Progress in studies of tetrandrine against hepatofibrosis

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Subject headings liver cirrhosis/therapy; liver cirrhosis/pathology; liver cirrhosis, expremental/therapy; tetrandrine/therapeutic use

Tetrandrine (Tet) is the main alkaloid isolated from the lumpy root of *Stephania tetrandra s. Moore*. Its molecular formula is $C_{33}H_{42}N_2O_6$ and its chemical structure belongs to a dibenzy-isoquindine. Modern pharmacological studies have proved that Tet is a Ca^{2+} antagonist, which acts mainly on the calcium channel of cell to block the cross-membrane transportation of calcium ions as well as their intracellular distribution and utilization. In recent years, the actions of Tet in preventing and treating hepatofibrosis have gradually attracted attention of more investigators. With the establishment of the technique of liver cell isolation and culture and advance in technology of molecular biology, studies of the antihepafibrotic effects of Tet have probed into the cellular, subcellular and molecular levels. This article is to give a brief review of such researches over the past few years.

EFFECT OF TETRANDRINE ON EXPERIMENTAL HEPATOFIBROSIS IN RATS

The effects of Tet on CC_{l4} induced rat hepatofibrosis model showed that the serum contents of hyaluronic acid (HA) and serum ALT activities among the Tet treated groups in different stages were all lower than those in the untreated control model ($P < 0.01$). At the end of the 3rd week, the degrees of liver cell degeneration and necroses and inflammatory cell unvasion in the treated group were all lower than those of the control model. At the end of the 12th week, the control model rats showed an increase in fibroblast proliferation to grade 2.8 with pseudolobule formation on HE and VG staining; however in the corresponding treatment group, the lobular structure was still fairly well preserved, although there were some proliferation of fibroblasts and increase in collagen. These indicate that Tet may improve liver function, reduce liver damage and inhibit the extracellular matrix (ECM) formation[1]. Tet could suppress the proliferation and transformation of fat storing cells (FSC or Ito cells), reduce the deposition of type IV collagen and remarkably decrease the number of FSCs in rat with hepatofibrosis, as compared with the saline treated controls ($P < 0.01$). Moreover, the area of rough endoplasmic neticulum of FSCs in the treated group was less than that of the control group ($P < 0.05$)[2,3]. In rats with liver cirrhosis and portal hypertension, Tet could reduce liver cell damage and fibroses, and lower the serum ALT, alkaline phosphatase (ALP) and total bilirubin (STB) to normal[4]. Tet could also lower the portal pressure and systemic arterial pressure in portal hypertensive rats[5]. In comparison with the traditional antifibrotic drug colohicine, Tet had more or less similar effects in reducing serum procollagen III peptide (P III P), HA and intrahepatic inflammation and had better effect than colohicine and less toxic side-effects[6] in suppression of proliferation and transformation of FSCs as well as the degree of collagen deposition.

CYTOLOGICAL STUDY OF THE ANTIFIBROTIC EFFECT OF TET

Liver fibrosis is a progressive proliferation of connective tissue in the liver parenchyma caused by various chronic liver diseases, and is an intermediate in the course of ultimate development to cirrhosis. Cells participating in liver fibrosis mainly include fibroblasts, Ito cells, hepatocytes, endothelial cells, Kupffer cells, etc. These cells, stimulated by cytokines of some immune active cells, may synthesize and secrete excessive amount of collagen and other extracellular matrix (ECM), which are deposited in the liver and thus lead to liver fibrosis.

