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• BRIEF REPORTS •

Effect of Sea buckthorn on liver fibrosis: A clinical study

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Abstract

AIM: To appraise the effect of sea buckthorn (Hippophae rhamnoides) on cirrhotic patients.

METHODS: Fifty cirrhotic patients of Child-Pugh grade A and B were randomly divided into two groups: Group A as the treated group (n=30), taking orally the sea buckthorn extract, 15 g 3 times a day for 6 months. Group B as the control group (n=18), taking vitamin B complex one tablet, 3 times a day for 6 months. The following tests were performed before and after the treatment in both groups to determine LN, HA, collagens types III and IV, cytokines IL-6 and TNFα, liver serum albumin, total bile acid, ALT, AST and prothrombin time.

RESULTS: The serum levels of TNFα, IL-6, laminin and type IV collagen in group A were significantly higher than those in the control group. After a course of sea buckthorn treatment, the serum levels of LN, HA, collagen types III and IV, total bile acid (TBA) decreased significantly as compared with those before and after treatment in the control group. The sea buckthorn notably shortened the duration for normalization of aminotransferases.

CONCLUSION: Sea buckthorn may be a hopeful drug for prevention and treatment of liver fibrosis.

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INTRODUCTION

Liver cirrhosis is a common chronic hepatic injury caused by chronic hepatitis B, ethanol consumption and metabolic disorders, etc. The patients often die of hepatic failure due to portal hypertension, bleeding of esophageal and gastric varices. Recent studies have shown that fat storing cells now called hepatic stellate cells (HSCs) are the main collagen producing cells in fibrotic liver. Under the influence of inflammatory cytokines, vitamin A- rich cells are activated, proliferating and transforming into myofibroblasts, producing extracellular matrix(ECM)[1,2]. Retinoic acid droplets and retinoic acid receptors (RAR) diminish. Recent studies have also shown that when retinyl esters and RAR contents are restored in HSC, HSCs would remain in the inactivated state. Hence HSCs are regarded as the therapeutic targets for prevention and treatment of hepatic fibrosis[3]. In this study, sea buckthorn (Hippophae rhamnoides,) was used in cirrhotic patients to determine its effect on the changes of fibrotic parameters, improvement of liver function and whether it could be used as a therapeutic antifibrotic agent.

MATERIALS AND METHODS

Subjects

Fifty patients aged 20-70 years were enrolled in this study with at least an elevation of two items of the following parameters, e,g, serum collagen types III and IV, laminin (LN), hyaluronic acid (HA). These patients were divided into treated group (group A, n=30, 25 hepatitis B cirrhosis and 5 alcoholic) and control group (group B n=20, 17 hepatitis B cirrhosis and 3 alcoholic). These two groups had similar demographic characteristics. All these patients had not taken any antifibrotic drug or immunomodulator or antiviral herbs in the past 6 months. Group A received sea buckthorn extract in fine granules (manufactured by Sichuan Pharmaceutical Co.LTD, China), 15 g, three times a day for 6 months. Group B received vitamin-B complex, 2 tablets once, 3 times a day for 6 months.

Measurement of cytokines, parameters of liver fibrosis and liver function tests

Cytokine: IL6 and TNFα were measured by enzyme-linked immunosorbent assay (ELISA). LN, HA, collagen types III and IV were measured by radioimmunoassay (RIA). Serum albumin (Alb), total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), conjugates, total bile acid (TBA), prothrombin time (PT) were measured by a biochemical autoanalyzer.

Statistical analysis

All data were analyzed with SAS software. The results were expressed as mean ± standard deviation, the rate of normalization of AST, ALT was analyzed by Chi-square test. LN, HA, Alb, TBA, PT, collagen types III and IV were analyzed by signed rank test (both pre-and posttreatment in the same group) and Wilcoxon rank test (between the two groups, pre-and post treatment for comparison). P value <0.05 was considered statistically significant.

RESULTS

Determination of TNFα, IL-6, LN, HA

The levels of TNFα, IL-6, LN, collagen type IV in the 50 cirrhotic patients were significantly higher than those in the controls (P<0.05). There were positive correlations between TNFα, IL-6 and LN, collagen type IV (Table 1).

Table 1 Measurements of TNFα, IL-6, LN, type IV collagen ($\bar{x}\pm s$)

Group	n	TNFα (ng/L)	IL-6 (ng/L)	LN (μg/L)	Type IV (ng/L)
A	30	19.6±3.2	15.1±2.8	374.1±31.2	250.9±22.6
B	20	6.7±1.2	3.8±1.1	99.4±6.8	51.8±4.6
t value		2.419	2.961	2.618	2.997
P value		<0.05	<0.05	<0.05	<0.05

Table 2 Normalization rates of AST,ALT ($\bar{x}$±s)

Group	AST (IU/L)		Normalization rate (%)	ALT (IU/L)		Normalization rate (%)
	Before treatment	After treatment		Before treatment	After treatment	
A	59.87±26.70	49.03±18.99	24/30(80)[a]	50.57±32.47	44.12±26.05	24/30(80)[a]
B	l54.75±20.21	47.85±23.53	10/18(56)	41.65±23.54	39.15±16.68	10/18(56)

[a]P<0.05 *vs* controls.

Table 3 Parameters of liver fibrosis ($\bar{x}$±s)

Parameters	Group	Before treatment	After treatment	Comparison of two groups	
				Stat Z	*P* value
III (ng/l)	A	428.43±196.02	149.43±75.91		
	B	423.56±251.41	169.80±138.94	0.0403	0.0394
IV (ng/l)	A	123.98±81.22	70.00±34.45		
	B	178.32±89.45	139.85±98.15	0.0393	0.0384
LN (μg/l)	A	210.91±165.12	136.51±105.56		
	B	211.56±188.91	156.00±100.00	0.0073	0.0070
HA (μg/l)	A	516.74±338.75	240.56±169.78		
	B	494.74±272.26	387.16±196.28	0.0148	0.0144

Wilcoxon rank test.

