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Research progress and prospect of Curcuma kwangsiensis on anti-liver fibrosis and promoting blood circulation and removing blood stasis based on W-P corpuscles

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Abstract

Hepatic fibrosis is a necessary stage for the development of chronic liver disease to cirrhosis. Up to now, no satisfactory drugs have been found to interfere with liver fibrosis. The disturbance of hepatic microcirculation is one of the important pathogenesis of chronic liver disease. Hepatic Sinusoidal Endothelial Cell (HSEC) is the most important cell which constitutes the hepatic microcirculation barrier. W-P corpuscles were found in HSEC of most patients with liver fibrosis, and W-P corpuscles became the site of synthesis and storage of cytokines such as von Wille brand factor and ET-1 which promote liver fibrosis, it can make the structure and function of HSEC maladjusted, cause hepatic microcirculation disorder and aggravate the process of hepatic fibrosis. However, previous studies found that Curcuma kwangsiensis, a special ethnic medicine of Guangxi, has definite effects of promoting blood circulation and removing blood stasis and anti-liver fibrosis, the further research idea is that "The effect of Curcuma kwangsiensis on promoting blood circulation and removing blood stasis and anti-liver fibrosis is produced by affecting the number of W-P corpuscles formed, the synthesis and storage of W-P corpuscles, and interfering with its exocytosis ability".

Introduction

The liver is the most important metabolic organ in the human body. Hepatic fibrosis refers to fatty change, inflammation and necrosis of liver cells after the liver is affected by various physical and chemical factors, a chronic disease in which excessive extracellular matrix and abnormal deposition of ECM components occur in necrotic areas. Almost any factor that can cause chronic damage to the liver can lead to liver fibrosis, such as chronic hepatitis B, chronic hepatitis C, steatohepatitis (including alcoholic or non-alcoholic), autoimmune liver disease, schistosomiasis liver disease, drug-induced liver disease [1]. Liver fibrosis is a typical pathological feature of chronic liver diseases, and it is a necessary pathological process for various chronic liver diseases to develop into cirrhosis, among which 25%-40% can develop into cirrhosis, the final stage of liver fibrosis [2]. There are more than 30 million patients with chronic liver diseases in our country, 2.1% of them develop into cirrhosis every year, and the probability of canceration after 10 years is more than 40%. The high mortality rate and the wide range of its patients are enough to cause our attention, which seriously affects the nationals quality of life and medical expenditure [3]. However, researchers have realized that liver fibrosis is a reversible pathological phenomenon in the early and middle stages. Professor Hans Popper, founder of contemporary hepatology, said: "Who can prevent or delay liver fibrosis, who will be able to cure most liver diseases." Professor Friedman put forward [4]:"Treatment of hepatitis B and C is anti-fibrotic, demonstrat-

ing the reversibility of hepatic fibrosis, which is more easily reversed than pulmonary fibrosis, cardiac fibrosis, and Scleroderma". So it is very important to prevent or intervene the course of liver fibrosis as early as possible to improve the quality of life and the prognosis of patients. But no satisfactory solution to this major global health problem has yet been found.

Hepatic microcirculation, hepatic sinusoidal endothelial cells and the occurrence and development of hepatic fibrosis

Microcirculation refers to the blood circulation and material exchange between arterioles and venules. Microcirculation disturbance can significantly affect the physiological function of each organ. Studies have shown that microcirculation disturbance is one of the important pathogenesis of chronic liver disease, improving the microcirculation of liver, it is very helpful to the recovery of liver function and can effectively prevent or delay the formation of liver fibrosis and cirrhosis [5]. The key barrier of hepatic microcirculation is called hepatic sinusoidal, which is one of the most permeable substance exchange structures in human body, and HSEC cells account for about 70% in hepatic sinusoidal, to maintain the normal microcirculation of the liver, blood and liver cells to ensure material permeation and exchange of important histological basis [6]. Unlike normal vascular endothelial cell, normal HSEC is characterized by a dense network of sieve-like apertures and no basement membrane barrier outside the cell wall -- substances can pass freely through the Disse cavity, exchange between blood and liver cells. Therefore, the number of fenestrae, the size of fenestrae and the presence or absence of extracellular basement membrane of HSEC cells regulate the blood flow of sinusoids and determine the process of substance exchange between hepatocytes and blood. Cytokines such as endothelin-1(ET-1) in the cytoplasm of HSEC can activate actin and myosin in microtubules and microfilaments, and directly cause the fenestrae to expand, shrink or close.

