Quantitative analysis of transforming growth factor beta 1 mRNA in patients with alcoholic liver disease

Wei-Xing Chen, You-Ming Li, Chao-Hui Yu, Wei-Min Cai, Min Zheng, Feng Chen

INTRODUCTION

Alcoholic liver disease (ALD) is the most common cause of liver disease in late stage in the developed world[1], and ranked as the second cause of hepatic cirrhosis in China now. The increased deposition of extracellular matrix in the liver is a key factor in the developing of the disease.

In recent years, the role of TGF-beta 1 in ALD has been much emphasized[2,3], but the study of expression of TGF-beta 1 in peripheral blood mononuclear cells (PBMC) from ALD patients has scarcely been performed. This study was aimed at quantitative analysis of the expression of TGF-beta 1 mRNA in PBMC of ALD patients through reverse transcription-polymerase chain reaction (RT-PCR) technique and dot blot.

MATERIALS AND METHODS

Subjects

The subjects was from the epidemiologic survey of ALD in Zhejiang province including 107 male alcoholics, aged 25~70 years(χ2=43.32±10.39), who had ingested more than 40g of alcohol a day for more than 5 years. According to the diagnostic criteria of Nanjing conference, there were 22 alcohol abuser without hepatic impairment, 30 alcoholic steatosis, 31 alcoholic hepatitis and 31 alcoholic cirrhosis. Percutaneous liver biopsy was performed in all cases. C and B hepatitis were ruled out by appropriate serological tests. 34 healthy subjects served as control, with age range from 26 to 68 years (χ2=45.60±10.08). There were no significant differences in their ages among the alcoholics and healthy controls.

Design and synthesis of primers

TGF-β1 primers and probe were synthesized by Gibco BRL Co. The forward primer, reverse primer and probe were 5’GGAGCACAACTATTTGCTGAG3’, 5’TCCAGCTCAAATGAGG3’ and 5’CTTGCCTATATTGACCTTCCCAGGAGAGAGCTC3’ respectively. β-actin primers were synthesized by the Academy of Microimmunogenetics Science of Lubeck University, and kindly provided by Prof. Chen Zhi. The sequence of the primers were 5’CTCCAGCTTCTTCTGCTG3’ (forward primer) and 5’TGGCGTCAGGAGGAAGCTAAT3’ (reverse primer). The Primers were designed according to the previously reported sequence as shown in Roulot’s report[4].

Samples preparation and RNA extraction

Blood samples 3ml were mixed with Ficoll fluid to isolate the PBMC and then 150μl Trizol Reagent (Gibco BRL Co.). The reaction was allowed to proceed at 41°C for 1 hour.

RT-PCR

The PCR mixture consisted of RT-PCR products 5μl, 10×PCR buffer 5μl, 20 pmol of each primer, 2μM dNTP, 5μl MgCl2 and Taq polymerase 1.25 unit(Gibco BRL Co.) to a total volume of 20μl. The reaction was allowed to proceed at 41°C for 1 hour.

RESULTS

The expression of TGF-beta 1 from all ALD patients was significantly greater than that in controls (1.320±1.162 vs 0.880±0.276, χ2<0.001). The differences of the expressions were significant between the patients from each groups (alcoholic steatosis (n=30); alcoholic hepatitis (n=31); and alcoholic cirrhosis(n=24). Using peripheral blood mononuclear cells (PBMC) as samples the gene expression of TGF-beta 1 was examined quantitatively by reverse transcription polymerase chain reaction (RT-PCR) and dot blot. There are 34 healthy subjects served as control.

CONCLUSION

TGF-beta 1 expression level can be a risk factor for alcoholic liver disease and might be related to the inflammatory activity and fibrosis of the liver in patients.

Chen WX, Li YM, Yu CH, Cai WM, Zheng M, Chen F. Quantitative analysis of transforming growth factor beta 1 mRNA in patients with alcoholic liver disease. World J Gastroenterol 2002;8(2):379-381
Dot blot
The probe was labelled using a DIG oligonucleotide 3′-end labeling kit (Boehringer Mannheim). The efficiency of the labeling reaction was checked by comparing with the labelled control-oligonucleotide by direct detection. 5 μl PCR products was dropped to on a nylon membrane. The membranes were hybridized at 60°C for 6h after being denatured, fixed, prehybridized and then washed and stained.

