

Effects of taurine on proliferation and apoptosis of hepatic stellate cells in vitro

Yue-Xiang Chen, Xing-Rong Zhang, Wei-Fen Xie and Shi Li

Shanghai, China

BACKGROUND: Hepatic fibrosis, a common response to chronic liver injury, is characterized by increased production of extracellular matrix components, whose major part is produced by hepatic stellate cells (HSCs). Taurine is a sulfur containing beta-amino acid rich in human body, and our previous experiments showed that it can inhibit the deposition of the extracellular matrix in the damaged liver. This work was to investigate the effects of taurine on proliferation and apoptosis of HSC and its possible mechanism.

METHODS: Cell proliferation was detected by the thiazole blue (MTT) colorimetric assay. Cell apoptosis and cell cycle were assessed via flow cytometry. The morphology of apoptotic cells was observed by phase-contrast fluorescent micrography after orange acridine staining, and the cAMP content was measured by radioimmunoassay. The expression of c-jun and c-fos was determined by the combination of immunocytochemistry and image analysis software.

RESULTS: Taurine dose-dependently inhibited the proliferation of HSCs at the concentration of 5-50 mmol/L, resulting in more cells in the G0/G1 phase and fewer in the S phase. Taurine markedly increased the synthesis of cAMP and suppressed the gene expression of c-jun and c-fos ($P < 0.01$) in addition to the inhibition of the proliferative effect of platelet-derived growth factor BB on HSC. However, taurine had no effect on induction of cell apoptosis.

CONCLUSIONS: Taurine can significantly inhibit the proliferation of HSC, causing a G0/G1-phase arrest. This effect on HSC proliferation is associated with the enhancement of the synthesis of cAMP and inhibition of the gene expression of c-jun and c-fos. However it can not induce the apoptosis of HSC.

(*Hepatobiliary Pancreat Dis Int* 2004; 3: 106-109)

KEY WORDS: taurine; hepatic stellate cell; proliferation; apoptosis; liver fibrosis

Author Affiliations: Department of Gastroenterology, Changzheng Hospital, Second Military Medical University, Shanghai 200003, China (Chen YX, Zhang XR, Xie WF and Li S)

Corresponding Author: Yue-Xiang Chen, MD, Department of Gastroenterology, Changzheng Hospital, Second Military Medical University, Shanghai 200003, China (Tel: 86-21-63610109 ext 73249; Fax: 86-21-63520020; Email: yuexchen1807@yahoo.com.cn)

This work was supported by a grant from the Science & Technology Development Foundation of Shanghai Health Bureau (No. 034112).

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Introduction

Hepatic stellate cells (HSCs) are a population of nonparenchymal liver cells known to be the major producer of proteins of the extracellular matrix in the liver. These cells play a crucial role in the development of liver fibrosis due to their over-proliferation or lack of apoptosis. Therefore, it is an important strategy for antifibrosis to inhibit the proliferation and/or to induce the apoptosis of HSC.^[1-3]

Taurine, the free amino acid rich in human body plays an important role in defense against injuries.^[4-6] Supplement of exogenous taurine can not only significantly prevent liver damage caused by a variety of harmful substances but also can inhibit the deposition of the extracellular matrix in the damaged liver so as to mitigate the degree of liver fibrosis.^[7-10] In order to further study the anti-fibrosis effect of taurine, this experiment was designed to observe the effect of taurine on proliferation and apoptosis of HSCs in vitro as well as its possible mechanism.

Methods

Materials

The rat hepatic stellate cell line (HSC-T6) of activated phenotype^[11] was a kind gift from Professor Scott L. Friedman, Division of Liver Diseases, Mount Sinai School of Medicine, New York, USA. Platelet-derived growth factor (PDGF) BB and taurine were purchased from Sigma, USA. Thiazole blue (MTT) from Amresco, USA, and DMEM medium from Gibco, USA. Radioimmunoassay kit for cAMP was provided by the Department of Experimental Medicine, Shanghai Second Medical University, Shanghai, China. Rabbit anti-human c-jun and c-fos monoclonal antibodies and ABC kit were purchased from Wuhan Boster Biological Technology Co., Ltd., China. Other reagents were purchased from Sigma, USA or imported from elsewhere.

Methods

Effect of taurine on the proliferation of HSC

Cell proliferation was measured by MTT colorime-

tric assay. Exponential phase cells (1×10^4 cells/well) were placed in the wells of a 96-well plate and incubated for 24 hours in 5% CO₂ at 37 °C. The medium was changed to serum-free DMEM for 24 hours to synchronize HSCs in the G₀ phase of cell cycle. Then the supernatant was discarded, while adding DMEM with different concentrations of taurine (0, 5, 10, 20, 30, 40, 50 mmol/L) and 2% bovine calf serum into each well for incubation for 48 hours. The trypan blue exclusion test showed the viability of the cells exceeded 95%. MTT (5 g/L) was added to the wells just 4 hours before the end of incubation. The optical density value was measured using a microplate reader at 550 nm with a reference wavelength of 690 nm.