In liver fibrosis, ECM deposits around the hepatic sinusoids, causing a continuous basement membrane between HSEC cells and hepatocytes, with the disappearance of the fenestrae and the activation of Hepatic Stellate Cells (HSC), these changes, called "Hepatic sinuses capillary" [7]. It is characteristic in the process of liver fibrosis and cirrhosis. They can significantly affect the liver microcirculation and become the main cause of clinical portal hypertension; Once the basement membrane is formed, it can bind and store a large number of cytokines, indirectly regulating the proliferation and activation of HSC, accelerating the process of liver fibrosis [8]. Majumder[9]found that hepatic sinuses capillary always precedes hepatic fibrosis and accelerates the progression of the disease. Multiple studies [10,11] have shown that HSEC injury is a necessary and critical condition for HSC activation and that fibrotic lesions can be partially reversed after removal of hepatic sinus capillary arization inducement. In conclusion, the changes of the structure and function of HSEC can significantly affect the microcirculation of liver, and then affect the development of hepatic fibrosis, which has become a new hotspot in the research of chronic liver diseases, it has also become a potential target of traditional Chinese medicine in the prevention and treatment of liver fibrosis.

The relationship between W-P corpuscles in HSEC and the occurrence and development of hepatic fibrosis

In 1964, Weibel and Palade discovered a large, short rod-shaped organelle in the cytoplasm of endothelial cells in rat pulmonary arteries, which came to be known as W-P corpuscles, derived from Golgi apparatus. W-P corpuscles are distinctive, secretory type of organelle to endothelial cells, it contains von Willebrand factor, p-selectin, ET-1, histamine, and Nitric Oxide synthetase (NOS), among other cytokines involved in coagulation and inflammation [12]. These cytokines are stored and released in W-P corpuscles, after being released into the cytoplasm, these cytokines act by rapidly activating coagulation factors, accelerating platelet adhesion, and contracting the fenestrae of endothelial cell, aggravate microcirculation of organs [13]. There are no W-P corpuscles in normal HSEC, but they can be detected in the cytoplasm of HSEC in many patients with chronic liver diseases. Investigators [14] observed liver tissue from 106 patients with chronic hepatitis B fibrosis, W-P corpuscles were found in 71 HSEC cells, and the number and diameter of the fenestrae in these HSEC cells were reduced, and the basement membrane was even formed. These results suggest that the HSEC cells with W-P corpuscles have transformed into general Endothelium and the hepatic sinusoidal structure has begun to transform into continuous capillary. The coagulation process of sinusoid was interfered and the fenestrae of sinusoid was constricted W-p corpuscles can disturb the microcirculation of liver by interfering the coagulation process of the hepatic sinuses and contracting the fenestrae of the hepatic sinuses; which is closely related to the formation and development of hepatic fibrosis.

The contents and functions of the W-P corpuscles are described as follows:

(1) Von Willebrand factor: Von Willebrand factor is a unique cytokine of endothelial cells, the W-P corpuscles itself is composed of vwf and its pre-cleavage sequence. The immune histochemical detection of von Willebrand factor (vwf) is considered to be a classical marker for the identification of W-P corpuscles and a common method for the identification of endothelial cells [15]. As a component structure of coagulation factor VIII, it can promote blood coagulation significantly: as a linker, the W-P corpuscles connect the specific receptor on platelet membrane with the tissue under vascular endothelium; To bind platelets to the site of vascular injury; to bind and stabilize factor VIII in circulating blood. When von Willebrand factor is secreted into the cytoplasm of HSEC by W-P corpuscles and expressed along the hepatic sinuses, it can make the blood in the hepatic sinuses in a pre-thrombotic state, easy to form micro-thrombus, and aggravate the microcirculatory disturbance of the liver [16].

(2) P-selectin: Synthesized by endothelial cells and stored in the resting state of HSEC, medial distribution of the membrane close to the W-P corpuscles. If it appears in the cytoplasm of HSEC, it is an early marker of cell activation and plays an important role in the early inflammatory reaction. When HSEC is activated or stimulated by thrombin [17], P-selectin is secreted from the medial membrane of the W-P corpuscles into the cytoplasm and rapidly migrates to the surface of HSEC cells, as a transmembrane glycoprotein, mediating the neutrophil to roll

and slow down, The neutrophil roll and slow down the flow rate, and adhere to the surface of HSEC cells, leading to microcirculation disturbance. In chronic inflammation of the liver, p-selectin can be repeatedly taken into the W-P corpuscles, secretion and release, participates in and exacerbates the inflammatory process throughout.