Quantitative analysis
Quantification of TGF-β1 mRNA was carried out using β-actin mRNA as an internal standard because the transcription levels of β-actin were very stable in all types of tissues. The dots on the membranes were quantitatively analyzed and pictures were taken using IS-1000 multifunction agarose imaging analysis system (Alpha Innotech Co.). The relative index (RI) of mRNA was calculated using a formula as RI=scan value of TGF-β1 mRNA dot/scan value of β-actin mRNA dot5. Here RI might represent the relative levels of TGF-β1 mRNA in PBMC.

Statistics
The data were presented as means±s, all the statistics were done with the software Statistical Package for the Social Sciences (SPSS) and the P value was considered significant when it was less than 0.05.

RESULTS
The expression of TGF-β1 from all ALD patients was significantly greater than that in the controls (1.320±1.162 vs 0.808±0.276, t=3.811, P=0.0001, Figure1).

The expression in patients with alcoholic hepatitis and alcoholic cirrhosis was significantly greater than that in alcohol abusers respectively (1.462±1.657, 1.329±0.610 vs 0.841±0.706, t=1.859, P=0.035, t=2.495, P=0.008). No significant differences of TGF-beta 1 mRNA expression were observed between alcoholic fatty liver males and alcohol abusers ((Figure3).

DISCUSSION
In Western societies roughly 50% of all cases of liver cirrhosis are related with alcohol abuse. The increased deposition of extracellular matrix in the liver is a key factor in the morbidity and mortality of alcoholic liver disease10. This increased fibrosis may be due to a superabundance of profibrogenic factors such as transforming growth factor-beta 1 and/or relative decrease in the factors that inhibit fibrogenesis such as collagenase or interferon20,21. Transforming growth beta 1 (TGF-beta 1) is believed to play a key role in enhancing fibrogenesis and inhibiting extracellular matrix degradation12,13. Acetaldehyde, the oxidative metabolite of ethanol damages cell membranes, initiates lipid peroxidation and forms noxious protein adducts, which result in the activation of Kupffer cells and perisinusoidal lipocytes/portal fibroblasts14-16. The activation of lipocytes and fibroblasts to a proliferative and collagen-producing myofibroblast-like phenotype is triggered by the release of TGF-beta 1 from the activated Kupffer cells. In the liver, TGF-beta 1 is primarily responsible for activation of lipocytes, which are the main source of extracellular matrix proteins. Their deposition play a key role in the development of alcoholic liver cirrhosis17,18.

A number of investigators have shown increase of TGF-beta 1 mRNA and protein levels in animal models of hepatic fibrosis20,21. A few studies also demonstrated increased levels of TGF-beta 1 mRNA and protein in animal models of ALD. Matsuoka et al22 examined Kupffer cells and hepatic lipocytes isolated from a rat model of alcoholic liver fibrosis. The collagen formation was increased significantly in the alcohol-fed group more than the control, which was completely inhibited by anti-transforming growth factor beta IgG. The major peak of the molecular weight was about 25000 which was revealed by high-performance liquid chromatography and demonstrated with Northern blotting and hybridization. Kamimura et al23 examined Kupffer cell gene expression of TGF beta 1 in the rat model of ALD. Kupffer cells were isolated from the model after 10 and 17 weeks of intragastric ethanol infusion. The protein and mRNA levels of TGF beta 1 were significantly increased by 143% and 204% at 10 weeks and 238% and 295% at 17 weeks respectively in the ethanol-fed rats.

