in heat evil ascending to the nose, damaging the nasal sinuses, obstructing qi and blood, and causing the accumulation of Yama evil toxins, thus leading to this disease [9-15]. The principle of Mongolian medical treatment for this disease is to kill the mucous insects, clear heat, and relieve pain. Mongolian medicine has shown significant efficacy in treating CARAS, particularly Mongolian Medicine Agar-8, which has garnered widespread attention from patients and medical professionals during CARAS treatment [16,17]. However, the material basis of Agar-8 in the treatment of CARAS remains unclear. This study, based on the efficacy of Agar-8 in treating CARAS, utilizes serum pharmacological analysis to screen for small molecules with differential metabolism in the blood, and preliminarily elucidates the targeted metabolism of these small molecules, as well as predicting their immunometabolic functions.

2. Experimental Materials and Methods

2.1 Experimental Animals

SPF-level SD rats (male), 180-200g, 40 in total, provided by the Beijing Laboratory Animal Research Center. The experiments were conducted in the laboratory of the Inner

Mongolia International Mongolian Hospital.

2.2 Model Establishment and Drug Administration Methods

Modeling was done using ovalbumin sensitization and nasal drip challenge method: After 14 days of intraperitoneal injection of ovalbumin, continuous nasal dripping with ovalbumin for 7 days was performed to establish the model. On the 7th day after the initial sensitization, ovalbumin was dripped into the nose, once a day, 10 μL each time, for 7 consecutive days. After that, 1g/L of ovalbumin was given intranasally every other day to maintain stimulation of the nasal mucosa. The number of nasal scratches and sneezes within 30 minutes of the nasal drip was observed every 7 days. The scoring method recorded the amount of nasal secretion, the number of sneezes, and the degree of nasal itching. The method was as follows: (1) Nasal discharge: reaching the anterior nostril, 1 point; exceeding the anterior nostril, 2 points; nasal discharge covering the face, 3 points. (2) Sneezes: within 4 times, 1 point; 5-10 times, 2 points; more than 10 times, 3 points. (3) Nasal itching: mild scratching of the nose, 1 point; frequent scratching of the nose, 2 points; continuous scratching of the nose, 3 points. Thirty minutes after nasal stimulation, the cumulative score method was used, with a total score exceeding 5 points indicating successful model establishment.

From the 15th day, the rats were divided into the treatment group and the control group, which were respectively given the water extract solution of Mongolian Medicine Agar-8. The model group rats were placed in a closed container (a self-made 50cm×30cm×20cm transparent plastic box with a lid) and given 2% Mongolian Medicine Agar-8 water extract for nebulized inhalation; saline was used as a substitute in the control group. The nebulization flow rate was set at 3 mL/min, with each nebulization session lasting for 20 minutes to induce asthma. The rats' responses were observed, including symptoms such as fur shaking, piloerection, nose scratching, coughing, sneezing, abdominal breathing, rapid breathing, and in severe cases, cyanosis of the lips and ears, neck extension, chest contraction, nodding movement, convulsions, collapse, and incontinence. After symptoms appeared, two rats from the normal group and two from the model group were randomly selected for pathological sectioning. Compared with the normal group, the model group showed a significant infiltration of eosinophils in the bronchial mucosa and submucosa, thickened bronchial walls, smooth muscle hyperplasia, narrowed lumen, and mild congestion and edema of the tissue mucosa, indicating successful model establishment.