(3) ET-1: ET-1 in the liver is mainly stored in W-P corpuscles. ET-1 is the most potent vasoconstrictor peptide known and the only substance that shrinks the capillary below 50 nm. It can cause significant contraction of the portal vein and hepatic sinusoids and the disappearance of HSEC cell fenestrae causes microcirculation disturbance, which has a very important relationship with the formation of portal hypertension [18], resulting in a decrease in hepatic blood flow, leading to liver cell turbid degeneration, promoting the development of liver disease. When the liver is damaged, HSEC cells produce large amounts of ET-1 and store it in the W-P corpuscles. The results of researchers such as Yang Wenjun confirm that [19] the storage and secretion of ET-1 is due to the dysfunction and injury of hepatic sinusoidal endothelial cell: the content of HSEC-derived ET-1 pre-mRNA increased by 60% in the animal model of liver injury. It was found that HSEC-derived ET-1 could activate HSC and cause HSC to contract intensively [20]--the activated HSC could encircle and squeeze the hepatic sinusoids, further reduce the diameter of hepatic sinusoids and aggravate the microcirculation disturbance.

(4) Histamine: W-P corpuscles are important storage sites for histamine in the liver. When the liver is damaged and repeatedly stimulated, histamine is released into HSEC cells, which promote the production of lymphocyte chemokines by CD8 + T cells, and when p-selectin has caused white blood cells in the blood to roll around and stick together, histamine can further increase the flow of rolling leukocytes, increase the permeability of blood vessels, and enhance the adhesion of leukocytes induced by lymphocyte chemokines, which is involved in aggravating the disorder of local microcirculation in the liver.

(5) Nitric Oxide synthetase (Nos): This enzyme catalyzes l-arginine deguanidyl to produce NO, and its ultra structure is localized in the W-P corpuscles. The NOS in the liver is mainly endothelial type (eNOS) and inducible type (iNOS), the physiological role of the two have great difference. Under normal conditions, only eNOS is expressed in the liver and the amount of iNOS is minimal [21]. The eNOS produces NO at the basal level, which plays a protective role in the liver. eNOS can antagonize the stimulating factor that damages the liver, regulate the perfusion of the liver, and prevent the adhesion of platelet and the formation of microthrombus. The iNOS was not expressed in physiological condition, but appeared after HSEC were repeatedly stimulated by lipopolysaccharide and cytokines, which could continue to produce a large amount of NO and produce the pathophysiological effect of liver injury, it can interfere liver biotransformation, reduce the metabolic rate of hepatocytes, induce hepatocyte apoptosis and DNA damage.

In conclusion, W-P corpuscles release stored cytokines into the cytoplasm of HSEC cells, which significantly affect the pathogenesis of blood coagulation, thrombosis, inflammation and so on. This secretory behavior is important for the microcirculation of the liver. A recent study [22] described its secretory process as the migration of W-P corpuscles toward the HSEC membrane under the stimulation of epinephrine and Ca2+, forming vesicles that release contents toward the intracellular cytoplasm in an exocytosis manner. cAMP-mediated exocytosis of W-P corpuscles, triggered by elevated adrenalin, has been reported to be the most important physiologic regulation of elevated levels of von Willebrand factor and p-selectin in the cytoplasm [23]. A recent study [24] published in the journal Nature found that down-regulation of the gene expression of Zyxin, which is a cell adhesion molecule in blood vessel Endothelium, significantly inhibited cAMP agonist-induced vwf secretion; Zyxin gene knockout mice showed a longer bleeding time and slower thrombosis. Live-cell imaging revealed that Zyxin protein could induce the remodeling of microfilaments molecules around vesicles under the membrane of endothelial cells, resulting in a ring-like "Scaffolding" structure, than the secretory behavior of vesicles is precisely regulated in time and space; it plays an important role in the process of blood vessel repair, blood coagulation and thrombosis. Recent work in the journal Blood [25] indicates that Myosin IIa is the strongest binding protein of Zyxin molecule and down-regulation of Myosin IIA gene expression can significantly inhibit cAMP agonist-induced vWf secretion; The endothelial Myosin IIA knock-out mice showed the phenotype such as prolonged bleeding time and slower thrombosis formation. The mechanism of rheostat-like regulation of W-P secretion was found: Myosin IIa binds to Zyxin protein in a skeleton-like manner, which can co-regulate the relationship between submembrane microfilaments and vesicles to initiate exocytosis. These studies not only found a new molecule Zyxin, which is a directly regulates the secretion of endothelial cells, but also found its binding protein Myosin IIa. This provides the research community with new molecular targets for the treatment of thrombotic diseases and microcirculation disorders, and for the first time, the interaction process between submembranous microfilamentous molecular networks and vesicles has been observed in living cells, a new skeleton model for regulating vesicle secretion is proposed, which provides a new idea and model for the study of the driving mechanism of stimulates vesicle secretion.

