Chin J Integr Med 2022 Jun;28(6):538-544

Chinese Journal of Integrative Medicine

Available online at link.springer.com/journal/11655
Journal homepage: www.cjim.cn/zxyjhen/zxyjhen/ch/index.aspx
E-mail: cjim_en@cjim.cn

Original Article

Active Components Formulation Developed from Fuzheng Huayu Recipe for Anti-Liver Fibrosis*

SUN Xin[1], TAN Ye[1], LYU Jing[1], LIU Hong-liang[1], ZHAO Zhi-min[2], and LIU Cheng-hai[1,2]

ABSTRACT **Objective:** To screen the active components from Fuzheng Huayu Recipe (FZHY) and redesign a new recipe composed of the active components, and validate the effect of active components formulation from FZHY against liver fibrosis. **Methods:** Thirty-two components from FZHY were evaluated for their activities against liver fibrosis respectively, with 6 kinds of cell models *in vitro*, including oxidative stressed hepatocyte in L-02, hypoxia injured/proliferative hepatic sinusoidal endothelial cells in SK-HEP-1 and human hepatic sinusoidal endothelial cells (HHSEC), and activated hepatic stellate cell in LX-2. The comprehensive activity of each component against liver fibrosis was scored according to the role of original herbs in FZHY and cell functions in fibrogenesis. Totally 7 active components were selected and combined with equal proportion to form a novel active components formulation (ACF). The efficacy of ACF on liver fibrosis were evaluated on activation of LX-2 and proliferation of HHSEC *in vitro* and in liver fibrosis model mice induced by dimethylnitrosamine (DMN). Totally 72 mice were divided into 6 groups using a random number table, including normal, high-dose ACF control (20 μmol/L×7 components/kg body weight), model, low-, medium-, high-dose ACF groups (5, 10, 20 μmol/L×7 components/kg body weight, respectively). Hematoxylin eosin and Sirius red stainings were used to observe inflammation and fibrosis change of liver tissue; scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were utilized to observe the effect of ACF on ultrastructure of hepatic sinusoids. **Results:** Fifteen components from FZHY showed higher scores for their activity on against liver fibrosis. Among them, 7 components including tanshinone ⅡA, salvianolic acid B, cordycepin, amygdalin, quercetin, protopanaxatriol, and schizandrin B were recombined with equal proportions to form ACF. ACF at 1, 2, 4 μmol/L showed strong inhibitory effects on activation of LX-2 and proliferation of HHSEC *in vitro* (all $P<0.01$). Compared with the model group, ACF attenuated liver collagen deposition, improved sinusoidal capillarization in a dose-dependent manner (all $P<0.05$). **Conclusions:** ACF exerts a satisfactory effect against experimental liver fibrosis and attenuates sinusoidal capillarization, which warrant a further research and development for herbal components formulation on liver fibrosis.

KEYWORDS active components formulation, Fuzheng Huayu Recipe, Chinese medicine, liver fibrosis, hepatic stellate cells, hepatic sinusoidal capillarization

Liver fibrosis, characterized as aberrant healing, excessive deposition of extracellular matrix proteins and sinusoidal capillarization or increased angiogenesis in liver, play a pivot role in chronic liver diseases (CLDs). Chronic liver diseases develop into cirrhosis even liver cancer and threaten the life of patients. The accumulated data showed that hepatic stellate cells (HSCs) and liver sinusoidal endothelial cells (LSECs) are important cellular basis for liver fibrogenesis.[1,2] HSCs is the potential cell for endothelial cell medium (ECM) production and LSECs usually is the early site for liver injury since they locate in hepatic sinusoid. They secrete cytokines to simulate each other, developing liver fibrosis and sinusoidal capillarization.[3] The intervention of HSCs and LSECs, attenuation of the sinusoidal capillarization could be ideal strategy to

*Supported by the Major Projects of the Ministry of Science and Technology of China (No. 2014ZX10005001), the National Natural Science Foundation of China (No. 81603467, 81730109) and the Third Batch of Open Projects of Shanghai Innovation Center of Traditional Chinese Medicine Health Service (No. ZYJKFW201811013)

1. Institute of Liver Diseases, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai (201203), China; 2. Shanghai Key Laboratory of Traditional Chinese Clinical Medicine, Shanghai (201203), China
Correspondence to: Prof. LIU Cheng-hai, E-mail: chenghailiu@hotmail.com

DOI: https://doi.org/10.1007/s11655-021-3293-x

develop agents against liver fibrosis.