2.3 Metabolite Extraction

At specific time points (1h, 2h, 3h, and 6h after administration), blood samples were taken. Add 1000 μL of water (IS=1000:10); vortex for 30 seconds, add 2 small steel beads, grind at 45 Hz for 4 minutes, ultrasonicate in an ice-water bath for 15 minutes; centrifuge the sample at 12000 rpm for 15 minutes at 4℃; remove the supernatant and filter it through a 0.22 μm membrane into a sample vial for testing; take 400 μL of the serum sample, add 40 μL of hydrochloric acid (2 mol/L); vortex for 1 minute, let it stand at 4℃ for 15 minutes; repeat vortexing and standing four times, then add 1.6 mL of acetonitrile; vortex for 5 minutes, centrifuge at 12000 rpm for 5 minutes, and dry 1800 μL of the supernatant with nitrogen; reconstitute with 150 μL of 80% methanol (IS=1000:10), vortex for 5 minutes, centrifuge at 12000 rpm for 5 minutes; take 120 μL of the supernatant into a sample vial for testing.

2.4 On-machine Testing

Analysis was conducted under the control of the Agilent ultra-high-performance liquid chromatography 1290 UPHLC. The chromatographic column used was the UPLC BEH C18 column (1.7μm2.1100 mm) purchased from Waters. The injection volume was 5 μL. The Q Exactive Focus mass spectrometer was used for data collection under the control of the Xcalibur software (Thermo Fisher Scientific) based on the FullScan-ddMS2 function. The detailed parameters were as follows: Sheath gas flow rate: 45 Arb, Aux gas flow rate: 15 Arb, Capillary temperature: 400℃, Full ms resolution: 70000, MS/MS resolution: 17500, Collision energy: 15/30/45 in NCE mode, Spray Voltage: 4.0 kV (positive) or -3.6 kV (negative).

2.5 KEGG Pathway Analysis of Metabolite

Targets and Disease Treatment Targets

The Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway database (www.kegg.jp/kegg/pathway.html) stores functional information about genes and genomes, including graphical representations of cellular biochemical processes such as metabolism, membrane transport, signal transduction, cell cycle, and conserved sub-pathways. By analyzing metabolic pathways significantly enriched in target proteins, it is possible to understand which pathways are affected by the active ingredients of traditional Chinese medicine to treat diseases. The results were rendered using the mapping module provided by the KEGG website for the rat database.

3. Results

3.1 Mass Spectrometry Detection and Analysis Results

Based on retention time, molecular ions, major fragment ions, and previously published articles and online databases, a total of 9729 compounds were preliminarily identified (Figure 1). Sixty-seven potential active metabolites of Mongolian Medicine Agar-8 were screened based on the simultaneous presence in the three drugs. The identified compounds are mainly classified as terpenes. The detailed information of the identified compounds is listed in Table 1. These results suggest that Mongolian Medicine Agar-8 has multiple pharmacological effects. We conducted a network analysis of the chemical components of Mongolian Medicine Agar-8 to determine its medicinal value.

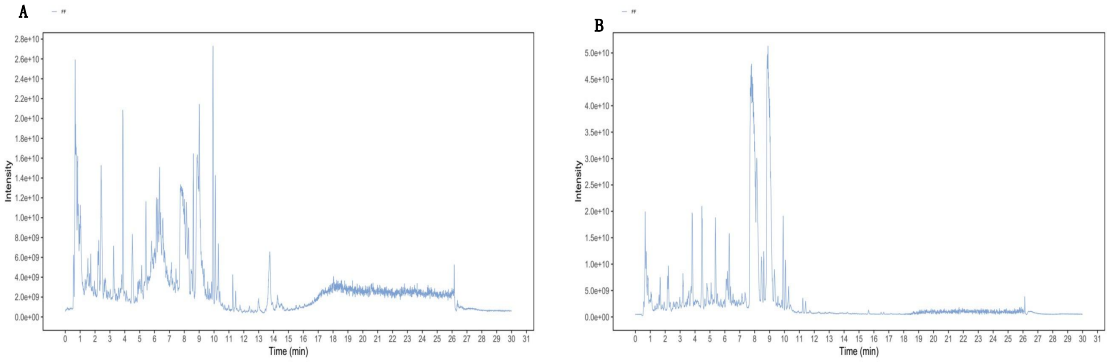

Figure 1. TIC Chromatograms of the Sample Detected by UHPLC-QE-MS. A. TIC Chromatogram in Positive Ion Mode B. TIC Chromatogram in Negative Ion Mode