These results suggested that whether W-P corpuscles appeared in HSEC and the number of W-P corpuscles, the ability of W-P corpuscles to synthesize and store cytokines (the expression levels of von Willebrand factor, p-selectin, endothelin-1, histamine and NOS); The exocytosis ability of W-P corpuscles to contents (the combination of Zyxin and Myosin IIA, as the driving force of exocytosis, directly regulates exocytosis) may become a new target for the treatment of liver fibrosis of blood stasis type; Through interventions in these links, It can directly affect the function of HSEC, regulate the microcirculation of liver, improve the permeability between liver cells and blood, and ultimately intervene even reverse the liver fibrosis.

Blood stasis syndrome in traditional Chinese medicine and liver fibrosis in Western medicine

According to the traditional Chinese medicine: "hepatic fibrosis is characterized by the syndrome of blood stasis, mass and stuffiess, which belongs to the category of pain, jaundice, accumulation and conglomeration.The role of the liver is storing blood , the effect is govern free flow of qi, the body of the liver belongs to yin but its function to yang. Therefore, blood stasis becomes a pathological product, which is easily formed in the course of chronic liver disease, and then becomes the cause of the disease, further aggravating liver fibrosis. The Institute of Hematology, Chinese Academy of Medical Sciences [26], pointed out that the essence of blood stasis is the proliferation and spoilage of fibrous connective tissue and microcirculation disorders. Many doctor of traditional Chinese medicine think that the disease should be "Treated from the blood stasis, the first initiative of dark stasis", the liver fibrosis

disease should be treated in the whole process of remove blood stasis blood stasis. Researchers such as Ying Wu [27] considered that "Blood stasis" is the main pathogenesis of liver fibrosis, and at the same time stagnation of liver, dampness-heat, phlegm-fluidity, pattern of dual deficiency of qi and blood are often combined. Researchers such as Lu zhiping [28] used "Xuefu Zhuyu decoction", which can promote blood circulation and remove blood stasis, to obtain definite therapeutic effect on several patients with liver fibrosis, and confirmed the close relationship between blood stasis and liver fibrosis. Stagnation of the circulation of vital energy and deficiency of vital energy hinder the rise and fall of normal activities of qi and the transportation of blood, which is the physiological essence of blood transmission; Researchers such as Liu Chenghai [29] statistically analyzed more than 3800 cases of liver fibrosis and pointed out that, pattern of qi stagnation and blood stasis, pattern of qi deficiency and blood stasis congealing are the main Syndrome types of traditional Chinese medicine of liver fibrosis. Researchers such as Song Fei [30] through the experimental study of integrated traditional Chinese and Western medicine confirmed that the physical signs of blood stasis syndrome appeared in the early stage of liver injury, with the formation and aggravation of liver fibrosis, the physical signs of blood stasis syndrome became more obvious; However, a series of changes occur in liver fibrosis, such as the changes of vasoactive factors (TGF-β1, VEGF, etc), ECM synthesis and secretion, the structural damage and functional disorder of HSEC, and so on, which cause the changes of liver morphology and blood flow, these are also consistent with the traditional Chinese medicine "Blood stasis syndrome" characteristics. It can be concluded that blood stasis syndrome is one of the essence of most liver fibrosis diseases, and the method of promoting blood circulation and removing blood stasis can effectively improve hepatic microcirculation disturbance. The further research on the treatment of liver fibrosis of blood stasis syndrome with drugs for promoting blood circulation and removing blood stasis will open up a new prospect in the research of liver fibrosis.

