Influence of compatibility of uncaria ramulus cumuncis with gastrodia rhizoma on liver gene expression in SHR rats

Yu Dong, Xing Wang*, Jia Mi and Juan Yang

School of Life Science and Engineering, Southwest Jiao-Tong University, Chengdu, China

ABSTRACT

Study the influence of compatibility of Uncaria Ramulus Cum uncis with Gastrodia Rhizoma on Liver Gene Expression in SHR Rats. Four SHR rat groups were established, including Blank, Uncaria decoction, Gastrodia suspension, Gastrodia and Uncaria decoction combined. After 10 days of continuous oral administration, mRNA in the liver of SHR rats was extracted, and then was hybrided with gene chip. After computerized scanning and analyzing, the changes in gene expression were observed. Fifteen target genes before and after compatibility of Uncaria Ramulus Cum uncis with Gastrodia Rhizoma were selected out.

Keywords: Uncaria Ramulus Cum uncis; Gastrodia Rhizoma; Compatibility; Liver; Gene expression

EXPERIMENTAL SECTION

1.1 Animal

SHR rats, SPF level, male, 14 weeks age, 40, body weight (270 ± 20) g (supplied by Shanghai SLAC Laboratory Animal CO. LTD, purchased by Sichuan Academy of Medical Sciences, Institute of Laboratory Animal). Animal Certificate: SCXK (Shanghai) 2008-0005. Animals were raised in IVC barrier (independent back to the Individually Ventilated Cages) system, 5 rats /box, temperature 22 ± 3 ℃, relative humidity 50% — 80%, and 12h light out / 12h dark (light intensity 150 Lux—300 Lux), drinking water using sterile water, feed was quality standards for the health of the whole price of grain feed, and fed by the irradiation.

1.2 Raw materials

The uncaria which is used to experiment is the stem with hamulus of Uncaria rhynchophylla and the hook with the same variety of plant stems, the place of origin is Anhui Bozhou, purchased from Sichuan lotus Pieces. The gastro-
dia which is used to experiment is the dry tubers of *Gastrodia elata Bl.*, the place of origin is the Emeishan of Sichuan, purchased from Sichuan lotus Pieces. All the herbs were identified by Associate Professor Song Liangke from the Southwest Jiao-Tong University, School of Life Science and Engineering, in accordance with the 2010 edition of Chinese Pharmacopoeia.

1.3 Reagent
TE buffer, 1 × sterile solution (pH 8.0) (U.S. AMRESCO Inc.); RNasey Mini Kit (Qiagen p/n 74904); Baseline-ZERO DNase (EPICENTRECat.Nos. DB0711K); Gene Expression Hybridization Kit (Agilent Part NO. 5188-5242, U.S.); Hybridization oven rotator for Agilent Microarray Hybridization Chambers (U.S. Agilent p/n G2530-60029); Agilent whole mouse genome Chip (U.S. Agilent Inc.).

1.4 Instruments
Z216MK desktop high-speed refrigerated centrifuge (Hermle German company); BioPulverizer™ System I (U.S. BioSpec company); Nanodrop ND-1000 (AmericanThermo Finnigan Corporation); Agilent Scanner microarray scanner (Agilent, USA); MICRO-4 hybrid oven (Hybaid UK company).

METHODS
2.1 Preparation of drug samples
Uncaria decoction: Take uncaria 20g, boiled with 15 times the volume of boiling distilled water, boiling 15min, concentrated into 0.1g crude drug per mL. Cool off for backup.

Gastrodia suspension: Take Gastrodia 15g, crashed into powder, over 200 meshesieve, add 10-fold distilled water Suspension to suspension (0.1g crude drug per mL). Cool off for backup.

Gastrodia and Uncaria Decoction: Take Gastrodia 15g, add 450mL of boiling distilled water for 1h, boiling 30min, then add 20g uncaria, boiling 15min, concentrated into 175mL (0.2g crude drug per mL). Cool off for backup.

To ensure the consistency of the samples, all the decoction were prepared in one time, refrigerated at 4 °C.

2.2 Animal experiments
Adaptive feeding for a week, SHR rats were divided into 4 groups randomly, 10 rats a group. Group A was the control group, administrated with normal saline for 3 mL / per; group B was administrated uncaria decoction for 3 mL / per; group C was administrated gastrodia suspension for 2.3 mL / per; group D was administrated gastrodia and uncaria decoction for 2.7 mL / per. All animals were given water freely. Intragastric administrated 1 time daily at 10 am for 10 days. After 30min of the last administration, the femoral artery was cut to bleeding to death. The liver of animals in each group were separated, quickly put into liquid nitrogen, then moved to -80 °C refrigerator for use.

2.3 Gene chips hybridization
Combined the liver tissue in each group preserved at item 2.2, using BioPulverizer™ to crash tissues in liquid nitrogen, added Trizlo reagent, extracting total RNA. mRNA extracted from each group was tagged as RNA probe by cy3 fluorescence, hybrided with 4 Agilent microarrays respectively, washing. Then used gene chip scanner to scan and get the chip chart used for data processing.