3.2 Screening and Identification of Differential Metabolites

A total of 67 differential oxidized metabolites were detected between the CARAS model group and the normal control group (Table 1), mainly including flavonoids, organic acids, amino acids, phenols, fatty acids, aromatic compounds, and oxidized metabolites of fatty acids. Among them, 7 metabolites met the screening criteria. Compared with the normal control group, 4 metabolites were elevated, including apigenin, fumaric acid, L-phenylalanine, and deoxycholic acid (Figure 2).

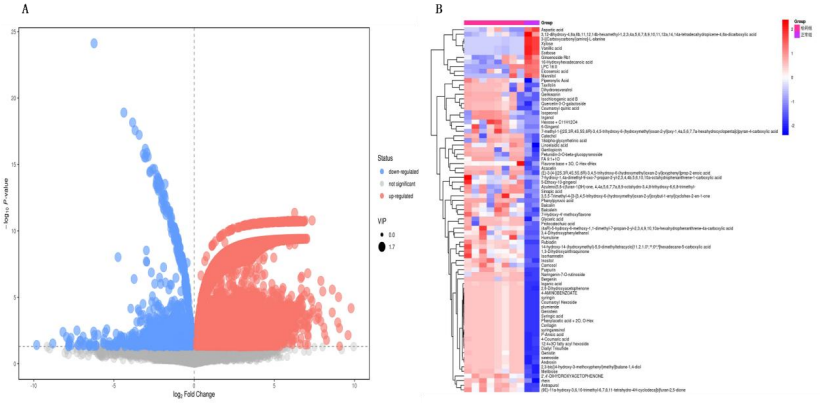

Figure 2. Analysis of Serum Pharmacotherapy Results
A. Volcano Plot B. Heatmap of Hierarchical Clustering Analysis