Effects of Tet on the growth and proliferation of fibroblasts

Fibroblasts can synthesize and secrete large amounts of collagen, fibronectin, HA, laminin (LN) and other components of ECM. Consequently, their growth and proliferation have innegligible effects on liver fibrosis. It was observed by means of flowcytometry that Tet in various concentrations (10, 20, 30, 40, 50 and 60 mg/L) had the following actions on the growth of 3T6 fibroblasts: remarkably delaying the progress of G1 to S stage, suppressing the increase of DNA contents in S stage while increasing the protein contents in G1 and G2, leading to an unbalance in cell growth till their

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Received 1998-06-25

death. Moreover, Tet showed a dose-effect relationship. The addition of Ca^{2+} and ATP proved that the effect of Tet on 3T6 growth was not related to a block of Ca^{2+} inflow[7,8]. Other studies reported that Tet in 1.0 mg/L could deter the cells from passage from G1 to S, Tet in 1.5 and 2.0 mg/L could remarkably increase G2+M cells, lower their RNA content, increase protein content, but cause no change in DNA content. These indicate that Tet may cause an unbalance of 6T3 growth and have a dose-dependent inhibition effect on their proliferation. The ^3H proline incorporation test indicated that Tet at 10 mg/L - 50 mg/L could remarkably inhibit collagen synthesis by 6T3 cells and the effect had a positive correlation with Tet concentration. Under reverse microscopy, the cells were found growing without well obvious morphological changes, indicating that the effects of Tet was not a direct cell-killing, but exerting an action at a molecular level. Tet may also suppress the proliferation of human embryonic lung fibroblasts in a concentration-dependent state, and the degree of suppression increases with a prolonged duration[9].

Influence of Tet on hepatocytic proliferation

Hepatocytes (or hepatic parenchymal cells) constitute over 90% of the liver. They can synthesize types I and II collagen, chondroitin sulfate, HA and some other ECMs. In the physiological state, its collagen synthesis is low, but under certain pathological conditions collagen synthesis is markedly promoted. The addition of CCl_4 to the *in vitro* hepatocyte culture caused cell damage and an elevation of intracellular Ca^{2+} concentration. As a result, Tet could increase the viability of the cell, stabilize intracellular Ca^{2+} concentration, reduce the release of lactic dehydrogenase and preserve the state of flow in cell membrane, thus protecting the cell against noxious agents[11]. Flow cytometric study of the action of Tet on the growth and proliferation of rat RBL liver cell demonstrated that with increasing concentrations of Tet from 10 mg/L - 60 mg/L, the progress from G1 to S was hastened, and the DNA contents in cells in S stage and protein contents in G1 and G2 stages were all increased with a positive concentration-effect relationship. The addition of Ca^{2+} and ATP into the above preparations indicated the promoting effect of Tet on hepatocyte growth and proliferation was not related to an influx of Ca^{2+} into the cells[12]. Nevertheless, there were also contradictory conclusions[10,13]. In addition, with 3H-TdR and 3H-proline incorporation tests, Tet in concentrations of 10 mg/L - 15 mg/L could markedly inhibit the synthesis of DNA and collagen of both human embryonic hepatocytes and rat liver cells, and the degree of inhibition was positively correlated with the concentration of Tet.

Effect of Tet on Ito cells

Ito cells are located in the perisinusoidal space of the liver and have the function of storing fat and vitamin A, and generating collagen and some other components of ECM. It is considered that Ito cells belong to a group of inactive fibroblasts which under certain circumstances may transform into myofibroblasts and fibroblasts, and thus play and important role in the development of liver cirrhosis. In the CCl_4 induced rat hepatic fibrosis model, Tet could remarkably inhibit the proliferation and transformation of Ito cell[2,6]. The addition of Tet to Ito cell culture markedly suppress the synthesis of DNA and collagen by the cell, and the degree of suppression is positively correlated with the concentration of Tet. Furthermore, Tet could block the action of platelet-derived growth factor (PDGF) in promoting cell proliferation and collagen synthesis of Ito cell. This suggests that in addition to a direct action of Tet on Ito cells, it may also exert an indirect anti-hepatofibrotic action through inhibiting the effect of PDGF[14].