To establish an animal model of liver fibrosis with Blood Stasis Syndrome (BSS) of integrated traditional Chinese and Western medicine

The etiology of liver fibrosis is complicated. All kinds of acute and chronic physical and chemical damage and the cause and pathogenesis of the disease of TCM can lead to liver fibrosis changes. In the experimental study of integrated traditional Chinese and Western medicine on anti-liver fibrosis, no matter which kind of single factor animal model is used, because of the limitation of the modeling method and the monotony of the pathogenic factors, it is difficult to reproduce the characteristics of human liver fibrosis comprehensively and accurately, especially the syndrome of blood stasis in Traditional Chinese Medicine (TCM), which limits the practicability and research value of these models [31]. Moreover, the traditional single-factor modeling method has the disadvantages of too long modeling period, too high animal mortality, and unstable fibrotic lesions. The animal model for pharmacological study of promoting blood circulation and removing blood stasis and anti-liver fibrosis should be based upon the theory of blood stasis syndrome of the theory of traditional Chinese medicine. At the same time, combined with modern Western medicine theory and technology for complex multi-etiology preparation. Researchers such as Peng Yue [32] have developed a method that combines Dimethylnitrosamine (DMN), push the (-)-Noradrenaline (NE) and Bovine Serum Albumin Injection (BSA), in addition, feeding with ethanol solution and high-lipid and low-protein. The animal model of liver fibrosis of blood stasis syndrome was prepared by above multifactorial compound induction. The model has obvious advantages in accuracy, it duplicates the traditional Chinese medicine blood stasis syndrome similar to human liver fibrosis. At the end of modeling, the rats had obvious symptoms of TCM blood stasis syndrome, such as bluish-purple ecchymosis of tongue, varicose veins of sublingual, darkening of eyeball, ecchymosis of tail, weight loss, dull hair and easy shedding; The pathological changes of liver fibrosis, such as fibrous tissue hyperplasia, pseudo-lobules formation, hepatic sinus dilatation and blood stasis were observed. The results showed that the model of liver fibrosis of blood stasis syndrome induced by complex factors had been established, and the results of pathological indexes detection and symptoms observation were in accordance with the pathological changes of liver fibrosis and the symptoms of TCM blood stasis syndrome, it provides a powerful model support for the study of the mechanism of promoting blood circulation and removing blood stasis of ethnic medicine.

Research progress of Curcuma zedoariae in Guangxi on anti-hepatic fibrosis and treatment of blood stasis syndrome

Curcuma kwangsiensis S.G.Lee et C.F.Liang is an authentic medicinal herb in Guangxi, with an annual output of about 1200-1500 tons, accounting for about 60% of the total output in China. It is the main variety of zedoary circulating in the medicinal materials market. The dry rhizome of Curcuma kwangsiensis is called "GUI curcuma", also called "Mao curcuma", which is a traditional Chinese medicine for promoting blood circulation and removing blood stasis, The nature and flavor of curcuma is acrid, bitter and warm, the curcuma can penetrate into the qi aspect of liver-spleen. This drug has a violent effect. Although it is the drug for the disease of qi aspect, but also has a better effect in the treatment of blood aspect. The curcuma have the function of disperse accumulation and relieve the pain. Therefore, curcuma can be used to treat the distending pain of the heart, abdomen and below the costal region, it can also be used to treat concretion, conglomeration, aggregation, and menstrual block due to stasis blood and so on. and it mainly contains volatile oil and curcumin The content of volatile oil (Curcumol; also known as curcumin epoxyalcohol) is rich, accounting for about 1% -2.5% of its weight, and it is mainly sesquiterpene lactones, which is the main active component of Curcuma kwangsiensis [33]. In the 2010 edition of Chinese Pharmacopoeia, the volatile oil of Curcuma was included as an antiviral and anticancer drug, and the content of Curcumol was used as the quality control index of Curcumae. It is reported that curcumol has many virtue such as promoting blood circulation and removing blood stasis, anti-tumor, reducing blood fat, protecting liver, anti-inflammation, cholagogue , anti-oxidation, anti-thrombus etc [34]; And it has a definite treatment for hepatitis, reversal of cirrhosis, protect the liver, anti-inflammatory and bacteriostasis, regulate immunity, as well as control of some advanced malignant tumors and other aspects of the role [35]. Pharmacological studies [36] based on the rat model of blood stasis have found that curcumol can significantly reduce the shear rate of whole blood viscosity in the rat model of blood stasis and significantly prolong the time of blood coagulation, it has the effect of promoting blood circulation and removing blood stasis in both the blood stasis model rats and the normal rats.

