• VIRAL HEPATITIS •

Detection of soluble TRAIL in HBV infected patients and its clinical implications

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Abstract

AIM: To detect the expression of soluble TRAIL (TNF-related apoptosis inducing ligand, TRAIL) in the peripheral blood of HBV infected patients and try to elucidate whether the expression level of sTRAIL have any correlativity with the clinical staging, the expression level of HBV markers and the degree of liver damage.

METHODS: 52 cases of HBV infected patients were investigated, including 8 HBV carriers, 30 chronic hepatitis B, 11 cirrhotics and 3 HBV infection related hepatocellular carcinoma. Expression of soluble TRAIL and markers of the hepatitis B were mearsured by enzyme-linked immunosorbent assay.

RESULTS: The expression level of sTRAIL in the peripheral blood of the HBV infected patients was significantly higher than that of healthy controls (1378.35±540.23 pg/ml *vs* 613.75±175.80 pg/ml, *P*<0.001). In the group of chronic hepatitis, the expression level of sTRAIL was coincident with the status of the disease and was significantly correlated with the level of ALT. In the group of cirrhosis and liver cancer, its expression level was significantly higher than that of the healthy persons and HBV carriers, but lower than that of the hepatitis B patients; meanwhile, the expression of sTRAIL did not have any correlativity with the functional indexes of the liver.

CONCLUSION: The soluble TRAIL in the HBV infected people may participate in the liver damage. Our results indicated that the expression level of soluble TRAIL may reflect the ravage of liver caused by host immune reaction to a certain degree.

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INTRODUCTION

TRAIL (TNF-Related Apoptosis Inducing Ligand), a new member of the TNF superfamily that could induce apoptosis, was first cloned and identified by Wiley in 1995[1]. TRAIL can potentially interact with five different receptors. These receptors include death receptor 4, death receptor 5, decoy receptor 1, decoy receptor 2[2-8], and a soluble receptor called osteoprotegerin[9].TRAIL can induce rapid and effective apoptosis of tumor cells while spare normal tissue cells successfully, which makes it a novel molecule that has the potential for treatment of malignant tumors. *In vivo* investigation of rTRAIL revealed that TRAIL could selectively kill tumor cells, but not normal cells, leaving the host organ systems unharmed[10,11]. However, since TRAIL is also an effective molecule participating in the immune surveillance, it is not known whether it takes part in the pathogenesis caused by hepatitis B virus and what role it may play in the HBV infection related inflammation. The expression level of soluble TRAIL in the peripheral blood of the HBV infected patients was detected and analyzed in our experiments. We tried to find out whether there is any relationship between the expression of soluble TRAIL and liver damage caused by hepatitis B virus.

MATERIALS AND METHODS

Cases and specimens

52 HBV infected patients with an average age of 41.9 years from Jinan Infectious Hospital and Shandong University Qilu Hospital were investigated, including 8 HBV carrier, 30 chronic hepatitis B patients, 11 cirrhotics and 3 HBV-related liver cancer. The diagnosis of hepatitis and cirrhosis were in accordance with the criteria emended in the Third National Conference of Infectious and parasitic held in Beijing 1995. 24 healthy blood donors with an average age of 31.4 years were taken as controls.

Major reagents

Soluble TRAIL ELISA detection kit was the product of Diaclone company in France with the detection range from 64 pg/ml to 3000 pg/ml. The HBsAg and HBeAg detection kits were the products of Lizhu Group.

Detection of soluble TRAIL in the peripheral blood

The standard specimen was diluted to 3000, 1500, 750, 375, 187.5, 93.5 pg/ml in turn. Specimens ,diluted standards and the negative control were put into the 96 wells plate coated with the antibody of TRAIL. The specimens were detected according to the procedures described in the protocol. A_{450} value was utilized to draw the standard curve and the expression level of sTRAIL was obtained from the curve.

Detection of Hepatitis B viral particles

Specimens, negative control and positive control were put into the ELISA kit coated with antibodies to HBsAg and HBeAg, respectively. The type B hepatitis markers were obtained from the ratio of A_{450} value of specimens to A_{450} value of negative control(S/N value).

Evaluation of liver function

The indexes that could reflect the function of the liver including alanine transaminase (ALT), albumin (ALB) and total bilirubin (Tbil) were assayed by the automatic biochemical analyzer equipment according to its protocol.

Statistical analysis

The results were expressed as means ± SD, and statistical analysis was performed with the analysis of variance (ANOVA). A value of $P<0.05$ was considered statistically significant.