2.4 Filter the differential expression gene
Through the analysis of Agilent GeneSpring GX software (version10.0), change the signal ratio of dose group and blank group into fold-change (Expression value is normalized by median normalization method but do not take the log2 numerical value, take absolute ratio of the two comparing value) to show the multiples change value of gene expression. The study set the screen standard—the threshold of differential gene expression as 1.5. The gene whose expression level is higher or equal to threshold will be regarded as differences.

RESULTS
After the data had been processed and filtered by GX software, the Uncaria—liver target genes were selected. There were 979 differentially expressed genes in the liver between uncaria group and the control group, 478 differentially expressed genes in the liver between combination group and the control group. Among them, there were 187 genes appear together. The fold change values of combination group—uncaria group were analyzed in these genes. There were 15 genes significantly different (fold chang>1.5) and included by the U.S. National Center for Biotechnology Information (NCBI), which were selected to be the target gene expressed in the liver after administration of uncaria. Gene list is in Table 1.
Table 1: The list of target genes of Uncaria in SHR rat liver

<table>
<thead>
<tr>
<th>Genbank Accession</th>
<th>Gene Symbol</th>
<th>Normalized Intensity (log2)</th>
<th>Fold Change</th>
<th>Regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM_031620</td>
<td>Phgdh</td>
<td>62.2</td>
<td>15.3</td>
<td>up</td>
</tr>
<tr>
<td>BC103490</td>
<td>LOC497860</td>
<td>92.6</td>
<td>5.2</td>
<td>up</td>
</tr>
<tr>
<td>NM_130752</td>
<td>Fgf21</td>
<td>439.8</td>
<td>4.9</td>
<td>up</td>
</tr>
<tr>
<td>NM_013079</td>
<td>Asns</td>
<td>224.0</td>
<td>4.9</td>
<td>up</td>
</tr>
<tr>
<td>NM_017136</td>
<td>Sdcl</td>
<td>399.2</td>
<td>2.9</td>
<td>up</td>
</tr>
<tr>
<td>NM_144755</td>
<td>Tshb3</td>
<td>628.4</td>
<td>2.9</td>
<td>up</td>
</tr>
<tr>
<td>NM_080886</td>
<td>Sc4mol</td>
<td>6034.6</td>
<td>2.8</td>
<td>up</td>
</tr>
<tr>
<td>NM_031841</td>
<td>Scd2</td>
<td>62.7</td>
<td>2.6</td>
<td>down</td>
</tr>
<tr>
<td>NM_181087</td>
<td>Cyp26b1</td>
<td>16.4</td>
<td>2.6</td>
<td>down</td>
</tr>
<tr>
<td>XM_235511</td>
<td>RGD1563996</td>
<td>65.6</td>
<td>2.5</td>
<td>down</td>
</tr>
<tr>
<td>NM_031598</td>
<td>Pha2g2a</td>
<td>70.3</td>
<td>2.1</td>
<td>up</td>
</tr>
<tr>
<td>XM_217167</td>
<td>RGD1311874</td>
<td>562.7</td>
<td>1.8</td>
<td>up</td>
</tr>
<tr>
<td>NM_139096</td>
<td>Lgals3bp</td>
<td>4083.4</td>
<td>1.6</td>
<td>down</td>
</tr>
<tr>
<td>NM_053838</td>
<td>Npr2</td>
<td>124.6</td>
<td>1.5</td>
<td>up</td>
</tr>
<tr>
<td>NM_001033691</td>
<td>Irf7</td>
<td>7502.6</td>
<td>1.5</td>
<td>up</td>
</tr>
</tbody>
</table>

The 15 target genes were mainly concentrated on the regulation of lipid metabolism, amino acid metabolism, liver protection and so on. And in the liver, the regulation of lipid metabolism and amino acid metabolism-related genes were closely related to gastrodia—uncaria combination for the therapy of hypertension and hyperactivity of liver Yang [6-8].

3.1 As shown in table 1, after administrated uncaria, the enzyme regulating the synthesis of serineproteases: the expression of 3-phosphoglyceric acid dehydrogenase (NM_031620) up-regulated.

3.2 As shown in figure 1, in the liver, the role of uncaria on the related genes are more evenly, some up-regulated, and some down-regulated, but almost all of these genes showed a significant up—regulated after compatibility. For example, uncaria had a clear pharmacological effects in the liver, because the expression of high-density lipoprotein (HDL) metabolismrelated phospholipase A II (NM_031598) was inhibited, which maintained HDL levels in the blood and played a role in anti-atherosclerosis, but these pharmacological effects disappeared after compatibility. Gastrodia and uncaria were both enhancing liver function, but the role was different. Summing up the group’s pre-study, uncaria can make fatty acid carrier protein (NM_012556) expression increased, and gastrodia can make the expression of long-chain fatty acid coenzyme A ligase (NM_024143), stearoyl coenzyme A △9 (NADH—△9) and desaturase SCD (NM_031841) increased. These genes are all related to exogenous lipid metabolism.