Table 1. Differentially Expressed Small Molecules Detected in Serum

ID	MS2 name	HMDB	KEGG COMPOUND ID	Sub.Class
1	Apigenin	HMDB0002124	C01477	Flavones
2	Digitoxin	HMDB0015468	C06955	Steroid lactones
3	Fumaric acid	HMDB0000134	C00122	Dicarboxylic acids and derivatives
4	L-Phenylalanine	HMDB0000159	C00079	Amino acids, peptides, and analogues
5	L-Valine	HMDB0000883	C00183	Amino acids, peptides, and analogues
6	Vanillin	HMDB0012308	C00755	Methoxyphenols
7	Chenodeoxycholic acid	HMDB0000518	C02528	Bile acids, alcohols and derivatives
8	Oleic acid	HMDB0000207	C00712	Fatty acids and conjugates
9	Gallic acid	HMDB0005807	C01424	Benzoic acids and derivatives
10	Palmitoleic acid	HMDB0003229	C08362	Fatty acids and conjugates
11	Tauroursodeoxycholic acid	HMDB0000874		Bile acids, alcohols and derivatives
12	Deoxycholic acid	HMDB0000626	C04483	Bile acids, alcohols and derivatives
13	Ellagic acid	HMDB0002899	C10788	Hydrolyzable tannins
14	Daidzein	HMDB0003312	C10208	Isoflav-2-enes
15	Gentisic acid	HMDB0000152	C00628	Benzoic acids and derivatives
16	Taurine	HMDB0000251	C00245	Organosulfonic acids and derivatives
17	Mannitol	HMDB0000765	C00392	Carbohydrates and carbohydrate conjugates
18	Diosmetin	HMDB0029676	C10038	O-methylated flavonoids
19	Abscisic acid	HMDB0036093		Sesquiterpenoids
20	Biochanin A	HMDB0002338	C00814	O-methylated isoflavonoids
21	Glyceric acid	HMDB0000139	C00258	Carbohydrates and carbohydrate conjugates
22	Licoricesaponin G2	HMDB0039318		Terpene glycosides
23	Glycitein	HMDB0005781	C14536	Isoflav-2-enes
24	Vanillic acid	HMDB0000484	C06672	Benzoic acids and derivatives
25	Palmitic acid	HMDB0000220	C00249	Fatty acids and conjugates
26	Vaccenic acid	HMDB0003231	C08367	Fatty acids and conjugates
27	Linoleic acid	HMDB0000673	C01595	Lineolic acids and derivatives
28	Linoelaidic acid	HMDB0006270		Lineolic acids and derivatives
29	Sebacic acid	HMDB0000792	C08277	Fatty acids and conjugates
30	Chlorogenic acid	HMDB0003164	C00852	Alcohols and polyols
31	Myristic acid	HMDB0000806	C06424	Fatty acids and conjugates
32	Glycocholic acid	HMDB0000138	C01921	Bile acids, alcohols and derivatives
33	Syringic acid	HMDB0002085	C10833	Benzoic acids and derivatives
34	Naringenin	HMDB0002670	C00509	Flavans
35	Uridine	HMDB0000296	C00299	
36	Malic acid	HMDB0000744	C00711	Beta hydroxy acids and derivatives
37	Methylsuccinic acid	HMDB0001844	C08645	Fatty acids and conjugates
38	Isoleucine	HMDB0033923	C16434	Amino acids, peptides, and analogues
39	Sinapic acid	HMDB0032616	C00482	Hydroxycinnamic acids and derivatives
40	Baicalin	HMDB0041832	C10025	Flavonoid glycosides
41	Ecgonine	HMDB0006548	C10858	
42	Pimelic acid	HMDB0000857	C02656	Fatty acids and conjugates
43	Citraconic acid	HMDB0000634	C02226	Fatty acids and conjugates
44	Succinic acid	HMDB0000254	C00042	Dicarboxylic acids and derivatives
45	Melibiose	HMDB0000048	C05402	Carbohydrates and carbohydrate conjugates
46	Glutamylphenylalanine	HMDB0029156		Amino acids, peptides, and analogues
47	Ginsenoside Rg5	HMDB0031830		Triterpenoids
48	Heptadecanoic acid	HMDB0002259		Fatty acids and conjugates
49	Octyl gallate	HMDB0033375		Benzoic acids and derivatives
50	Deoxyloganic acid	HMDB0037028	C11647	Terpene glycosides
51	Isosakuranin	HMDB0029481		Flavonoid glycosides
52	alpha-Solanine	HMDB0034202	C10820	Steroidal glycosides
53	L-Tryptophan	HMDB0000929	C00078	Indolyl carboxylic acids and derivatives
54	Glabrolide	HMDB0034515		Triterpenoids
55	4-Hydroxybenzoic acid	HMDB0000500	C00156	Benzoic acids and derivatives
56	Nicotinic acid	HMDB0001488	C00253	Pyridinecarboxylic acids and derivatives
57	Umbelliferone	HMDB0029865	C09315	Hydroxycoumarins
58	Glimepiride	HMDB0014367	C07669	Benzenesulfonamides
59	Ginkgolide J	HMDB0029605	C07604	Terpene lactones
60	Quercetin	HMDB0005794	C00389	Flavones
61	Protocatechuic acid	HMDB0001856	C00230	Benzoic acids and derivatives
62	rutamarin	HMDB0030666	C09308	Furanocoumarins
63	Rosmarinic acid	HMDB0003572	C01850	Hydroxycinnamic acids and derivatives
64	Genistein	HMDB0003217	C06563	Isoflav-2-enes
65	Kaempferide	HMDB0037441	C10098	O-methylated flavonoids
66	Ginsenoside Rb1	HMDB0035892		Steroidal glycosides
67	Suberic acid	HMDB0000893	C08278	Fatty acids and conjugates

3.3 KEGG Pathway Analysis of Metabolite Targets and Disease Treatment Targets

Using the clusterProfiler R software, the differentially expressed genes screened from each comparison group were compared with

the KEGG database. The results showed that they were mainly enriched in the phenylalanine, tyrosine, and tryptophan biosynthesis pathways (Figure 3).