RESULTS

Comparison of the expression level of sTRAIL in the chronic hepatitis patients and that of the healthy control

The expression level of sTRAIL was much higher in the HBV infected group than that of the healthy controls (1378.35 ± 540.23 pg/ml vs 613.75 ± 175.80 pg/ml, $P<0.001$).

Study on the correlation between the expression of sTRAIL and the clinical staging of hepatitis B patients

There was significant difference in sTRAIL expression among the groups of hepatitis patients divided by the stage and severity of the illness. The expression level of sTRAIL became higher gradually with the change of severity of the ailment in the group of chronic hepatitis patients. In the group of liver cirrhosis and liver cancer, the expression level of sTRAIL was significantly higher than that of the healthy controls and the HBV carriers, but lower than that of chronic hepatitis patients group. The statistical analysis of the expression level of sTRAIL and the status of the HBV infected patients was referred to Table 1.

Table 1 Statistical analysis of the relevance of expression of sTRAIL and clinical staging of hepatitis B

	n	sTRAIL($\times 10^{-9}$μg/ml)
HBV carriers	8	876.88±369.59
Chronic hepatitis	30	1494.97±533.24
Mild chronic hepatitis	9	1138.90±424.90
Moderate chronic hepatitis	12	1558.65±489.87[a]
Severe chronic hepatitis	9	1812.78±499.99[b]
HBV infectious related cirrhosis	11	1375.00±526.08[c]
HBV related infectious liver cancer	3	1561.00±449.56[d]

[a]$P<0.01$ vs mild chronic hepatitis group; [b]$P<0.001$ vs mild chronic hepatitis group; [c]$P<0.05$ vs severe chronic hepatitis group; [d]$P<0.05$ vs severe chronic hepatitis group

Study on the relationship between the expression of sTRAIL and clinical indices of the hepatitis B patients

In all the HBV infected patients, the expression level of sTRAIL was positively correlated with the ALT ($r=0.425$, $P<0.01$). But not with the level of Albumin and Tbil. Further study indicated that in hepatitis patients, the expression level of sTRAIL was positively correlated with the value of ALT and AlB, but not between sTRAIL and any of the clinical indices in the group of HBV carrier and cirrhosis.In the HBV infection related liver cancer group, it could not be analyzed because the number was too small. The related results referred to Table 2.

Table 2 Analysis of corelation between the expression of sTRAIL and clinical indices of hepatitis B

	sTRAIL	clinical indices		Correlativity coeffficient
HBV carrier	876.88±369.59	ALT	28.38±5.09	
		Tbil	17.73±3.34	
		ALB	39.5±1.20	
Chronic hepatitis	1494.97±533.24	ALT	173.61±135.32	$r=0.496(P<0.01)$
		Tbil	66.79±61.73	
		ALB	32.95±5.86	$r=-0.392(P<0.05)$
HBV infectious related cirrhosis	1375.00±526.08	ALT	150.36±132.72	
		Tbil	82.83±118.00	
		ALB	34.01±5.79	
HBV infectious related liver cancer	1561±449.56	ALT	85.67±48.76	
		Tbil	58.81±24.43	
		ALB	36.63±10.07	

Relationship between sTRAIL and the expression level of HBVmarkers

Our results indicated that the expression level of sTRAIL was positively correlated with the level of HBeAg ($r=0.3,P<0.05$), but it not with the level of HBsAg. The statistical analysis please referred to Table 3 and Table 4.

Table 3 Analysis of correlation between the expression of HBsAg and that of sTRAIL

Group divided by S/N	n	Expression of HBsAg(S/N)	Expression of sTRAIL in peripheral blood ($\times 10^{-9}$μg/ml)
<2.1(-)	2	0.5±0.7	1852.50±123.74
>2.1 and <10(+)	0		
>10 and <30(++)	0		
>30 and <50(+++)	4	43.25±3.78	1693.75±547.76
>50(++++)	46	61.48±5.19	1330.30±537.80

Table 4 Analysis of the correlation between Expression of HBeAg and that of sTRAIL

Group divided by S/N	cases	The expression of HBeAg(S/N)	Expression of sTRAIL in peripheral blood ($\times 10^{-9}$μg/ml)
<2.1(-)	14	0.86±0.51	1166.81±448.84
>2.1 and <10(+)	9	5.47±2.88	1088.33±528.27[a]
>10 and <30(++)	19	20.79±5.39	1590.26±474.81[ab]
>30 and <50(+++)	4	36.06±4.82	1655.00±534.48[a]
>50(++++)	6	54.69±4.49	1730.83±652.64[bc]