STUDY OF ANTIHEPATOFIBROTIC EFFECTS OF TET AT SUBCELLULAR LEVEL

Liver (parenchymal) cells contain mitochondria (Mc), microsomes (Ms), lysomes (Ls) and other organelle in abundance. The functions of Mc and Ms are not limited to energy metabolism and protein synthesis, but also participate in modulating the intracellular free Ca^{2+} ion concentration. Impairment of the latter function may be one of the important factors leading to liver cell damage, eventually causes liver fibrosis. The monoamine oxidase (MAO) in the mitochondrion is an enzyme participating in collagen synthesis; and an elevation of serum MAO activity may be a sensitive parameter of liver fibrosis. N-acetyl-β-D-glucosaminidase (NAG) is another Mc enzyme involved in decomposing matrix proteoglycans and this is also related to liver fibrosis. Elevated serum NAG indicates an activity of both synthesis and decomposition of matrix. Our experiment in CCl_4 treated rats showed that small dose of Tet could lower the activities of mitochondria MAO and NAG, and increase the active uptake but reduce the passive release of Ca^{2+} by Mc. Moreover, Tet enhanced the flow over the inner membrane as well as the flow in the middle of lipid-bilayer of Mc, and there was an increase in sulfhydryl group content[10,11]. These suggest that Tet may protect the Mc against injury and also may have an antihepatofibrotic effect.

MOLECULAR BIOLOGICAL STUDY OF ANTIHEPATOFIBROTIC ACTION OF TETRANDRINE

In the 3T6 fibroblast culture, molecular hybridization of nucleic acid was done with the cDNA probe. The results demonstrated that Tet could remarkably suppress the expression levels of types I, III and IV procollagen mRNAs of 3T6 cells ($P < 0.05 - 0.01$), suggesting the inhibiting effects of Tet on the expressions of types I , III and IV collagen genes being at the level of transcription. In liver fibrosis, Tet could also lower the expression levels of mRNAs of PDGF, PDGF receptor β (PDGFR β) and transforming growth factor β1 (TGF β1), suggesting that Tet indirectly reduces collagen synthesis through suppression of gene expressions of TGF β1, PDGF and other hepatofibrosis-related growth factors.

CLINICAL OBSERVATIONS OF THE EFFECTS OF TET ON LIVER CIRRHOSIS AND CHRONIC LIVER DISEASE

In 33 patients with liver cirrhosis, who received oral Tet for 3 months, there was obvious improvement in liver function, including the IV tryptophan load test; after 18 months continuous Tet therapy, serum P III P level fell remarkably ($P < 0.01$)[19]. In another series of 54 cirrhosis cases, oral Tet therapy for 18 months resulted in marked decrease of serum P III P, HA and types I and III collagen in the liver tissue ($P < 0.05 - 0.01$). There was statistically significant difference in comparison with the glucurone treatment group as a control ($P < 0.01$). Patients in the Tet treatment group showed different degrees of improvement in liver and kidney function[20]. In 115 cases of chronic liver disease treated with oral Tet for 6 months, their serum HA and P III P levels were remarkably lower than those before treatment ($P < 0.01$). Among them, liver fibrosis disappeared in 14 (15.4%) cases, much reduced in 54 (58.1%) cases and slightly reduced in 19 (20.4%) cases. In addition, there were reductions in inflammatory cell infiltration and numbers of Ito cells in the Tet treated patients, as compared with the glucurone controls ($P < 0.001$).

In conclusion, the antihepatofibrotic effect of tetrandrine has been well proven by the results of experimental and clinical studies. With the ever-increasing in-depth studies about the pharmacodynamics, pharmacokinetics, reasonable combinations of drugs, the best preparation form and dosage of the drug and courses, tetrandrine is expected to be an effective antihepatofibrotic agent.