A number of studies have suggested a role for TGF-beta 1 in human liver disease24-26. In our study, the expression of TGF-beta 1 was significantly greater in all the ALD patients than in the controls and the expression in the patients with the alcoholic steatosis, alcoholic hepatitis and alcoholic cirrhosis was significantly greater than...
their expressions in the controls respectively. No significant differences of TGF-beta 1 mRNA expression were observed between alcohol abusers and the controls. The results demonstrated that the expression of TGF-beta 1 was related with ALD. Our study also showed that the expression of patients with alcoholic hepatitis and alcoholic cirrhosis was significantly greater than their expression in alcohol abusers respectively, and no significant differences of TGF-beta 1 mRNA expression were observed between alcoholic steatosis men and alcohol abusers. These results indicated that the expression of TGF-beta 1 was higher in more active and advanced ALD patients. Santos et al. also demonstrated similar results in liver specimens.

The presence of TGF-beta 1 expression could be recognized as a risk factor for active and advanced alcoholic liver disease.

REFERENCES

1 Kumar S, Stauber RE, Gavaler JS, Basista MH, Dindzans VJ, Schade RR, Rabinozvit M, Tarter RE, Gordon R, Starzi TE, ValThiel DH. Orthotopic liver transplantation for alcoholic liver disease. Hepatology 1995;11:159-164
2 Malizia G, Brun EM, Peters MC, Rizzo A, Broekelmann TJ, McDonald JA. Growth factor and procollagen type I gene expression in human liver disease. Gastroenterology 1995;108:145-156
3 Czaja MJ, Weiner FR, Flanders KC, Giambone MA, Wind R, Biempica L, Zern MA. In vitro and in vivo association of transforming growth factor-beta 1 with hepatic fibrosis. J Cell Biol 1989;108:2477-2482
4 Roulot D, Durand H, Coste T, Rautureau J, Strosberg AD, Benarous R, Marullo S. Quantitative analysis of transforming growth factor-alpha and messenger RNA in the liver of patients with chronic hepatitis C: absence of correlation between high levels and severity of disease. Hepatology 1995;21:298-304
5 Yang BB, Li FJ. Quantitative analysis of TGF-a and EGFR mRNA in laryngeal carcinoma tissues. Chin Med J 1999;112:1088-1092
6 Breitkopf K, Lahme B, Tag CG, Gressner AM. Expression and matrix deposition of latent transforming growth factor beta binding proteins in normal and fibrotic rat liver and transdifferentiating hepatic stellate cells in culture. Hepatology 2001;33:877-396
7 Bai WY, Yao XF, Feng LY. Current situation in studies of hepatic fibrosis. Shijie Huaren Xiaohua Zazhi 2000;8:1267-1268
8 Blazejewska S, Preaux AM, Mallat A, Brocheriou I, Mavier P, Dhumeaux D, Hartmann D, Schuppan D, Rosenbaum J. Human myofibroblastlike cells obtained by outgrowth are representative of the fibrogenic cells in the liver. Hepatology 1995;22:788-797
9 Milani S, Herbst H, Schuppan D, Stein H, Surrenti C. Transforming growth factors beta 1 and beta 2 are differentially expressed in fibrotic liver disease. J Hepatol 1991;139:1221-1229
10 Krull NB, Zimmermann T, Gressner AM. Spatial and temporal patterns of gene expression for the proteoglycans biglycan and decorin and for transforming growth factor-beta 1 revealed by in situ hybridization during experimentally induced liver fibrosis in the rat. Hepatology 1993;18:581-589
11 Liang DD, Wei YL, Li QF. Molecular mechanism of transforming growth factor-beta 1 on Ito cells. Shijie Huaren Xiaohua Zazhi 1999;7:980-981
12 Fang C, Lindros KO, Badger TM, Ronis MJ, Ingelman-Sundberg M. Zonated expression of cytokines in rat liver: effect of chronic ethanol and the cytochrome P450 2E1 inhibitor, chlorimethiazole. Hepatology 1998;27:1304-1310
13 Matsuoka M, Tsukamoto H. Stimulation of hepatic lipocyte collagen production by Kuffer cell-derived transforming growth factor beta: implication for a pathogenetic role in alcoholic liver fibrogenesis. J Gastroenterol 2000;35:365-370
14 Santos RM, Norton P, Esposti SD, Zern MA. TGF-beta 1 isoforms in alcoholic liver disease. J Gastroenterol 1998;33:383-389