FEATURE ARTICLE

Inhibition of Pathological Angiogenesis of Chinese Medicine against Liver Fibrosis

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ABSTRACT Pathological angiogenesis of liver which includes liver sinusoidal capillarization due to lose of fenestraes of liver sinusoidal endothelial cells (LSECs) and formation of new vascular, is a crucial mechanism responsible for origination and development of liver fibrosis and closely involves in the development of cirrhosis and hepatic cancer. Anti-neovascularization medicine such as sorafenib can decrease portosystemic shunts, improve splanchnic hyperdynamic circulation, lower portal hypertension, while it can not be applied in clinic due to its serious toxic and side reactions. Chinese herbal formula can effectively inhibit pathological angiogenesis of liver, improve microcirculation of liver, and decrease the probability of gastrointestinal hemorrhage in cirrhotic patients. Different Chinese herbal formula are of different characteristics on inhibiting pathological angiogenesis in liver fibrosis, which partly explains synergistic effect of different compatibility of Chinese materia medica and opens up good vista for Chinese medicine against liver fibrosis through inhibiting angiogenesis.

KEYWORDS pathological angiogenesis, liver fibrosis, Chinese medicine

It is a common consensus that liver fibrosis is reversible. However, the reversion is limited in improvement of pathological staging of liver fibrosis and it is still a medical dilemma to reverse liver fibrosis completely up to now. The key issue is to repair the abnormal vascular structure of the liver accompanying liver fibrosis which is responsible for prognosis of cirrhosis. Blood supply and special structure of the liver sinusoid is the vascular biological basis of portal hypertension. Different recruitment of pro-regeneration pathway and pro-fibrosis pathway in vascular microenvironment during liver injury plays an important regulating effect on hepatic cells regeneration and fibrosis.[1] There is evident vascular destruction and reconstruction including sinusoidal capillarization (a main form of vascular formation in the liver-intussusception) and formation of vascular anastomosis in the early stage of cirrhosis (liver fibrosis). Capillary in normal liver tissue is liver sinusoid which is a kind of pseudo-capillary of rather high permeability to guarantee the smoothness of microcirculation and mass exchanges between hepatic cells and blood, because of large amount of fenestraes in liver sinusoidal endothelial cells and discontinuity of basement membrane. During chronic liver injury, liver sinusoidal endothelial cells (LSECs) lose fenestraes to form continuous basement membrane underlying the endothelium, and then buds to form new vasculars.[2] Changes mentioned above result in microcirculatory disturbance, microenvironment destruction, hepatic cells injury, hepatic stellate cells (HSCs) activation, and eventually portal hypertension and cirrhosis. Hepatic sinusoidal capillarization is the main form of angiogenesis in cirrhosis.[3]

As one type of perisinusoidal cells in the liver, activated HSCs are the key cells responsible for extracellular matrix production and possess the contracting function. Nitric oxide (NO) regulates angiotasis in the liver through maintaining HSCs resting state and promotes vascular distension.[4] Injured LSECs transmit signal to HSCs and vascular endothelial growth factor (VEGF) receptor (VEGFR)-2 might be the key regulator of endothelial cells response to the injury; activated HSCs stimulate endothelial cells to secrete cytokines, and simultaneously up-regulate LSECs activated receptors' pathway.[5] So, pathological angiogenesis closely involved in occurrence of cirrhosis and liver cancer.

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Portal hypertension is a critical complication of cirrhosis. Researches suggest that angiogenesis (splanchnic neovascularization) stimulated by VEGF and other vascular originated cytokines, such as platelet derived growth factor (PDGF), is the main mechanism responsible for portal hypertension, splanchnic hyperdynamic circulation, portosystemic shunts formation. It is verified in recent researches that anti-neovascularization medicinal, such as sorafenib and sunitinib can decrease portosystemic shunts, improve splanchnic hyperdynamic circulation, lower portal hypertension, which might be related to decreased VEGF and PDGF expressions and splanchnic neovascularization.[6-8] However, this kind of medicine can not be applied in clinic due to its serious toxic and side reactions.

