Effect of alpha 2b interferon on inducement of mIL-2R and treatment of HCV in PBMC from patients with chronic viral hepatitis C

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AIM: To study the level of membrane interleukin-2 receptor (mIL-2R) on surface of peripheral blood mononuclear cells (PBMC) and the therapeutic efficacy of alpha 2b interferon on the treatment of HCV-RNA in PBMC of patients with chronic hepatitis C and to compare the negative rates of HCV-RNA in PBMC, HCV-RNA and anti-HCV in serum.

METHODS: Before and after treatment of alpha 2b interferon, the level of mIL-2R of patients with chronic hepatitis C was detected by biotin-streptavidin (BSA). The therapeutic group (26 cases) was treated with alpha 2b interferon (3 MU/d) and control therapeutic group (22 cases) was treated with routine drugs (VitC, aspartic acid). The total course of treatment with alpha 2b interferon and routine drug was six months and per course of the treatment was three months. The levels of HCV-RNA in PBMC, HCV-RNA and anti-HCV in serum were detected before and after a course of the treatment.

RESULTS: Before and after treatment of alpha 2b interferon and routine drugs, the levels of mIL-2R in silence stage were (3.44±0.77) % and (2.95±0.72) %, the levels of mIL-2R in inducement stage were (33.62±3.95) % and (30.04±3.73) %. There was a significant difference between two groups (P<0.01-P<0.05). After treatment of alpha 2b interferon with 3 MU/d for two courses of the treatment, the total negative rates of HCV-RNA in the PBMC and HCV-RNA, anti-HCV in serum were 42.31 % (11/26), 57.69 % (15/26), 65.38 % (17/26) respectively. After the treatment of routine drug, the negative rates of HCV-RNA in PBMC and HCV-RNA, anti-HCV in serum were 13.64 % (3/22), 22.73 % (5/22), 27.27 % (6/22) respectively. There was high significant difference in the group treated with alpha 2b interferon and the group treated with routine drugs (P<0.01-P<0.05).

CONCLUSION: The mIL-2R can be induced by alpha 2b interferon during the treatment. The alpha 2b interferon has a definite effect on the treatment of HCV-RNA in PBMC.

The curative effect of alpha 2b interferon is better than that of the routine drugs.

Wang J, Xiang GJ, Liu BX. Effect of alpha 2b interferon on inducement of mIL-2R and treatment of HCV in PBMC from patients with chronic viral hepatitis C. World J Gastroenterol 2003; 9(4): 751-754
http://www.wjgnet.com/1007-9327/9/751.htm

INTRODUCTION
Treatment of interferon on the chronic viral hepatitis C has been shown to have a good curative effect on inhibition of HCV replication, reduction in transmission level in serum and liver cells[1-8], but the improvement in clinical condition was not obvious and its effective rate was only 50 %-13]. The curative effect of interferon was based on virostatic replication of HCV in serum and some improvement of liver function. The literatures show that the peripheral blood mononuclear cells (PBMC) have large different active immune cells and membrane interleukin-2 receptor (mIL-2R) is an important symbol of active T cells, and the immune function of PBMC will be inhibited after being infected by HCV and the scavenging effect limited[18,19]. In order to study the effect of interferon on treatment of HCV in PBMC and the effect of interferon on inducement to mIL-2R, forty-eight patients with typical chronic hepatitis C were observed.

MATERIALS AND METHODS
Clinical data
Forty-eight patients with chronic viral hepatitis C were selected from the Second Miner Hospital of Huainan and our teaching hospital during 1997/03-2000/05. The total number of patients was 48 (male 27, female 21) and range of age was from 18 to 57 years old (average 37.3). The clinical diagnosis was based on the modified diagnosis criterion being affirmed on the Chinese viral hepatitis conference in Xian (2000). The patients all needed the following qualifications: (1) The positivity of anti-HCV in serum for more than six months. (2) The HCV-RNA in peripheral blood mononuclear cells and serum were positive. (3) The patients had been eliminated the infection of hepatitis A, B, D, E and G viruses. (4) There were no any systemic treatment of antiviral medicine, immunomodulator, cortical hormone for the patients. The normal control was 20 healthy students (male 12, female 8) with range age from 20 to 23 years old (average 22.5).