3.3 As shown in figure 2, in the target genes screened from the “uncaria – liver”, Fgf21, Asns, and Lgals3bp genes were related to liver dysfunction and liver cancer. Fibroblast growth factor FGF21 significantly protect alcohol-induced liver damage and fatty liver, which could promote fat using and burning, inhibit fat synthesis to improve cell energy metabolism, inhibit liver fibrosis process to correct the effects of obesity on liver. On the other hand, FGF21 has the function of regulation glycometabolism and lipid metabolism as insulin. Asparagine synthetase...
ASNS is a specific protein spots in hepatoma carcinoma cell line, and the content is significantly lower in hepatoma carcinoma cell, compared with normal liver cell line [9].

After SHR rats had been administrated uncaria singly, soluble galectin-binding protein LGALS3BP (NM_139096) was reduced significantly in the liver. LGALS3BP is a scavenger receptor inhibitor, and when its expression is down, the activity of scavenger receptor would be enhanced, causing liver disease. In addition, LGALS3BP is also a tumor marker, and the expression levels were much lower in hepatoma carcinoma cell line, compared with normal liver cell line [10-12]. So uncaria may be toxic to the liver. However, after gastrodia and uncaria compatibility, the gene downward trend had been reversed obviously, and Fgf21, Asns even showed a significant increase.

![Liver disease-related genes expression trends](image)

**DISCUSSION**

4.1 The influence of before and after compatibility on Liver Gene Expression of regulating amino acid metabolism

Serine protease is a protease family, their function is to crack the peptide bond of macromolecule protein, and make it to small molecule protein. The activation was realized by a group of amino acid residues change in active center. One of them must be serine. In mammals, serine protease plays an important role especially in digestion, blood coagulation and complement system. There are three serine proteases in pancreatic secretion. Several activated coagulation factors are serine proteases. The study shown, the function of liver protection of uncaria were through influencing the expression of serine protease and protein kinase, regulating amino acid metabolism level and blood coagulation system of model animals.

4.2 The influence of before and after compatibility on Liver Gene Expression of regulating blood lipid metabolism

According to the study, the genes in Table 1 were related to exogenous lipid metabolism. The up-regulated genes can enhance the exogenous lipid metabolism function of liver, reduce the blood lipid and protect cardiovascular system. After compatibility, these genes were significantly up-regulated. It indicated that the pharmacological function was concentrated on liver after compatibility. This result was in accord with the “Medicinal guide by the compatibility” theory of traditional Chinese medicine.

4.3 The influence of before and after compatibility on Liver Gene Expression of liver protection

From previous study, uncaria used alone was harmful to liver. Through pathological observation, cluster analysis and target genes analysis, we found three points. ① Uncaria used alone hasn’t obvious liver protection. ② Uncaria used alone can make hepatic disease. ③ The FGF21 (Fibroblast growth factor) expression was down-regulated when SHR rats administrated uncaria. ASNS(Asparagine expression synthetase) was down-regulated. LGALS3BP(Soluble galactose lectin binding protein) expression was significantly down-regulated. These may be the reason of decreasing liver function and cancerization. From the points we can make conclusion, uncaria used alone can make hepatic disease, probably the cancerization of liver. After compatibility with gastrodia, The expression trends of Fgt21, Asns and Lgals3bp were reversed obviously. Among them, Fgt21 and Asns were up-regulated. This means that the compatibility has the function of “detoxification”. If the further evidence of uncaria’s toxicity and liver protection wanted, the toxicological experiment should be done.

The study used Agilent GeneSpring software to extract gene hybridization signal, transfer to expression value. After
a series of screen, the “Disease—Drug” gene expression difference chart was established. The study screen the target genes associated with drug channel distribution, through GEO database of NCBI to explain and cluster. From the result, the compatibility of uncaria and gastrodia has the obviously influence of regulating blood lipid metabolism, amino acid metabolism and liver protection, it has close relationship to the therapy of hypertension and Hyperactivity of liver Yang.

Gene Chips can determine the gene expression of target tissue, demonstrate the whole target gene after administrated Chinese Pharmacy. Provide clues to study the functional mechanism. The study reflected the functional mechanism of Chinese Pharmacy qualitatively and quantitatively in molecular biology level. The analysis method used can explore the “channel distribution” theory of traditional Chinese medicine. The explanation of “channel distribution to liver” of uncaria can contribute to the Modernization of uncaria.

CONCLUSION

After the compatibility of Uncaria Ramulus Cum unci s with Gastrodia Rhizoma, the gene expression related with lipid metabolism, amino acid metabolism, and liver protection are changed significantly. It indicates that the related effects of Uncaria on liver are strengthened and decrease the toxicity after the compatibility.

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