Table 4 Changes of TBA, PT, Alb ($\bar{x}$±s)

Parameters	Group	Before treatment	After treatment	Before treatment		Before/after treatment		Comparison of two groups before/after treatment	
				Stat(Z)	*P* value	Stat(t)	*P* value	Stat (S)	*P* value
TBA (ng/l)	A	38.70±27.50	22.83±12.28			189.32	0.0001		
	B	40.50±34.02	38.55±22.60	0.751	0.743	35.55	0.1922	545.0	0.0003
PT (sec)	A	14.57±0.97	13.50±0.73			7.443	0.0001		
	B	15.15±0.93	14.40±0.74	4.35	0.048	5.252	0.0001	2.21	0.1415
Alb (g/l)	A	34.07±9.35	35.13±7.13			1.205	0.238		
	B	27.45±7.41	27.40±6.25	7.02	0.010	0.053	0.958	0.48	0.4887

Signed rank test and Wilcoxon rank test.

ECM parameters and liver function tests

Remarkable changes were found in AST and ALT after sea buckthorn treatment. The rate of normalization was 80 % in the treated group and 56 % in the control group (P<0.05). No difference was found in serum albumin and prothrombin time. In group A, serum LN, HA, total bile acid (TBA), collagen types III and IV were decreased after treatment as compared with group B. There was a significant difference between the two groups (P<0.05). (Tables 2-4).

DISCUSSION

Sea buckthorn is a plant growing in severely cold region of South-west China, its fruit juice has been taken as a tonic by the local Mongolians and Tibetans. It contains a great deal of vitamins, amino acids and trace elements, which are beneficial to human health[4]. Recent studies have shown that sea buckthorn contains lots of vitamin A precursors including β carotene and unsaturated fatty acids. Zhao *et al*[5] reported that sea buckthorn could protect the liver from damage by CCl_4. A combination of an antiviral drug and sea buckthorn in treating patients with chronic hepatitis B could shorten the duration for the normalization of serum ALT. The rate of turning negative of HBeAg and HBsAg was 52.16 % and 16.67 %, respectively[6].

In the normal liver, HSCs are mainly involved in the storage of vitamin A. In addition, they synthesize extracellular matrix components, matrix degrading metalloproteinases, cytokines, and growth factors[7,8]. Following acute or chronic liver injury, HSCs are activated and undergo a process of transdifferentiation, leading to a myofibroblastic phenotype. The activated HSCs are characterized by a loss of vitamin A droplets, increase of proliferation, release of proinflammatory, profibrogenic, and promitogenic cytokines and migration to the sites of injury with increased production of extracellular matrix components and alterations in matrix protease activity and provision for the fundamental needs of tissue repair[9]. In acute or self-limited liver damage, these changes are transient, whereas in case of persistent injury, they lead to chronic inflammation with an accumulation of extracellular matrix, resulting in liver fibrosis and ultimately cirrhosis. Several growth factors and cytokines are involved in HSCs activation and proliferation, of which transforming growth factor β (TGFβ), platelet derived growth factor (PDGF), TNFα and IL-6 are probably the most important ones[10].

TNFα is not only an anticancer factor, but also participates in the process of immunologic reaction and inflammation. The synthesis of collagen and some extracellular matrices were elevated 3 fold and 2.6 fold, respectively when rat HSCs were incubated with TNFα (5.0 nmol/l) for 24 hours[11]. IL-6 is a cytokine which has many biologic functions, such as promoting cell proliferation and differentiation, regulating immune

function. Wang *et al*[12,13] reported IL-6 increased in the peripheral blood of an early animal model of liver fibrosis, and the peripheral blood level of IL-6 in cirrhotic patients was remarkably higher than that in those without[14].

HSCs represent 5-8 % of all human liver cells. They have long cytoplasmic processes which run parallel to the sinusoidal endothelial wall. The second order branches sprout out from the processes, and embrace the sinusoids. Some HSCs are in close contact with nerve endings, some of which contain neuropeptides such as substance P, neuropeptide Y, somatostatin, and calcitonin gene-related peptide[15,16].

Our previous study showed[17] that retinoic acid receptor (RAR) and cAMP of primarily cultured HSCs were reduced, as compared with those in freshly isolated HSCs. The contents of RAR and cAMP of cultured HSCs were increased after treated with all-transretinoic acid (10^{-5} Mol/L).

Based on the recent articles and our results, we may deduce that the resting HSCs are activated by TNFα and IL-6 released by Kupffer cells during the process of acute or chronic inflammation, then TNFα and IL-6 in turn stimulate Kupffer cells to release TGFβ and PDGF. Eventually, HSCs proliferation and synthesis of ECM, along with the loss of vitamin A droplets, will transform themselves into myofibroblast producing liver fibrosis[18,19].

The present study showed that sea buckthorn could reduce the serum levels of laminin, hyaluronic acid, TBA, collagen types III and IV in patients with liver cirrhosis, indicating that it may restrain the synthesis of collagen and other components of ECM. We are attempting to restore vitamin A and RAR contents of HSCs, so as to keep HSCs in a quiescent status and to prevent progression of liver fibrosis. Sea buckthorn may be a hopeful drug for prevention and treatment of liver fibrosis, but further well controlled clinical trials are required.

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