With multi-components and comprehensive effect of muliti-targets and multi-levels, Chinese materia medica is of rather good efficacy on liver fibrosis. In the recent 10 years, Chinese medicine (CM) has made a great progress against liver fibrosis in evaluation of clinical efficacy, action mechanism research and substance basis research, etc., which not only protrude the advantage of CM against liver fibrosis, but also suggest that Chinese herbal formula can effectively inhibit pathological angiogenesis of the liver, improve microcirculation of the liver, and decrease the probability of gastrointestinal hemorrhage in cirrhotic patients. Action mechanism research indicates that different Chinese herbs formulas are of different characteristics on inhibiting pathological angiogenesis in liver fibrosis, which partly explains synergistic effect of different compatibility of Chinese materia medica and opens good vista for CM against liver fibrosis through inhibiting angiogenesis.

Fuzheng Huayu Capsule (扶正化瘀胶囊, FZHYC) Effectively Improves Microcirculation of Cirrhotic Liver and Lowers Probability of Oesophageal Variceal Bleeding in Patients with Liver Cirrhosis

A multi-center randomized trial is conducted in patients with post hepatitis B cirrhosis diagnosed as oesophageal varices with stomachoscopy examination. After 2-year follow-up, the trial is suspended if the end point parameters arise (esophageal variceal bleeding and liver cancer). Results indicate that for patients with mild esophageal varices, the cumulative non-bleeding probability in FZHYC group and the placebo control group is 96.30% and 77.01%, respectively; for patients with moderate and severe esophageal varices without history of bleeding, the cumulative non-bleeding probability in FZHYC group, propranolol group and FZHYC plus propranolol group is 56.99%, 76.13% and 87.55%, respectively; for patients with moderate or severe esophageal varices and history of bleeding, the cumulative non-bleeding probability propranolol group and FZHYC plus propranolol group is 23.53% and 44.87%, and median non-bleeding time is 8.00±2.56 months and 22.00±1.38 months.[9] This trial suggests that long term of administration of FZHYC can improve microcirculation in cirrhotic liver and significantly decrease variceal bleeding probability; for patients with moderate or severe esophageal varices and history of bleeding, compared to that in patients simply received administration of propranolol, median non-bleeding time in patients with administration of propranolol and FZHYC prolonged 2 times.

Interstitial matrix metalloproteinase (MMP)-13 protein expression and activity decreases significantly in rats with dimethylnitrosamine (DMN)-induced liver fibrosis, while protein expression of plasminogen activator inhibitor-1 (PAI-1) increases significantly, protein expression of tissue inhibitor of metalloproteinase-1,2 (TIMP-1,2) increases, portal pressure increases, diminishing of fenestrae in LSECs and continuity of basement membrane. FZHYC can significantly decrease protein expression of plasminogen activator inhibitor (PAI)-1 and TIMP-1 and 2, promote activity of MMP-13, lower serum hexadecenoic acid level, alleviate endothelial cells injury, decrease hepatic sinusoid capillarization and portal pressure.[10]

Liver angiogenesis is evaluated by liver tissue microvascular imaging analysis and microvessel density (MVD) labeled by CD31. FZHYC can decrease hepatic microvasculature, down regulate VEGFR2 protein expression and inhibit alkaline phosphatase activity in zebrafish.[11]

Further studies[12-14] find that *Cordyceps Mycelia* extract (CME) can not only effectively inhibit DMN induced cirrhosis in rats, but also reverse developed cirrhosis in rats. CME can inhibit sharp increase of MMP-2 and -9 activities in the early stage of DMN challenging, initiation of liver fibrosis and formation of liver fibrosis and portal hypertension.

During the development of DMN-induced liver

fibrosis in rats, phenotype shift of LSECs results in permeability decrease due to the mode of permeability changes from predominant-fenestrae transport to predominant-caveolae transport. CME can prevent endothelial cells injury and improve LSECs permeability, inhibit HSCs activation, alleviate or even reverse capillarization in rats with DMN-induced liver cirrhosis.[12-14]

Effect of CM Formulas with Different Actions on Pathological Angiogenesis in Liver Fibrosis and Cirrhosis

To CM, different therapeutic method is applied to treat cirrhosis patients with different clinical findings. Formulas with action of tonifying qi, nourishing yin and expelling blood stasis are usually used in clinic to treat cirrhosis.