Treatment medicine
The alpha 2b interferon was produced by High Science and Technology of Anke Biology in Anhui Province.

Treatment methods
The patients with chronic hepatitis C were divided into two groups with treatment of alpha 2b interferon 3MU (26 cases)
and routine medicine (22 cases) respectively. The total course of treatment lasted sixty months, and per course was three months. Alpha 2b interferon was given intramuscularly injection (im) qd for two weeks and then alt dieb for two courses of treatment. The routine treatment group (22 cases) was given with Vit C, Aspactic acid, etc for six months.

**Reagents**

The diagnostic reagent of anti-HCV was purchased from Huamei Bioengineering Company of Shanghai, No.980811. The diagnostic reagent of HCV-RNA with RT-PCR was purchased from Shanghai Zhongya Gene Institute, No. 980805. The diagnostic reagent of mL-2R was purchased from Immunology Institute of Shanghai. The lymphocytes separation medium was purchased from the Second Reagent Factory of Shanghai, No.970505. The reagent of PRMI 1640 culture was produced by Sigma (USA).

**Instruments**

The analysis instrument of Spector-I was made in USA; MDF-135 CO₂ incubator was made in Japan; The instrument for gene amplification (Hema-8000) was made in Hema Company of China.

**Methods**

The total volume of 5 ml peripheral venous blood from patients with hepatitis C before breakfast was taken before and after treatment, and distributed a sterile Eppendorf tube and an anticoagulant tube (heparin) respectively. For detection of anti-HCV, the process was performed strictly according to the direction, and with two blank, two negative and two positive pores as controls in each test. The average titer was examined by the analyser Spector-I. The average OD titer of test sample ≥2.1 times of average OD titer in negative control was considered to be positive. Detection of HCV-RNA in PBMC with RT-nested-PCR: After the heparin anticoagulant blood mixed with the equal volume Hank’s liquid without Ca²⁺ and Mg²⁺, lymphocytes separation medium was used to separate the PBMC. The cells were washed twice with Hank’s liquid without Ca²⁺, Mg²⁺ and diluted to (1-3)×10⁷/ml before detection of HCV-RNA. A positive and a negative control were set up at the same time for comparison in each test. The RT-nested-PCR was made by reverse transcription and primer selected from non-coded region and part of C region of HCV. The specific amplified fragment length was 248 bp. The synthesis parameters for cDNA were 94°C 40 s, 55°C 40 s, 72°C 1 min, for 30 circles. The amplification parameters for cDNA were 94°C 50 s, 55°C 40 s and 72°C 90 s, 35 circles, including initial denaturation for 4 min at 94°C and last extension for 5 min at 72°C. The amplification product was run for electrophoresis on gel with 2% EB. The result that was uniform to the positive control was considered to be positive. Detection of mL-2R in silence and inducement stages: 10 µl suspension of the PBMC was smeared on the slide and left dry naturally and fixed with acetone for fifteen minutes or twenty minutes. The 10 µl anti-Tac antibody was mixed with the membrane of smears. The cells were grown in continuous culture (37°C, 50 ml·L⁻¹ CO₂ in atmosphere) for thirty minutes. The immune sheet glass pores were measured after staining with the color-developing agent and several washings with TBS. The total number of 200 PBMC was counted and its positive cells were statistically analyzed with the help of high power lens. The positive criterion was that the color of cytoplasm or cell membrane was brown.

**Statistical analysis**

Statistical analysis included analysis of Chi-square (χ²) and t test.