Although some anti-fibrotic drugs had completed phase Ⅱ or Ⅲ clinical trials,[4,5] they mainly target one or more signaling mediators of liver fibrosis, the efficacy and safety are not satisfactory. Chinese medicines (CMs), with multi-components and multi-targets, play a prominent role in the field of anti-liver fibrosis. Clinical research showed that Fuzheng Huayu Capsule (FZHY, 扶正化瘀胶囊) possesses satisfactory therapeutic effects on alleviating liver fibrosis due to chronic hepatitis B by histological examination of liver.[6] Also, a phase Ⅱ, randomized, placebo-controlled, double-blind, multicenter study was conducted to assess the anti-fibrotic activity of FZHY in chronic hepatitis C patients with hepatic fibrosis, and the results showed that FZHY was well tolerated and effective.[7] Experimental researches also revealed that FZHY could prevent liver fibrosis via multiple pathways, including protecting hepatocytes from oxidative stress and apoptosis, inhibiting activation of HSCs, as well as regulating metabolism of extracellular matrix (ECM).[8] Our earlier study showed that FZHY could inhibit intrahepatic sinusoidal capillarization in liver fibrotic mice, by reducing the hypoxia inducible factor 1 (HIF1)–vascular endothelial growth factor (VEGF) signaling pathway to prevent LSECs dedifferentiation.[9]

However, active substances in FZHY and their pharmacological targets for liver fibrosis are still elusive. In the current study, the active components from FZHY were evaluated using hepatic cell lines and the representative ones were selected according to the comprehensive scores of activities and role of herb from which the components derived, and the new formulation composed of the active components were redesigned, and its efficacy on liver fibrosis and sinusoidal capillarization was validated. The presented formulation was featured by clear ingredients and targets, and the proposed method provided a new approach for development of anti-fibrosis drugs.

METHODS

Drugs

The Chinese herbs and 32 chemical components of FZHY are listed in Appendix 1. Those ingredients were purchased from Shanghai Winherb Medical Technology Co., Ltd. (Shanghai, China), with the actual purity ⩾99%.

Reagents

ECM and endothelial cell growth supplement (ECGs) were purchased from ScienCell Research Laboratories Inc. (Cat No. 1001 and 1052, San Diego, CA, USA). Dulbecco's modified Eagle medium (DMEM), RPMI-1640 medium, minimum essential medium (MEM), and fetal bovine serum (FBS) were provided by GIBCO (Cat No. 12100046, 23400021, 11700077 and 10099; Grand Island, NY, USA). Dimethyl sulfoxide (DMSO) and hydrogen peroxide (H_2O_2) were purchased from Sinopharm Chemical Reagent Co., Ltd., (Cat No. 0231 and 10011218, Shanghai, China). Methylthiazol-diphenyl tetrazolium bromide (MTT) and Cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_2O$) were commercially provided by Sigma-Aldrich Corp. (Cat No. M5655 and C8661; St Louis, MO, USA). Recombinant human transforming growth factor-β 1 (rhTGF-β 1) was purchased from R&D Systems, Inc. (Cat No. 240, Minneapolis, MN, USA). VEGF-A and platelet-derived growth factor B (PDGF-B) were provided by ProSpec-Tany TechnoGene Ltd. (Cat No. CYT-338 and CYT-491; Rehovot, Israel). Cell counting kit-8 (CCK-8) was purchased from Shanghai Yeasen Biotechnology Co., Ltd. (Cat No. 40203ES88, Shanghai, China).

Cell Culture

Human liver cell line HL-7702 (L-02; Cell Bank of Type Culture Collection, Chinese Academy of Sciences, Shanghai, China) was cultured in RPMI-1640 medium containing 10% fetal bovine serum (FBS). Human hepatic stellate cell line LX-2 (provided by Prof. XU Lie-ming, Institute of Liver Diseases, Shanghai University of Traditional Chinese Medicine, Shanghai, China) was cultured in DMEM containing 10% FBS. Human liver ascites adenocarcinoma endothelial cell line SK-HEP-1 (ATCC® HTB-52™; American Type Culture Collection, Manassas, VA, USA) was cultivated in MEM containing 10% FBS. Human hepatic sinusoidal endothelial cells (HHSECs; ScienCell™ Research Laboratories Inc., Carlsbad, CA, USA) were cultured in ECM, which was supplemented with 5% FBS and 5% ECGs. All cell lines were cultured in the condition of 5% CO_2 at 37 ℃ (Thermo Fisher Scientific, Waltham, MA, USA). Establishing cellular model was performed as follows: oxidative damage model of L-02 was induced by 1 mmol/L H_2O_2 for 30 min, activated model of LX-2 was induced by 0.1 nmol/L rhTGF-β 1 for 24 h, hypoxia injury model of SK-HEP-1 was induced by