[a]$P<0.05$ vs the negative group; [b]$P<0.01$ vs the negative group; [c]$P<0.05$ vs the mildly positive group(+)

DISCUSSION

It was believed in the past that the HBV related liver damage was caused by hepatocytic necrosis. With the development of investigation methods, more and more researchers realized that

the hepatic dysfunction was caused by apoptosis rather than necrosis[1,12,13]. Many apoptosis inducing molecules have been found to participate in the progress. FasL was the first apoptosis inducing ligand found to play a role in apoptosis elicited by HBV. With the cloning and definition of TRAIL, it was found that many apoptosis reactions caused by combination of FasL were indeed induced by FasL and TRAIL, furthermore, TRAIL played a key role in many reactions[12-14].

FasL was proven to participate in the pathogenesis of HBV infection related disease[15-17], but it had not been elucidated whether TRAIL also played a role in the HBV infection related illness. Our results indicated that the expression level of soluble TRAIL in the circulating blood of the HBV infected patients was significantly higher than that of the healthy controls. In the patients whose livers were severely damaged,the expression level of sTRAIL was even higher, which suggested that sTRAIL might be activated by the HBV infection and played a role in the immune reaction elicited by HBV leading to liver damage.

It was reported that soluble TRAIL could induce massive and rapid apoptosis of the tumor cells at pmol concentration but spared the normal tissue cells. Soluble TRAIL may exert its function by binding with the death receptors in the liver cells after secreting into the circulating blood. TRAIL may act as an effective immune molecule after HBV infection, its mechanism may be explained in two ways. On the one hand, the HBV infected cells may be eliminated by the apoptosis inducing effect of sTRAIL; on the other hand, when the apoptosis reaction induced by sTRAIL is excessive, it may cause massive destruction of the liver tissue and lead to persistence of HBV infection related inflammation.

Our results indicated that sTRAIL was greatly upregulated in chronic hepatitis and was positively correlated with the liver damage. However, in cirrhotic and carcinoma patients, the level of sTRAIL fell somewhat. This indicated that the body immune surveillance would be activated after HBV infection, thereafter TRAIL and many other immune molecules could eliminate the virus from the body. On the other hand, HBV itself still replicates and become persistent in the body. The liver damage is caused mainly by the associated immunologic reaction rather than by the virus itself. It indicated that the immune reactions elicited by HBV would determine the outcome of the infection to a certain degree. Since TRAIL was an immune surveillant molecule widely expressed throughout the body, which probably participated in the HBV infection related immune reaction. Our results indicated that in case of chronic infection, liver damage was positively correlated with the expression of sTRAIL which suggested that sTRAIL may participate in the immune destruction elicited by HBV. This is the first report in the world concerning the role of the sTRAIL plays in HBV infection and only FasL was reported to have some effect on the HBV infection in the past[15-19].

To further analyze the mechanism of the upregulation of sTRAIL,secretion of HBsAg and HBeAg were detected. It showed that the expression of sTRAIL had no correlation with the secretion of HBsAg, but was positively correlated with the secretion of HBeAg. This suggested that HBeAg may play an even more important role in the activiation of TRAIL than HBsAg. HBeAg is a secreted nonparticulate version of hepatitis B core Ag (HBcAg), and its function is not completely known. It was reported that HBeAg may have an immunoregulatory function in promoting viral persistence[20-22]. Since HBeAg was reported to induce T-cell tolerance to HBV infected cells[21], we propose that the upregulation of sTRAIL correlated to the secretion of HBeAg maybe some immune regulation elicited to rectify the virus evasion caused by HBeAg.

HBV carrier also had an upregulated expression of sTRAIL compared with the healthy control, which indicated that the immune system of these HBV carrier was also activated though they had not yet shown any symptoms of the disease. If this activation did not break the balance between HBV replication and the immune destruction, the hepatitis virus would persist in the body for a long time. However, it also indicated that the HBV carrier already had slight or undetectable immune destruction before the appearance of clinical symptoms, they were at risk of developing into the chronic hepatitis. Once the balance between the viral replication and immune destruction being broken, hepatitis symptoms would appear.

It is the first report that HBV infection may activate the expression of sTRAIL and the HBV infected hepatocytes can be killed by the upregulated expression of sTRAIL. The inappropriate upregulation of sTRAIL can lead to the liver damage and the spreading of the virus.Our results indicated that the exprssion of sTRAIL could reflect the liver damage to a certain degree which may help estimate the status and prognosis of the disease.

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