Effect of taurine on the proliferative effect of PDGF-BB

PDGF-BB (10, 20 ng/ml) or PDGF-BB combined with taurine (20, 40 mmol/L) was added at the same time. After 48-hour incubation, the MTT assay was performed to detect cell proliferation.

Effect of taurine on the cell cycle of HSC

The cells in logarithmic growth phase were seeded into 6-well tissue culture plates at 1×10^5 cells/well and incubated for 24 hours. The medium was subsequently replaced with serum-free DMEM and incubated for one more 24 hours. Afterward, taurine (0, 5, 20, 40 mmol/L) was added and incubated for 48 hours. Each sample was taken in triplicate. The cells were then trypsinized, washed by PBS, fixed by resuspension in 70% ice-cold ethanol, and stored at -20 °C overnight. The fixed cells were washed twice again in PBS, and stained with propidium iodide in the dark for flow-cytometric analysis. The data were analyzed by the Mcycle software for cell cycle distribution.

Effect of taurine on the apoptosis of HSC

The cells in the logarithmic growth phase were placed in a 6-well plate and incubated for 48 hours in a DMEM medium containing 10% bovine calf serum and taurine (0, 5, 10, 20, 50 mmol/L). Then they were stained with acridine orange for visualizing the morphologic changes of apoptosis and assessing the apoptotic rate. The same method was utilized to treat the cells, and after a 48-hour incubation with taurine, flow-cytometric analysis was performed and apoptotic fractions were determined by the Mcycle software.

Effect of taurine on the intracellular cAMP of HSC

The cells were seeded into the wells of a 48-well plate, incubated for 48 hours and then washed three times with Earle's balanced salt solution containing 25 mmol/L of HEPES and 0.05% bovine serum albumin. Isopropylidene xanthine (0.5 mmol/L) was added into the wells for incubation of 10 minutes, followed by co-

incubation with Earle's buffer solution containing taurine (0, 5, 20, 40 mmol/L) for another 2 minutes. The supernatant was discarded, while adding cold hydrochloric acid (0.1 mmol/L) into the wells for storage at 4 °C for 20 minutes. Finally the supernatant was collected for measuring the content of cAMP by radioimmunoassay. The left cell lysate was dissolved by NaOH and stained by Coomassie brilliant blue for protein quantitative analysis.

Effect of taurine on the expression of c-jun and c-fos of HSC

The cells in the logarithmic growth phase were suspended in solution and plated on glass coverslips. After the cell growth reached the desired density, taurine (0, 20, 40 mmol/L) was added into the DMEM supplemented with 2% bovine calf serum (BCS) and incubated for 48 hours. Samples were then rinsed in PBS and fixed by acetone acid, followed by avidin biotin complex (ABC)/diaminobenzidine (DAB) detection and counterstaining of nuclei with Mayer's hematoxylin. Physiological saline (0.9% NaCl) was used as a negative control, whereas the human tumor section provided by Wuhan Boster Company, Wuhan, China served as a positive control.

Statistical analysis

The data were expressed as mean \pm standard deviation. Student's *t* test was used to test individual differences and the chi-square test was used to assess the apoptotic rate of HSCs. A *P* value of less than 0.05 was considered statistically significant.

Results

Effect of taurine on the proliferation of HSC

No toxic and adverse effects of taurine were found on HSC, but an inverse correlation between the proliferation of HSC and the concentrations of taurine in dosage. There were significant differences among the groups of different concentrations of taurine ($P < 0.05-0.01$) and they were dose-dependent (Table 1). In addition, taurine markedly inhibited the proliferative effect of PDGF-

Table 1. Effect of taurine on the proliferation of HSC

Taurine (mmol/L)	Optical density
0 (control)	0.90 \pm 0.04
5	0.84 \pm 0.03 *
10	0.77 \pm 0.03 **
20	0.69 \pm 0.03 **
30	0.61 \pm 0.03 **
40	0.57 \pm 0.02 **
50	0.49 \pm 0.03 **

Compared with the control group: * : $P < 0.05$, ** : $P < 0.01$.

Table 2. Effect of taurine on the proliferative effect of PDGF-BB

PDGF-BB (ng/ml)	Optical density
0 (control)	0.89±0.03
10	1.17±0.04 *
20	1.37±0.03 **
10+20 mmol taurine	0.80±0.04 ^Δ
10+40 mmol taurine	0.67±0.04 ^Δ

Compared with the control group: * : $P < 0.05$, ** : $P < 0.01$; ^Δ : compared with PDGF-BB 10 ng/ml, $P < 0.01$.

Table 3. Effect of taurine on the cell cycle of HSC

Taurine (mmol/L)	Cell cycle		
	G0-G1 (%)	G2-M (%)	S (%)
0 (control)	56.2±1.71	5.3±0.92	38.5±0.79
5	60.7±1.56 *	4.1±1.30	35.2±1.51 *
20	63.5±0.98 **	5.2±1.18	31.3±2.16 **
40	68.2±1.37 **	5.6±0.53	26.2±1.28 **

Compared with the control group: * : $P < 0.05$, ** : $P < 0.01$.