0.8 mmol/L $CoCl_2$ for 24 h, excessive proliferation model of SK-HEP-1 was induced by 0.5 nmol/L VEGF-A plus 0.5 nmol/L PDGF-B for 24 h, hypoxia injury model of HHSEC was induced by 0.5 mmol/L $CoCl_2$ for 24 h, and excessive proliferation model of HHSEC was induced by 2% ECGs for 48 h.

Drug Administration

All chemical components were dissolved in serum-free medium containing 0.1% DMSO. For evaluating the toxicity of each component or combination, cells were adjusted to the density of 5×10^3 cells/mL, and 100 mL per well was added into 96-well plate after serum was starved; then, the cells were incubated in medium for 48 h. After that, the cells were incubated with CCK8 at 37 ℃ for 2 h. The spectrophotometric absorbance of samples was measured at 450 nm using a microplate reader (BioTek Instruments, Inc., Winooski, VT, USA). The cells treated with drugs were subjected to MTT for 3 h at 37 ℃, and then, incubated with DMSO at room temperature for 30 min.[10] Spectrophotometric absorbance of samples was measured at 570 nm. All experiments were repeated at least for 3 times.

Comprehensive Quantitative Assessment Scheme Based on Multicellular Models

Inhibiting activation or proliferation was taken as a major factor into consideration, while the protective effect assumed as an auxiliary factor in hepatic fibrosis and angiogenesis. A scoring scheme was implemented on multicellular model *in vitro*, as shown in Appendix 2. According to principle of "monarch, minister, assistant and guide" in the CM, 7 active components of FZHY were selected, including 2 from monarch herb *Radix Salviae* Miltiorrhizae, 1 from minister herbs *Cordyceps* and *Semen* Persicae, 1 from assistant herbs *Pollen Pini*, and 1 from *Gynostemma Pentaphyllum*, and 1 from envoy herb *Fructus Schisandrae* Chinensis.

Evaluation of Efficacy of New Active Components Formulation *in vitro*

The selected components were directly mixed in an equal proportion and taken it as a new active components formulation (ACF) of anti-hepatic fibrosis components. To assess the efficacy of new ACF, LX-2 activation model induced by 5 ng/mL rhTGF β 1 and HHSEC proliferation model induced by 2% ECGs were subsequently components employed. New ACF was set to 3 different concentrations: low-, medium-, and high-dose (1, 2, 4 μmol/L × 7 components) ACF, within the maximum non-toxic concentration range for each drug. The drug administration method and duration of incubation were adopted as mentioned above.[10] Cell viability was detected by a CCK-8 assay kit.

Development of DMN-Induced Liver Fibrosis Models in Mice

Five-week-old male Institute of Cancer Research (ICR) mice (25 ± 1 g, specific pathogen-free) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). They were housed in a specific pathogen-free and controlled environment (20 ± 2 ℃, 55% ± 5% humidity, lighting 12 h a day). All 72 mice were divided into 6 groups using a random number table (n=12 for each group): normal group, high-dose ACF control group, model group, low-, medium-, and high-dose ACF treatment groups. For model mice, 5 mL/kg body weight DMN (0.1% v/v in 0.9% Nacl Solution) was intraperitoneally injected into mice daily for 3 weeks.[11] ACF (7 components were mixed in equal proportions) was set at low-, medium-, and high-dose (5, 10, 20 μmol/L/kg body weight) according to the maximum non-toxic concentration of the drug in mice. Administration of 3 mentioned doses was performed by intragastric gavage daily starting from week 5–7. The drugs were dissolved in double-distilled water (DDW), containing 0.5% sodium carboxymethyl cellulose. The mice in nomal group only received DDW. The high-dose ACF control group was treated with the maximum dose of ACF without modeling to monitor the safety and toxicity of the drug. The animals were anesthetized by sodium pentobarbital and sacrificed by cervical dislocation after 24 h after the last administration. All animal experiments were performed in accordance with Guide for the Care and Use of Laboratory Animals published by the Laboratory Animal Center, Shanghai University of Traditional Chinese Medicine, Shanghai, China.