Huangqi Decoction (黄芪汤) with Qi Tonifying Action Can Significantly Inhibit HSCs Activation and Contraction

Huangqi Decoction is superior to Yinchenhao Decoction (茵陈蒿汤) in inhibiting α-smooth muscle actin (SMA) expression in DMN-induced fibrosis in rats at progressive stage. Furthermore, Huangqi Decoction also promote expression of antioxidant protein [Prdx6, Hsp70, catalase and total superoxide dismutase (T-SOD), etc.] in the liver tissue of DMN induced fibrosis in rats at advanced stage.[15,16]

Formulas with Invigorating Blood and Expelling Blood Stasis Can Improve Microcirculation in Cirrhotic Liver

Formulas with invigorating blood and expelling blood stasis can promote activity of interstitial collagenase, enhance apoptosis of HSCs and degradation of fiber deposition, and inhibit sinusoidal capillarization and angiogenesis in the liver. Xiayuxue Decoction (下瘀血汤) can regulate the imbalance of MMPs and TIMPs, and improve activity of MMPs in the liver tissue of porcine serum induced fibrosis in rats.[17] Xiayuxue Decoction can also inhibit high expression of MMP-2 and MMP-9 to alleviate basement membrane destruction, decrease protein expression of CD31, von willebrand factor, VEGF, VEGFR-2, decay accelerating factor (DAF), α-SMA to inhibit angiogenesis in CCl4-induced cirrhotic liver.[18] Further study showed that extract of Yinchenhao Decoction can significantly enhance activated HSCs apoptosis while promote proliferation of hepatic cells, inhibit differentiation of liver sinusoidal endothelial cells, improve pathological changes of sinusoidal capillarization in the liver.[19] Xuefu Zhuyu Decoction (血府逐瘀汤) is also of rather good effect on angiogenesis in CCl4-induced fibrosis in mice, and the effect is similar to sorafenib.[20]

Yiguan Decoction (一贯煎) with Nourishing Yin Action Can Inhibit Angiogenesis in Fibrotic Liver

Yiguan Decoction can improve biotransformation function of the liver with chronic injury. Microarray analysis of liver tissue of CCl4-induced fibrosis in rats revealed that toxicity clearing function of cytochrome P450 is lowered, polypeptide 13 (CYP3A13), arginine vasopressin receptor 1A (AVPR1A), protein synthesis related gene expression such as afamin, alpha-2u globulin, globin, alpha and beta-glo, etc., is down regulated significantly, metabolism-related gene expression of amino acid or its derivatives and ammonia also is down regulated significantly, while gene expression of lymphotoxin A (LTA), MMP-23, RNA binding motif protein 3 (RBM3), thrombospondin 2 (TSP2), AP1 gamma subunit binding protein 1 (AP1GBP1), growth hormone releasing hormone receptor (GHRHR), and amiloride binding protein 1 (ABP1), etc., is up-regulated significantly. Yiguan Decoction can also significantly up regulate gene expression of CYP3A13, AVPR1A, beta-glo, etc., and simultaneously down regulate significantly gene expression of LTA, MMP-23, RBM3, TSP2, AP1GBP1, GHRHR, ABP1, etc.[21] Yiguan Decoction can improve hypoxia of the liver, protect liver endothelial cells and inhibit angiogenesis in CCl4-induced liver fibrosis in rats, the underlying mechanism is to improve hypoxia of the liver, decrease abnormal gene expression of hypoxia inducing factor-1 alpha (HIF-1α), CD31 and VEGF; furthermore, its action on lowering expression of HIF-1α mRNA and protein in fibrotic liver tissue is superior to that of sorafenib. Yiguan Decoction can also promote generation of dimethylaminohydrolase in hepatic cells to degrade asymmetric dimethyl arginine (AMDA), increase endothelial nitric oxide synthase activity to promote NO synthesis to decrease vascular tone in the fibrotic liver.[22]

There might be different microvascular changes in liver fibrosis induced by different causes, which explains the different development form and prognosis of liver fibrosis induced by different causes.[23] It is widely acknowledged that CM is of great advantage against liver fibrosis but its mechanism on microangiopathy is still needed further investigation. The most difficult problem that we must face is how to carry out feasible clinical research. Cirrhosis is the outcome of liver fibrosis and microangiopathy in cirrhosis plays a critical role in portal

hypertension and liver cancer. CM should cooperate with Western medicine and more effort should be put to explore the potential of CM against liver fibrosis.

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