BB (Table 2).

Effect of taurine on the cell cycle of HSC

The cells treated with taurine demonstrated an increased percentage of cells in the G0/G1 phase and a decreased percentage of cells in the S phase. In the cells in the G2-M phase, however, no marked difference was observed (Table 3), suggesting that taurine is able to block the cells in the G0/G1 phase from entering the S phase.

Effect of taurine on the apoptosis of HSC

Apoptotic morphological changes were assessed by staining of acridine orange and fluorescence microscopy. As the concentration of taurine increased, the apoptotic rate of HSC was 2.91%, 2.69%, 3.14%, 2.53% sequentially, and that of the control group was 3.0%. The difference between the two groups was not of statistical significance ($P > 0.05$). Flow cytometry showed that no apoptotic peak was found in either the control group or the taurine-treated group. The apoptotic indices in the taurine-treated group were 2.66%±0.23%, 2.71%±0.45%, 2.38%±0.33%, 3.09%±0.79% respectively. There was no significant difference between the two groups (2.69%±0.59%, $P > 0.05$).

Effect of taurine on the intracellular cAMP of HSC

The content of intracellular cAMP in HSCs treated with taurine (5, 20, 40 mmol/L) increased markedly by 21.4%, 86.9%, 123.3% ($P < 0.01$) respectively in comparison with that of the control group. This indicated that taurine can promote the synthesis of cAMP of HSC.

Effect of taurine on the expression of c-jun and c-fos

Table 4. Effect of taurine on the expression of c-jun and c-fos of HSC

Taurine (mmol/L)	Mean gray density	
	c-jun	c-fos
0 (control)	0.71±0.08	0.69±0.05
20	0.44±0.06 *	0.45±0.09 *
40	0.22±0.02 *	0.29±0.04 *

* : Compared with the control group, $P < 0.01$.

of HSC

The expression of c-jun and c-fos was markedly inhibited by taurine at the concentrations of 20, 40 mmol/L. As displayed by the image analysis software, the mean density (gray value) of c-jun decreased by 38.0% and 68.6% respectively compared with that of the control group ($P < 0.01$). Similarly, the gray density of c-fos decreased by 35.8% and 57.9% ($P < 0.01$, Table 4).

Discussion

Taurine is a sulfur-containing beta-amino acid that possesses many bioeffects.^[4-6] Our previous work showed that taurine can protect hepatocytes from injury, and mitigate the fibrosis of the liver.^[7-9] However these mechanisms remain unclear. HSCs reside in the perisinusoidal space of Disse and are considered the major source of increased extracellular matrix components in chronic liver diseases. Following liver injury, HSCs undergo a phenotypic transformation with acquisition of myofibroblast-like features by increased proliferation and synthesis of extracellular matrix components, and play a pivotal role in the formation of fibrosis.^[12] Inhibiting the activation and proliferation of HSC is regarded as one of the potential targets for therapy of liver fibrosis.^[1,2] This study demonstrated that taurine can not only inhibit the proliferation of HSC in a dose dependent manner leading to arrest of the cells in the G0/G1 phase but also can suppress the proliferative effect of PDGF-BB on HSC, suggesting that the antifibrosis effect of taurine is associated with its antiproliferative effect on HSC.

The mechanism by which taurine inhibits the proliferation of HSC is not fully understood. It has been reported that cell proliferation is regulated by cAMP and the increased cAMP can inhibit the cell proliferation.^[13-15] In this study, we found that taurine can increase the intracellular cAMP level of HSC, which is in accordance with our previous findings that taurine can markedly elevate the cAMP level in fibroblasts.^[16] This gives us a hint that the inhibitory effect of taurine on proliferation of HSC is correlated with the enhancement of the synthesis of cAMP. C-jun and c-fos proteins, the two important members of the transcription factor AP-1 family, are encoded by c-jun and c-fos proto-oncogenes respec-

tively and play a part in the regulation of cell growth and differentiation. There is a positive correlation between the expression intensity of c-jun, c-fos and the cell proliferation activity.^[17,18] This experiment showed that the protein expression of c-jun, c-fos in HSC is significantly inhibited by taurine, which indicates that taurine can block the c-jun, c-fos pathway, subsequently inhibit the proliferation of HSC.

Apoptosis is the process of programmed cell death. In the liver, the over-proliferated HSCs are mainly eliminated by apoptosis instead of reversing their phenotype.^[19,20] Recently it has been reported that liver fibrosis of rat can be spontaneously reversed by inducing apoptosis of HSC.^[21,22] So, the induction of HSC apoptosis may be an important countermeasure to treat liver fibrosis.^[1,2,23] In this study, we used the methods of flow-cytometry and acridine orange staining for investigating the effect of taurine on apoptosis of HSC, but the results demonstrated that taurine did not induce apoptosis of HSC. This finding suggests that the attenuation of liver fibrosis by taurine is to a major extent based on its inhibitory effect on the proliferation of HSC.

Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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Received November 12, 2003

Accepted after revision December 4, 2003