Histological Examinations

Liver tissues were fixed in 4% formaldehyde solution, embedded in paraffin, sliced in 4 μm, dewaxed with xylene and multistage ethanol to water, stained with Picric acid-Sirius red or hematoxylin eosin (HE) dye,[9] neutral gum sealed sheet and photographed with Olympus IX70 inverted microscope (Olympus, Japan). The area of Sirius red was calculated by

Image-Pro Plus software (Media Cybernetics, USA).

Hydroxyproline Content in Liver Tissue

About 100 mg of fresh liver tissue was extracted from each mouse and hydroxyproline (Hyp) content was determined by Jamall's hydrochloric acid hydrolysis method.[12]

Scanning and Transmission Electron Microscopes

The livers of mice were perfused with 0.9% Nacl Solution via the portal vein and fixed with 2.5% glutaraldehyde. Liver tissues were cut into small pieces and fixed in 4% osmium for 1 h. Then, the tissues were processed for sequential alcohol dehydration and infiltrated with t-butyl alcohol. After freezing, the tissues were vacuum-dried and coated using ion sputter Hitachi E-1030 (Hitachi, Tokyo, Japan) for analysis by SEMS-4100 (Hitachi). The tissue samples were immersed in acetone and carefully dissected into pertinent regions of interest from the anterior apex, and kept with one dimension of the tissue at 13 mm. After incubation in acetone, the tissues were put in mixtures of acetone and 618 resins with increasing concentration gradients. The specimens were then oriented and positioned in labeled molds and placed in an oven for curing. The hardened blocks were trimmed and prepared for 1.0–1.5 μm semi-thin sections. Sections were mounted on microscope slides and stained with lead citrate for 1 min at 80 ℃. The stained ultrathin sections were observed under a transmission electron microscope (FEI Tecnai G2 Spirit, USA).[9]

Statistical Analysis

All data were presented as mean ± standard deviation ($\bar{x} \pm s$). The differences among the groups were assessed by non-parametric one-way analysis of variance (One-way ANOVA), further pairwise comparison was performed by LSD test, and all data were statistically analyzed by using SPSS 21.0 software (IBM, Armonk, NY, USA). Statistical significance was set at $P<0.05$.

RESULTS

Comprehensive Scoring of 32 Components of FZHY and Taking Tanshinone ⅡA as an Example

In multicellular experiments, tanshinone ⅡA totally received 23 points as follows: 1 point was assigned for significantly affected cell activity in a dose-dependent manner (all $P<0.01$, but lower than the model group) without a protective influence on the oxidative damage of L-02; 7 points were assigned for inhibiting the activation of LX-2 cells in a dose-dependent manner (all $P<0.01$), while optical density (A) value of 20 μmol/L group was lower than the normal group; 2 points were assigned for dose-dependently effects on the cell viability of 2 SK-HEP-1 cellular model although there were no active influences on hypoxic injury (all $P>0.05$) or the proliferation (all $P<0.01$, but lower than the normal group); 3 points were assigned for 2 concentration protective effect on the hypoxia injury of HHSEC ($P<0.05$ *vs*. model group) and it trends in dose-dependent changes; besides, 10 points were assigned for its 3 concentrations showed dose-dependent inhibition on HHSEC proliferation (all $P<0.01$). Based on the above-mentioned scoring methods, the comprehensive scoring of tanshinone ⅡA was 23 points (Figure 1).

Similar to the comprehensive scoring method of tanshinone ⅡA mentioned above, 32 components of FZHY were scored through multicellular model (Appendix 3). Guided with prescriptions monarch compatibility theory of CM, 7 components were screened: tanshinone ⅡA (23 points), salvianolic acid B (26 points), cordycepin (25 points), amygdalin (18 points), quercetin (27 points), protopanaxatriol (21 points), and schizandrin B (18 points). These components were selected to form a new ACF.