**RESULTS**

The forty-eight patients with chronic hepatitis C were divided into two groups and treated with alpha 2b interferon (26 cases) and routine drugs (22 cases) respectively. The results had been shown that the high negative rates of anti-HCV and HCV-RNA in serum were in the group with treatment of alpha 2b interferon. There was very significant difference before and after treatment of alpha 2b interferon (P<0.01-P<0.05, Table 1). The negative rate of HCV-RNA between in PBMC and in serum was similar (P>0.05, Table 2). The level of mL-2R in situation of silence and inducement stages after treatment with alpha 2b interferon was higher than that after treatment with routine drugs (P<0.05, Table 3).

**Table 1** The detective results of HCV-RNA in serum and PBMC after treatment with alpha 2b interferon (n, %)

| Group                | HCV-RNA in PBMC | HCV-RNA in serum | Anti-HCV |
|----------------------|-----------------|-----------------|----------|
|                      | Negative rate   | Negative rate   | Negative rate | Negative rate |
| Interferon treatment |                |                |            |             |
| Interferon treatment | 26              | 11              | 42.3¹      | 57.69        | 17          | 65.30      |
| Routin treatment     | 22              | 3               | 13.64      | 5            | 22.73       | 6          | 27.27      |

P<0.05, P<0.01 vs interferon treatment.

**Table 2** The results of negative rate of HCV-RNA in serum and PBMC

| HCV-RNA in serum | HCV-RNA in PBMC | Total | χ² | P |
|------------------|-----------------|-------|----|---|
| +                | 7               | 4     | 11 |  |
| -                | 8               | 7     | 15 | >0.05 |
| Total            | 15              | 11    | 26 |  |

**Table 3** The level of mL-2R before and after treatment of interferon (n, x±s, %)

| Group                | mL-2R (in silence) | mL-2R (inducement) |
|----------------------|---------------------|--------------------|
|                      | Before treatment    | After treatment    | Before treatment | After treatment |
| Interferon treatment | 26 2.63±0.70       | 2.95±0.72¹        | 30.34±3.55       | 33.62±3.95¹    |
| Routin treatment     | 22 2.43±0.78       | 2.95±0.72¹        | 30.03±3.87       | 30.04±3.73²    |
| Normal control       | 20 4.5±1.48        | 37.42±4.10        |                 |                |

P<0.05, P<0.01 vs before and after treatment; P<0.05, P<0.01 vs interferon treatment.

**DISCUSSION**

There are three kinds of interferon, α, β, γ. Interferon alpha is the most active one. Alpha 2b interferon can not enter into the host cells and kill the virus directly, but can induce the production of protein kinase (2', 5' AS) in the infected cells.
The protein kinase and 2', 5' AS can be produced after being infected by virus in cells. The degradation of virus-RNA can be made by endogenous ednonuclease induced with activated 2', 5' AS so that the necessary enzyme activity for synthesis of ribose is killed, the synthetic protein of virus can be decreased, the growth of hepatitis virus C is inhibited[30-33].

The reports have been shown that interferon has definite curative effect of the treatment of chronic hepatitis C and elimination of HCV-RNA, anti-HCV in serum. Therefore, HCV-RNA is an important index to evaluate condition of the patient's[34]. HCV-RNA in PBMC is a direct evidence of the existence of extrahepatic or latent infection, and is one of important reasons causing the chronicity of viral hepatitis C[28-31]. The results of Table 1 had shown that the rate of change HCV-RNA into negative in PBMC and serum after treatment of alpha 2b interferon was similar between two groups (P>0.05). The curative effect was higher in the treatment group with alpha 2b interferon than that with routine treatment (P=0.05-P<0.01). This result had showed that the inducing capability of alpha 2b interferon in lymphocytes of the patients was strong, and the high effect against HCV would be taken by activated lymphocytes obviously. Although the negative rate of HCV-RNA in PBMC was lower than that in serum, there was no significant difference (P>0.05). The results had shown that alpha 2b interferon had high effect on HCV-RNA both in PBMC and in serum. There were four cases of chronic hepatitis C with negative in serum and positive in PBMC. It indicated that with the T lymphocytes activation, multiple cell factors were released, that inhibited the replication of free HCV-RNA in serum, but not the HCV-RNA in PBMC. The cellular immune function was disorder in different extent in patients with chronic hepatitis C and so was their response to interferon and anti-HCV[32-37]. Otherwise, the low level of membrane interleukin-2 receptor (mIL-2R) on the surface of PBMC decreased the activity in chronic hepatitis C, which limited the induction of protein kinase and the activity of 2', 5' AS to eliminate HCV-RNA[38].

