Effect of liniment levamisole on cellular immune functions of patients with chronic hepatitis B

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INTRODUCTION
It has been reported that cellular immune functions are abnormal in patients with hepatitis B [1]. To explore the effects of liniment levamisole on cellular immune functions of patients with chronic hepatitis B, we measured the levels of CD3⁺, CD4⁺, CD8⁺, and the ratio of CD4⁺/CD8⁺ cells as well as the expression level of mIL-2R in PBMCs in patients with chronic hepatitis B before and after the treatment with liniment levamisole. The results support that levamisole can improve cellular immune functions of patients with chronic hepatitis B.

MATERIALS AND METHODS

Patients
According to the diagnostic criteria modified during the 10th National Conference on Viral Hepatitis and Hepatopathy 2000, 76 patients with chronic hepatitis B (45 males and 21 females) aged 25-64 years (average 48.25 years) were enrolled in this study and randomly divided into treatment group (n=38, 27 males and 11 females, aged 32-64 years and averaged 51.8 years) and control group (n=38, 18 males and 10 females, aged 25-58 years and averaged 44.7 years).

Treatment methods
Vitamins B and C, silymarin compound and other liver-protecting drugs were used in 76 patients. The treatment group was primed with liniment levamisole at a dose of 500 mg twice a week for 3 mo (a course of treatment). Five milliliters of liniment levamisole was smeared on the surface of skin inside the four limbs near the body. The area of the skin should be as large as possible and the smeared skin was washed within 24 h to assure absorption of the drug. No antiviral agent or immunomodulator was used in the two groups.

Samples
Five milliliters of peripheral vein blood was collected at 8:00 a.m. from each patient with hepatitis B and 2.5 mL was put into a sterile test tube and 2.5 mL into an anticoagulant test tube with heparin.

Separation of PBMC and detection of T cell subsets, mIL-2R
After the heparinized blood was mixed with an equal amount of anticoagulant. The mixture was incubated at 37°C for 30 min and the PBMCs were then separated by Ficoll-Paque density gradient centrifugation. The T cell subsets were detected by biotin-streptavidin (BSA) technique and the total expression level of mIL-2R in PBMCs was measured by flow cytometry.

RESULTS
After one course of treatment with liniment levamisole, the levels of CD3⁺, CD4⁺, and the ratio of CD4⁺/CD8⁺ increased as compared to those before the treatment but the level of CD8⁺ decreased. The total expression level of mIL-2R in PBMCs increased before and after the treatment with liniment levamisole.

CONCLUSION
Liniment levamisole may reinforce cellular immune functions of patients with chronic hepatitis B.

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Key words: Liniment levamisole; Chronic hepatitis B; Cellular immune function; T lymphocyte subsets; mIL-2R

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volume of Hanks’ liquid without Ca^{2+} and Mg^{2+}. PBMCs were harvested from heparinized whole blood by centrifugation on Ficoll-Hypaque sedimentation gradient and diluted to (1-3)×10^6/L cell suspension with RPMI 1640 culture liquid. Ten microliter suspension of PBMCs was smeared on sheet glass pores so that the cells with CD3^+ , CD4^+ , CD8^+ and the rest phrase of mIL-2R could be detected. Of the PBMC suspension, 0.5 mL was mixed with RPMI 1640 culture liquid. The cells were grown in continuous culture (37 °C, 50 mL/L CO_2 in atmosphere) for 72 h and mIL-2R could be measured by antibodies against the membrane of T cells.

**Immunocytochemical BSA technique**

Different monoclonal antibodies (mAb) against CD3^+ , CD4^+ , CD8^+ and Tac with biotin and SA-HRP were smeared on different sheet glasses. The smears were left to dry naturally and fixed with acetone for 15-20 min. The cells were incubated in continuous culture (37 °C, 50 mL/L CO_2 in atmosphere) for 30 min. The immune sheet glass pores were measured after being stained with color-developing agents and washed with Tris buffer solution (TBS). The total number of PBMCs was counted and the positive cells were analyzed with the help of high power lens.

**Reagents and instruments**

Antibodies against T-lymphocyte subsets were provided by Shanghai Jing’an Medical Institute; Ficoll-Hypaque sedimentation gradients were offered by Shanghai Second Reagent Factory. Carbon dioxide incubator (MDF-135) was from Japan.

**Statistical analysis**

Statistical analysis was performed using *t* test to determine the difference between the two groups.

**RESULTS**

Before the treatment, there was no significant difference in the levels of CD3^+ , CD4^+ , CD8^+ , mIL-2R and the ratio of CD4^-/CD8^- between the two groups. After one course of treatment, the levels of CD3^+ , CD4^+ and the ratio of CD4^-/CD8^- increased, while the level of CD8^- decreased. The total expression level of mIL-2R in PBMCs increased before and after the treatment with liniment levamisole.

**DISCUSSION**

This study demonstrated that levamisole could exert its immunopotentiating activity in patients with chronic hepatitis B. As an immune stimulant, levamisole could be connected with receptors of thymopentin on the surfaces of immunologic cells and induces inhibited T lymphocytes into immunologically competent cells, which take part in cell-mediated immune, induce interferon production and prime the lymphocytes and macrophages. According to clinical experiments, levamisole has therapeutic effectiveness and boosts immunity in a variety of infectious diseases and some cancers. Krastev et al. treated 25 viremic patients with chronic HBV infection with LMS and found that LMS may benefit some patients with chronic ongoing viral replication including patients who are contraindicated for TNF-α. It was reported that LMS acts by resetting the immune balance toward a type 1 response via the induction of IL-18.

We have previously shown that decreased CD3^+ and CD4^- levels and CD4^-/CD8^- ratio as well as increased CD8^- level occur in patients with chronic hepatitis B. We have also shown that the level of mIL-2R in PBMCs is lower than that in normal controls. In the present study, all these parameters including the levels of CD3^+ , CD4^- , CD8^- , mIL-2R and the CD4^-/CD8^- ratio became normal in the levamisole-treated patients. This reversion of aberrant cellular immunity may improve symptoms of chronic hepatitis B patients.

Liniment levamisole is made from levamisole, which is applied on the surface of skin and can penetrate into the liver, spleen, lungs, kidneys and other internal organs through the skin. We suggest that liniment levamisole should be used in the treatment of patients with chronic hepatitis B.

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