Efficacy of New ACF From FZHY *in vitro*

Compared with the model group, ACF

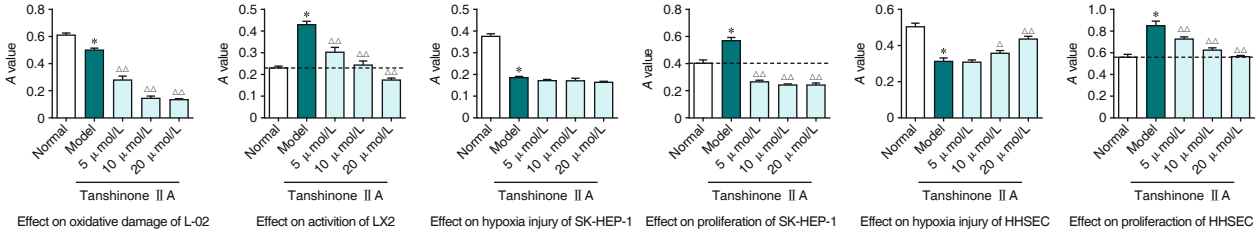

Figure 1. Cellular Pharmacological Activity of Tanshinone ⅡA ($\bar{x} \pm s$, n=4)

Notes: *A*: optical density; HHSEC: human hepatic sinusoidal endothelial cells; $^{*}P<0.01$ *vs*. control group; $^{\triangle}P<0.05$, $^{\triangle\triangle}P<0.01$ *vs*. model group

Chin J Integr Med 2022 Jun;28(6):538-544

significantly inhibited the activation of LX-2 induced by TGF-β 1 (P<0.05), in addition to proliferation of HHSEC induced by ECGs at a dose-dependent manner (all P<0.01, Figure 2).

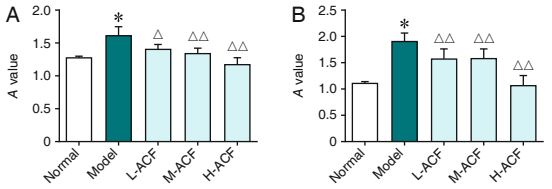

Figure 2. Effects of New ACF on Activation of LX-2 Model and Proliferation of HHSEC Model ($\bar{x} \pm s$, n=6)

Notes: A: Effect on activition of LX-2 induced by TGF β 1; B: Effect on proliferation of HHSEC induced by ECGs. *A*: optical density; TGF β 1: transforming growth factor-β 1; H-ACF, M-ACF, L-ACF: high-, medium- and low-dose active components formulation, respectively. HHSEC: human hepatic sinusoidal endothelial cells; ECGs: endothelial cell growth supplement; *P<0.01 *vs.* control group; △P<0.05, △△P<0.01 *vs.* model group

Validating Efficacy of New ACF *in vivo*

The fibrotic model demonstrated by massive infiltration of inflammatory cells, collagen deposition and high expression of Hyp. Compared with the model group, ACF showed reduced inflammation and a dose-dependent inhibition of Hyp content and collagen deposition (P<0.05 or P<0.01; Figures 3A–3D).

The liver sinusoidal endothelial cell fenestrae observed by TEM and SEM remarkably decreased in model mice. The intercellular space disappeared and continuous basement membrane formed under the endothelium. After treatment, the sinusoidal capillarization was notably improved by ACF at different dosages (Figure 3E).