REFERENCES

1 Fan LY. Kong XT, Gao Feng. Studies on tetrandrine against experimerital liver fibrosis. *Med J Chin PLA*, 1994; 19(6): 444-446
2 Wang YJ, Sun ZQ, Li DG, Wang YJ, Che JT, Qi F, et al. Effcets of tetrandrine on collagen IV and fat-storing cells in experimental hepatic fibrosis. *J New Dig*,1994;2(2):78-79
3 Quan QZ, Sun ZQ, Li DG, Wang YJ, Che JT, Qi F. et al. Experimental and clinical studies of Ca^{2+} antagonists in antihepaticfibrosis. *J New Dig*, 1994; 2 (4): 214-217
4 Mu Yi, Shen YZ, Chu YF. Effects of tetrandrine on gastric mucosal and hepatic lesions in cirrhotic portal hypertensive rats. *China J Dig*, 1997;16(4): 213-216
5 Liu TB, Lin HC, Huang YT. Portal hypotensive effects of tetrandrine and veraparmil in portal hypertensive rats. *J Pharm Pharmacol*, 1997;49(1):345-347
6 Sun ZQ, Wang YJ, Quan QZ, Zhang ZJ, Comparative study of antifibrotic effects of teiranchine and colchicine. *Chin Pharmacol Bull*, 1996;12(4); 345-347
7 Liu YL, Li DG, Lu HM, Xu QF, The effers of calcium antagorists on 3T6 fibroblasts. *Beijing Med J*, 1996;18(1);26-29
8 Liu YL, Li DG, Lu HM. Effects of tetrandrine on RBL cells and 3T6 fibroblasts. *Intergr Trad West Medi Liuer Dis*, 1994;4(4)21-23
9 Liu XS, Li DG, Lu HM, Yu QF. Effects of terandrine and verapamil on the proliferation of fibroblasts. *J New Dig*, 1997;5(2);82-83
10 Fan LY, Kong XT, Gao Feng, Gao CF, Hou Jian. Effects of tetrandrisne on the DNA and collagen synthesis of the fibroblasts and human calf hepllocytes. *Chin J Chin Hepatol*, 1995;11(1);25-26
11 Chen XH, Hu YM, Liao YQ. Pretective Effect of tetrandrine on CCL_4-injured Hepatocyte. *Acta Pharmacol Sinica*, 1996;17(14);348-350
12 Liu YL, Li DG, Lu HM, Xu QI. Effects of tetrandrine on hepatocytes proliferation. *Shanghai Second Med Uni Acta*, 1995;15(3);212-215
13 Fan LY. Kong XT, Gao CF. Effects of tetrandrisne on the DNA and collagen of rat hepatocytes and fat-storing cells. *Chin J Dig*, 1994;14(5);281-283
14 Tian ZB, Liu SL, Li DG, Lu HM. Blocking action of tetrandine on the cell proliferation induced by PDGF in human lung fibroblasts and liver Ito cell of rats. *Natl Med J China*, 1997;77(1);50-53
15 Li DG, Liu YL, Lu HM, Jiang ZM, Xu QF. Effects of tetrandrine on mitochondria of hepatofibrosis rats. *Chin J Dig*, 1994;14(6);339-341
16 Liu YL, Li DG, Lu HM, Jiang ZM, Xu QF. Subcellular study of calium antagonists in treating hepatic fibrosis. *J New Dig*, 1996;4(1);3-5
17 Liu XS, Effects of Ca^{2+} antagonists on the collagen gene expression of rats in experimental hepatofibrossis. *Doctor's Degree Thesis* (to be published)
18 Tian ZB, Effects of growth factor on the pathogenesis of hepatic fibrosis. *Doctor's Degree Thesis* (to be published)
19 Li DG, Xia WX, Lu HM, Li XH. Jiang ZM, Quan QZ. et al. Significance of serum procollagen III peptide in reflecting the rtheapeutic effects of calcium-channel blockers on hepatic fibrosis, *Chin J Int Med*, 1990;29;453-456
20 Quan QZ, Xu JUN, Li DG, Xu Jun, Qi Feng et al. Effects of tetrandrine on serum hyaluronate and tye III procollagen peptide in patients with cirrhosis. *Shanghai Med J*, 1993;16(10);562-564