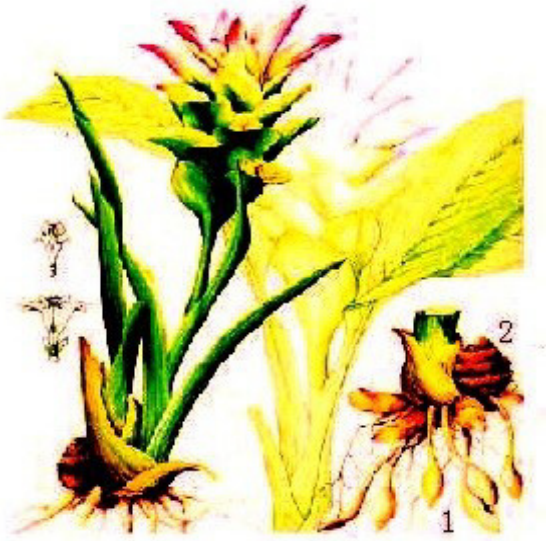

Figure 1: Picture of the original plant of Curcuma kwangsiensis.

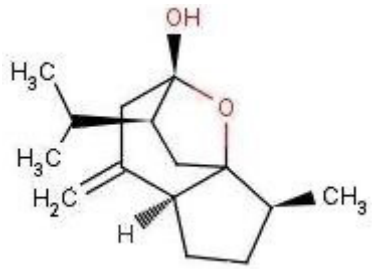

Figure 2: Chemical structural formula of curcumol.

In recent years, the applicant's research group has systematically carried out the research work of promoting blood circulation and removing blood stasis and anti-liver fibrosis of curcuma, an ethnic medicine. It has received two grants from the National Natural Science Foundation of China (81660705, 81960751) in the past five years. In this paper, we briefly introduce the previous research results: (1) The decoction of Curcuma kwangsiensis can obviously inhibit the acute liver injury induced by CCL4 in rats, decrease the levels of ALT and AST, and increase the level of plasma albumin, and has the effect of protecting liver and reducing enzyme, inhibiting the Lipid peroxidation of liver tissue, improving the synthesis of liver protein, these play a role in protecting the liver [37]. The decoction of Curcuma kwangsiensis can also reduce the contents of Ha, LN and PCIII in plasma, masson's staining observation confirmed that it can reduce the fibrous hyperplasia in liver tissue [38]. (2) Based on the study of human hepatic stellate cell line (HSC-LX2), the core cell of hepatic fibrosis, the target site and molecular mechanism of curcuma action on this cell were explored from proliferation rate, apoptosis, collagen synthesis and secretion, and the expression of pro-fibrotic cytokines [39,40]. The results showed that curcuma could significantly inhibit the proliferation rate of HSC-LX2 cells, stlocke cells enter the proliferative cycle, and induce apoptosis; The drug could decrease the synthesis and secretion of α-SMA, collagen type I and collagen type III, the fibrotic components of HSC-LX2 strain; And it can also reduce the expression level of fiber-promoting factors such as TGF- β 1 and VEGF; At the same time, the expression of MMP1 and MMP13, which degrade the fibrotic components, was increased. These results confirmed the anti-fibrotic effect of curcumol by inhibiting the cell viability and secretory functions of HSC-LX2 .In a study of human hepatic sinusoidal endothelial cells (HSECs), it [41] was observed that Curcumol could reopen the hepatic sinusoidal fenestrae of closed HSEC cells, by intervention in the hepatic sinus capillary arization phenomenon, the total area of the fenestrae through which material is transmit increased obviously and the continuous basement membrane formed outside the HSEC cell decreased; The expression of cytokines which cause fibrosis and microcirculation disorder was decreased, such as ET-1, TGF-β1, CTGF, ColIV was decreased. The above results confirmed that curcumol has the effect of promoting blood circulation and removing blood stasis could, which could significantly improve the microcirculation of the liver by inhibiting the capillary of hepatic sinusoids, and partly explained the mechanism, by which curcumol effect the structure and function of HSEC. Some valuable phenomena were also found in the study: Curcumol can significantly reduce the number of W-P corpuscles formed in HSEC cells; At the same time, the expression level of ET-1 in HSEC cytoplasm decreased, (ET-1 was mainly stored in W-P corpuscles and exocytosis to HSEC cytoplasm after pathological stimulation). These have become the subject group's follow-up thinking point. What is the relationship between the decrease of ET-1 in cytoplasm and the disappearance of W-P corpuscles? Are they related to the intervention of intrahepatic microangiopathy, hepatic sinusoidal capillary, and intrahepatic microcirculation disturbance? These problems are urgently needed to be solved by the research group. The hot spot of liver disease research in recent years indicates that the occurrence of hepatic microcirculation disturbance, to a great extent, it is related to the formation of W-P corpuscles in HSEC cells during liver fibrosis, which contain a lot of von Willebrand factor, p-selectin and ET-1, all of them can participate in the pathophysiological process of hepatic sinusoids becoming smaller, fenestrae disappearing, platelets and leucocyte chemotaxis and adhesion to form thrombus, blood coagulation speeding up and so on, cause the liver microcirculation obvious disorder, forms the blood stasis syndrome, and then lead to clinical cirrhosis with portal hypertension. Therefore, in order to intervene in hepatic microcirculation disturbance and the explanation of the promoting blood circulation and removing blood stasis effect of anti-hepatic fibrosis drugs, the study on the formation of W-P corpuscles, the contents of cytokines in the corpuscles, and the exocytosis function of the corpuscles is essential and very valuable. This topic is based on the new scientific research hotspot of liver disease, to the early research result further thorough forecast.