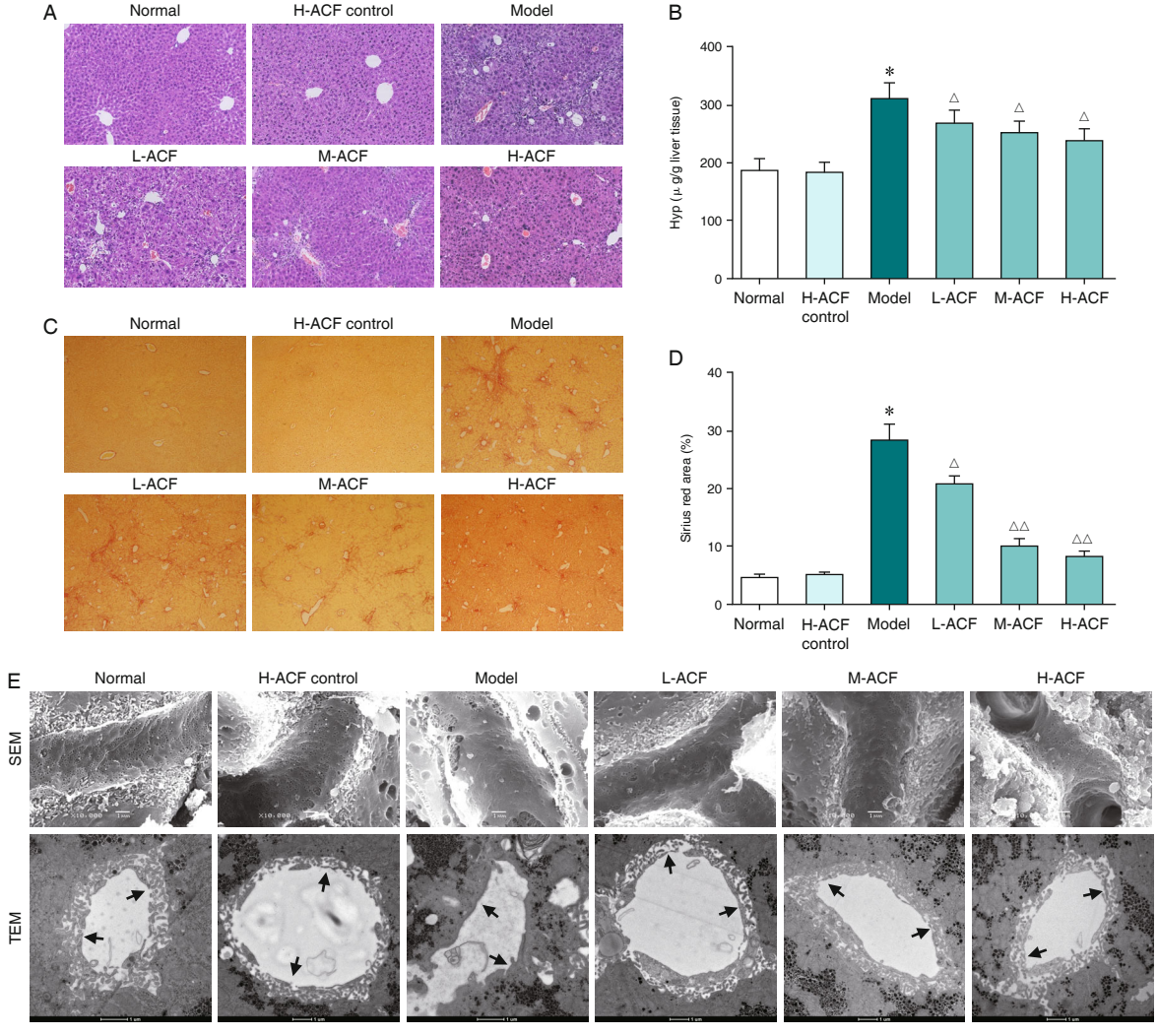

Figure 3. Validating Efficacy of New ACF *in vivo*

Notes: A: Liver tissue section stained by HE, 400× (n=8); B: Hyp content in liver tissue (n=8); C: Liver fibers stained by sirius red, 200× (n=8); D: The proportion of Sirius red staining area (n=8); E: The structure of hepatic sinusoidal lumen observed by SEM and change of basement membrane observed by TEM (n=6); *P<0.01 *vs*. normal group; △P<0.05, △△P<0.01 *vs*. model group

DISCUSSION

The liver fibrosis destroys hepatic structure and impairs liver function, leading to cirrhosis and decompensated status. The occurrence and development of liver fibrosis have extremely complex pathophysiological mechanisms, involving multi-cells signaling molecular pathways, including hepatocyte inflammation, HSC activation and LSECs injury.[13] The anti-fibrosis drugs designed to the single pathological target have not success yet. CM contains complex components and has multi-cells, multi-targets, and multi-pathways anti-liver fibrosis effect.[14] Clinical trials had confirmed that FZHY can significantly improve the liver histological regression,[6] approved by liver biopsy histological examination. However, the regression rate of FZHY was about 52% after 6 months treatment. Exploring patent medicine "reasonable nucleus"–components with action to specific target, and recombination of several components with multiple targets, is one of the strategies for developing new generation product.