Anti-HCV is an important index for the diagnosis of HCV and is one of the evidences for evaluating the curative effect on the treatment of hepatitis C. The anti-HCV diagnostic kits of the second generation were used in our laboratory testing for core antigen, NS1, NS2 and had high specificity and sensitivity[39,40]. The rate of negative change of HCV-RNA was higher in alpha 2b interferon treatment group than that in routine treatment group (P<0.01). Among the twenty-six cases, there were six cases with anti-HCV(-) in serum and HCV-RNA (+) in PBMC, two cases with anti-HCV(-) in serum and HCV-RNA(+) in serum. This result showed that even the anti-HCV in serum became negative, HCV-RNA not stopping replication completely, some HCV-RNA could still be detected in some patients[41]. The probable reasons were: (1) After being treatment of alpha 2b interferon, the level of anti-HCV in serum decreased obviously and could not be detected by routine test. (2) The degree of variation of HCV is high, the hydrophilic peptid chain on core protein is subjective to escape the attack of CTL and keep the infection persist in chronic state. (3) The variant antigen of HCV can not match the antibody produced. mIL-2R is an important symbol of active T cells and plays key role on biologic effect of IL-2 and its level can reflect the course of activity of T cells and immune state[38-41]. The levels of mIL-2R in silence stage detected by BSA were lower in patients with hepatitis C than those in normal controls (P<0.01). The probable reasons are: (1) The mIL-2R on surface of some T cells can be restrained by HCV. (2) The degree of variation of HCV is high, the hydrophilic peptid chain on core protein is subjective to escape the attack of CTL and keep the infection persist in chronic state. (3) The variant antigen of HCV can not match the antibody produced. A lot of soluble interleukin-2 receptor (sIL-2R) can be released after replication of HCV-RNA in PBMC so that the expression of mIL-2R was restrained. It is not only a kind of manifestation of disorder and low cellular immune to HCV but also one of reasons of chronic hepatitis C. Some T cells receptor (TCR) on surface of some Tc cells can combine with the complement (HCV-MHC-I) and some perforation proteins can be released so that many liver cells will be injured. The sIL-2R, a kind of restrained factor, as well as mIL-2R all can combine with IL-2 competitively and induce infection of HCV from activity to chronicity. With the inducement of PHA, the level of mIL-2R in silence and inducement stages was obviously increased. This result showed that the mIL-2R could be induced by PHA and had strong compete ability against sIL-2R in serum.

After inducement of PHA, the level of mIL-2R of patients in inducement stage was higher than that in silence stage, and lower than that in normal controls (P<0.01). The results of our study showed that the effect of PHA was infirm for patients with hepatitis C and was similar to the reports published. The level of mIL-2R in silence and inducement stages were higher after treatment of alpha 2b interferon than that before treatment of alpha 2b interferon (P<0.01). The active T cells and high level of mIL-2R can be induced by alpha 2b interferon during the treatment. The results showed that alpha 2b interferon not only can induce the protein kinase and 2', 5' AS but also stimulate T cells and induce the effect of Tc cells against infected cells.

In conclusion, some active T cells and mIL-2R can be induced during the treatment of alpha 2b interferon. The patients with viremia are sensitive to treatment of alpha 2b interferon that have good effect on negative change of HCV-RNA in PBMC and serum and anti-HCV in serum. If the treatment time of alpha 2b interferon prolongs, the negative rate of anti-HCV and HCV-RNA will increase simultaneously. In treatment of HCV with alpha 2b interferon, the value of negative change of HCV-RNA in serum alone is limited. In well equipped hospital both HCV-RNA in serum and PBMC can be examined at the same time.

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Edited by Zhang JZ