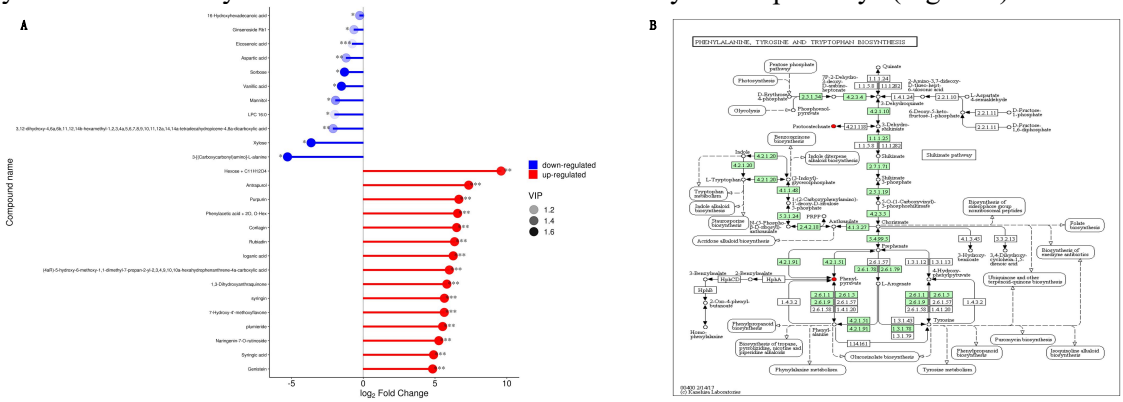

Figure 3. KEGG Pathway Map. The Metabolites Marked in Light Red/Light Blue are Differential Metabolites; the Lines Indicate the Direction of Metabolic Reactions

4. Discussion

CARAS refers to a respiratory allergic reaction disease that occurs concurrently in both the upper respiratory tract (allergic rhinitis) and the lower respiratory tract (asthma). CARAS is characterized by long-term recurrent attacks, stubborn conditions, and difficult recovery, especially in elderly patients and those with chronic illnesses and weak bodies, making it hard to eradicate. Over time, it may develop into pulmonary distension (chronic obstructive pulmonary disease, COPD) [18].

In clinical practice, they have found that Mongolian Medicine Agar-8 has a short treatment course, good efficacy, a high cure rate, and a low recurrence rate for CARAS. Additionally, Mongolian medicine is known to enhance physical fitness and improve immunity, which significantly helps in the recovery and prevention of CARAS. This study used metabolomics to analyze the components of Agar-8 that enter the blood in the CARAS model and the Mongolian medical syndrome. This paper described the effective components of Agar-8 that enter the blood of CARAS model rats through metabolomics. This study established a serum pharmacochemical fingerprint of Agar-8 for CARAS. On this basis, a comparative analysis was conducted on the serum pharmacochemical fingerprints of the treatment group, the blank control group, and the model group. The results identified 67 differential oxidized metabolites in the blood, mainly including flavonoids, organic acids, amino acids, phenols, fatty acids, aromatic compounds, and oxidized metabolites of fatty acids. Among them, 7 metabolites met the screening criteria. Compared with the normal control group, 4 metabolites were elevated, including apigenin, fumaric acid, L-phenylalanine, and deoxycholic acid [19-21]. Therefore, apigenin, fumaric acid, L-phenylalanine, and deoxycholic acid may be the pharmacodynamic substances of Agar-8 in the treatment of CARAS, laying the foundation for elucidating the material basis and mechanism of its action in the body.

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