Nowadays, most of researches evaluated the effect of herbal component on single cell. It is easy to know the pharmacological activity of components on individual cell type, but it is difficult to understand their comprehensive activity on the pathological process involving multiple cells, such as fibrosis which involved in hepatocytes, HSCs, LSECs, etc. In the current research, we tried to establish a scoring system *in vitro* for evaluating the comprehensive effect of herbal component on liver fibrosis, according to the role of definite hepatic cell type in fibrogenesis and degrees of its action on each cell. The scoring system need further confirmation and modification through much more researches with positive drugs.

In 2001, Prof. ZHANG Gui-jun from Beijing University of Chinese Medicine proposed the "pharmacodynamics theory of CM components". Based on CM theory of "monarch, minister, assistant and guide", in addition to the effects and indications of CM prescription, the chemical components were achieved and simplified. The effects of CM prescription were previously studied.[15] In 2005, according to the composition principle and main effects of CM prescription, Zhang, et al[16] observed the effective chemical components of each CM prescription and the most appropriate dosage of chemical components was indicated by modern pharmacology. There are two crucial issues for new formulation of active components, one is candidate components. Another is the ratio of chosen components. Selection of components depend on its scoring of activity, in our study, we evaluated 32 active components in FZHY in total and chose 3 non-toxic concentrations of each component within 20 μmol/L according to previously research *in vitro*.[17,18] Considered the role of herb in whole recipe, more components from "monarch herb" *Radix Salviae Miltiorrhizae* were selected. We performed the ratio comparation of 3 components from Danggui Buxue Decotion (当归补血汤) by calculating their combination index.[19] As a novel ACF of FZHY, it exhibited strong inhibitory effects on activation of LX-2 and proliferation of HHSEC at all the 3 dose (1, 2, 4 μmol/L×7 components) *in vitro*. Additionally, ACF dose-dependent attenuated liver collagen deposition, improved the sinusoidal capillarization on DMN mice model *in vivo*. Although it is preliminary work, it provided the basis for further investigation with different ratio.

Hepatic sinusoidal capillarization, characterized by LSECs injury and defenestration, basement membrane formation and HSCs activation, is a pathological feature of liver fibrosis, which initiate earlier lobular fibrosis and ultimately leads to cirrhosis.[20,21] HSCs and LSECs are the main cytological bases of liver fibrosis formation and progression. On the one hand, they are involved in the occurrence of hepatic sinusoidal capillarization, caused hepatic lobules hypoxic damage to initiate fibrosis. On the other hand, it is a cellular factor for refractory regressing of fibrosis. Thus, the intervention of HSCs and LSECs, sinusoidal capillarization could be ideal strategy to develop agents against liver fibrosis. Our previous results indicated that FZHY decrease sinusoidal capillarization.[9] Results of this study demonstrated that ACF of FZHY with equal proportion of tanshinone ⅡA, salvianolic acid B, cordycepin, amygdalin, quercetin, protopanaxatriol, and schizandrin B dose-dependently exerted against liver fibrosis effect and sinusoidal capillarization *in vitro* and *in vivo*.

In conclusions,15 active components (scoring ⩾ 15 points) including tanshinone ⅡA, salvianolic acid B, cordycepin, amygdalin, quercetin, protopanaxatriol and schizandrin B were found from FZHY against liver fibrosis. Among them, 7 components were selected

according to "comprehensive scoring" *in vitro* based on hepatic multiple cell lines and original herb role in the whole recipe, and combined as a new ACF, which had a good effect against experimental liver fibrosis and sinusoidal capillarization in fibrotic liver. The results warranted a further research and development for ACF from FZHY on liver fibrosis, as well as provided a possibility for screening active components of herbal products.

Conflict of Interest

Authors declare that they have no conflict of interest.

Author Contributions

Liu CH designed the project; Tan Y, Sun X and Liu HL performed the experiments; Sun X, Lyu J and Zhao ZM analyzed the data; Sun X and Lyu J drafted the manuscript; Liu CH critically revised the manuscript. All the authors read and approved the manuscript for publication.

Electronic Supplementary Material: Supplementary material (Appendixes 1–3) are available in the online version of this article at http://dx.doi.org/10.1007/s11655-021-3293-x.