Conclusion and outlook

Based on the summary of the mechanism of liver fibrosis, the mechanism of promoting blood circulation and removing blood stasis, the structure and function of HSEC, and the pharmacological research progress of curcuma, it has been confirmed that curcumol, an active ingredient of Guangxi characteristic ethnic drug, has definite anti-fibrotic effect, and it has been further observed that zedoary turmeric can inhibit the capillary arization of hepatic sinusoids and improve hepatic microcirculation, it is further suggested that the drug has the effect of promoting blood circulation and removing blood stasis. So, we can explore this further, the further hypothesis was put forward that “The effect of promoting blood circulation, removing blood stasis and resisting liver fibrosis shown by curcuma was produced by influencing the number of W-P corpuscles formed, the synthesis and storage of W-P corpuscles contents, and interfering with its exocytosis ability”.

The idea of further research is as follows: we can establish the rat model of liver fibrosis of blood stasis type and the hu-

man hepatic sinusoidal endothelial cell model activated by lipopolysaccharide. After the curcuma intervention, the two models were observed as follows: (1) The number of W-P corpuscles in HSEC cells was observed and counted, and combine with the expression level of vWf (specific protein of W-P corpuscles) in HSEC was determined. Then determine the quantity level of W-P corpuscles. (2) To detect the expression levels of vwf, p-selectin, ET-1, histamine and NOS in HSEC cells, and to reflects the ability of W-P corpuscles to synthesize and store cytokines. (3) To measure the driving force of exocytosis by detect the binding level of Zyxin protein and Myosin IIa protein,which triggered exocytosis after the combination of the two corpuscles. The final results of exocytosis of W-P corpuscle were obtained by the location and distribution of vWf, p-selectin, endothelin-1, histamine and NOS inside and outside of the corpuscles. The exocytosis ability of W-P corpuscles is described comprehensively by combining the driving force of exocytosis process with the final results. (4) after purifying the proteins from Zyxin and Myosin IIa by GST-pull down technique, we can confirm the pure binding relationship between the two proteins proteins, and measure their binding level (binding amount); At this point, add zedoary, it can be observed whether the drug has a direct blocking effect on the binding of two proteins, and it can be explored whether the target of the drug action occurs on the basis of interfering with the binding of Zyxin to Myosin IIa; and its blocking level (blocking amount) can be measured.Through the above observation, detection, analysis, comparison, from the tissue and cell level, to comprehensively understand the specific intervene effects of curcuma on the formation of W-P corpuscles, the synthesis and storage of W-P corpuscles, and the exocytosis of W-P corpuscles in chronic liver diseases. The results can fully explore the mechanism and target sites of the anti-liver fibrosis and promoting blood circulation and removing blood stasis. It can provide more abundant scientific basis for the further development and utilization of curcumol, an active ingredient of Guangxi characteristic ethnic drug for the treatment of liver fibrosis diseases, and it can provide new targets site and new ideas for the targeted treatment of liver cirrhosis and portal hypertension with traditional Chinese medicine and Western medicine.

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