REFERENCES

1. Mercedes F, David S, Jordi B, Isabelle C, Massimo P, Jaume B. Angiogenesis in liver disease. J Hepatol 2009;50:604-620.
2. DeLeve LD. Liver sinusoidal endothelial cells in hepatic fibrosis. Hepatology 2015;61:1740-1746.
3. Thabut D, Shah V. Intrahepatic angiogenesis and sinusoidal remodeling in chronic liver disease: new targets for the treatment of portal hypertension? J Hepatol 2010;53:976-980.
4. Friedman Scott L. Hepatic fibrosis: emerging therapies. Dig Dis 2015;33:504-507.
5. Lee Youngmin A, Wallace Michael C, Friedman Scott L. Pathobiology of liver fibrosis: a translational success story. Gut 2015;64:830-841.
6. Liu P, Hu YY, Liu C, Xu LM, Liu CH, Sun KW, et al. Multicenter clinical study on Fuzhenghuayu Capsule against liver fibrosis due to chronic hepatitis B. World J Gastroenterol 2005;11:2892-2899.
7. Hassanein T, Box TD, Tong MJ, Pozza R, Rossaro L, Glenn JS, et al. A phase Ⅱ, randomized, placebo-controlled, double-blind, multicenter study to assess the antifibrotic activity of Fuzheng Huayu in chronic hepatitis C patients with hepatic fibrosis. Hepatol Int 2014;8:S308-S309.
8. Liu CH, Hu YY, Xu LM, Liu C, Liu P. Effect of Fuzheng Huayu Formula and its actions against liver fibrosis. Chin Med 2009;4:38-53.
9. Liu HL, Lv J, Zhao ZM, Xiong AM, Tan Y, Glenn Jeffrey S, et al. Fuzhenghuayu Decoction ameliorates hepatic fibrosis by attenuating experimental sinusoidal capillarization and liver angiogenesis. Sci Rep 2019;9:18719.
10. Zhao ZM, Liu HL, Sun X, Guo T, Shen L, Tao YY, et al. Levistilide A inhibits angiogenesis in liver fibrosis via vascular endothelial growth factor signaling pathway. Exp Bio Med 2017;242:974-985.
11. Lv J, Tan Y, Zhao ZM, Huang K, Shen L, Tao YY, et al. Fuzheng Huayu components formula exerted anti-liver fibrosis through inhibiting angiogenesis. World Chin Med (Chin) 2015;10:186-191.
12. Jamall IS, Finelli VN, Que Hee SS. A simple method to determine nanogram levels of 4-hydroxyproline in biological tissues. Anal Biochem 1981;112:70-75.
13. Friedman Scott L. Evolving challenges in hepatic fibrosis. Nat Rev Gastroenterol Hepatol 2010;7:425-436.
14. Liu CH, Zhao ZM, Lv J. Advances in the understanding and treatment of liver fibrosis in traditional Chinese medicine. J Clin Hepatol (Chin) 2019;35:728-733.
15. Cui XW, Zhang GJ. Component Chinese medicine and Chinese medicine modernization. Lishizhen Med Mater Med Res (Chin) 2009;20:1290-1291.
16. Zhang BL, Wang YY. Basic research on key scientific issues of TCM prescription-modern Chinese medicine was developed by the combination of components. Chin J Nat Med (Chin) 2005;3:258-261.
17. Huang K, Zhao ZM, Liu HL, Sun X, Lv J, Tao YY, et al. Evaluation of cryptotanshinone inhibition of angiogenesis in human hepatic sinusoidal endothelial cells. Acta Pharmaceut Sin (Chin) 2016;51:1257-1262.
18. Zhu CW, Lv J, Zhao ZM, Tao YY, Liu CH. Effect of schisantherin A inhibit liver sinusoid endothelial cell function and action against liver fibrosis relating to angiogenesis. China J Tradit Chin Med Pharm (Chin) 2016;41:279-284.
19. Guo T, Liu ZL, Zhao Q, Zhao ZM, Liu CH. A combination of astragaloside Ⅰ, levistilide A and calycosin exerts anti-liver fibrosis effects *in vitro* and *in vivo*. Acta Pharmacol Sin 2018;39:1483-1492.
20. Xu MM, Wang XH, Zou Y, Zhong XS. Key role of liver sinusoidal endothelial cells in liver fibrosis. Biosci Trends 2017;11:163-168.
21. Greuter T, Shah VH. Hepatic sinusoids in liver injury, inflammation, and fibrosis: new pathophysiological insights. J Gastroenterol 2016;51:511-519.

(Accepted August 3, 2020; First Online September 28, 2021)
Edited by TIAN Lin