PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

World J Gastroenterol 2004;10(23):3531-3533
World Journal of Gastroenterology
 ISSN 1007-9327

• BRIEF REPORTS •

Effects of selenium on peripheral blood mononuclear cell membrane fluidity, interleukin-2 production and interleukin-2 receptor expression in patients with chronic hepatitis

Shui-Xiang He, Bing Wu, Xin-Ming Chang, Hong-Xia Li, Wen Qiao

Shui-Xiang He, Xin-Ming Chang, Hong-Xia Li, Wen Qiao, Department of Gastroenterolgy, First Hospital of Xi'an Jiaotong University, Xi'an 710061, Shaanxi Province, China
Bing Wu, Editorial Department of Journal of Xi'an Jiaotong University, Xi'an 710061, Shaanxi Province, China
Correspondence to: Dr. Shui-Xiang He, Department of Gastroenterolgy, First Hospital of Xi'an Jiaotong University, Xi'an 710061, Shaanxi Province, China. hesx123@163.net
Telephone: +86-29-5324001 **Fax:** +86-29-85221659
Received: 2003-12-19 **Accepted:** 2004-01-12

Abstract

AIM: To study the effect of selenium on peripheral blood mononuclear cell (PBMC) membrane fluidity and immune function in patients with chronic hepatitis.

METHODS: PBMCs were pretreated with selenium (1.156×10^{-7} mol/L) for 6 h *in vitro* or extracted directly from patients after administration of selenium-yeast continuously for 8-12 wk (200 μg/d), and then exposed to Con-A for 48 h. The membrane fluidity, interleukin-2 (IL-2) production and interleukin-2 receptor (IL-2R) expression in PBMCs and malondialdehyde (MDA) concentration in medium and lipid peroxide (LPO) in plasma were determined.

RESULTS: The PBMC membrane fluidity, IL-2 production and IL-2R expression in patients with chronic hepatitis were significantly lower than those in healthy blood donators (particle adhesive degree R, 0.17±0.01 *vs* 0.14±0.01, $P<0.01$; IL-2, 40.26±9.55 *vs* 72.96±11.36, $P<0.01$; IL-2R, 31.05±5.09 *vs* 60.58±10.56, $P<0.01$), and the MDA concentration in medium in patients with chronic hepatitis was significantly higher than that in healthy blood donators (1.44±0.08 *vs* 0.93±0.08, $P<0.01$). Both *in vitro* and *in vivo* administration of selenium could reverse the above parameters.

CONCLUSION: Supplement of selenium can suppress lipid peroxidation, and improve PBMC membrane fluidity and immune function in patients with chronic hepatitis.

He SX, Wu B, Chang XM, Li HX, Qiao W. Effects of selenium on peripheral blood mononuclear cell membrane fluidity, interleukin-2 production and interleukin-2 receptor expression in patients with chronic hepatitis. *World J Gastroenterol* 2004; 10(23): 3531-3533
http://www.wjgnet.com/1007-9327/10/3531.asp

INTRODUCTION

Hepatic viruses attack immune cells thus leading to cell immune functional disorders, which is one of the major mechanisms of chronic hepatitis[1-4]. It is currently believed that the damage of immune cells caused by lack of the minor elements of selenium, proliferation of free particles and peroxidization of lipid are probably associated with cell immune functional disorders in patients with chronic hepatitis[5-9]. The use of anti-oxidants could contribute to improving the psychopathologic process of chronic hepatitis, suppress cell immune functional disorders and accelerate the recovery of liver functions[10-112]. The minor elements of selenium have anti-oxidizing effects, and have been utilized in the immune regulation of many diseases, but the effect of selenium treatment on peripheral blood mononuclear cell (PBMC) functions in patients with chronic hepatitis is still unclear[13-15]. We found during our early work that using selenium in the periphery could improve the inlerleukin-2 production of PBMCs and IL-2R expression in patients with chronic hepatitis[16]. By examining the changes of PBMC membrane fluidity, interleukin-2 (IL-2) and its receptor system in patients with chronic hepatitis before and after using selenium, We further explored the effects and mechanism of selenium treatment on the cell immune functions in patients with chronic hepatitis.

MATERIALS AND METHODS

Patients

A total of 45 patients with chronic hepatitis were inpatients of our hospital from 1995 to 1997, among them 38 were males and 7 females, aged 21-52 years. The HBsAg of 30 patients with chronic hepatitis and 15 patients with cirrhosis was positive. The diagnosis was in accordance with the readjust standards of the National Academic Conference of Viral Hepatitis held in Shanghai in 1990. Besides, ten healthy blood donators were chosen as the control group.

Peripheral experiment

Elbow venous blood was taken from fasting healthy blood donators. Then PBMCs were routinely separated and floated in the RPMI-1640 culture medium. Then cell density was adjusted to 1×10^6/L and 1 mL of cell suspension was added into each well of 24-well culture plates, and randomly divided into control group, tBHP damage inducing group, and selenium treatment group. In addition, PBMCs were taken and divided into 4 patient control group and 5 patient selenium group. $NaSeO_2$ (final density, 1.156×10^{-7} mol/L) was added into groups 3 and 5, each of the rest groups was added with the same amount of culture liquid followed by incubation for 6 h, and con A (5 mg/L) were added. Meanwhile, lipoperoxide inducing agents tBHP (20 μmol/L) were added into groups 2 and 3, and cultured at 37 ℃ for 48 h in a humidified atmosphere containing 50 mL/L CO_2.

Clinical treatment and survey

The patients were divided into two groups. The normal group was given bifendate pills and Fufangyiganling. Some individual patients were given compound ammonium glycyrrhetate injection and Qinkailing injection intravenously. Selenium treatment group, on the basis of the routine treatment, was given selenium yeast or selenium amylase (200-300 μg/d) orally for 8 to 12 wk. At the beginning of and after the treatment, blood was collected from the patients to separate PBMCs and to measure the blood plasma.

Measurement of induced IL-2 production and its activation

Each group of PBMCs was added with con-A (5 mg/L, Sigma) and cultured at 37 ℃ in a humidified atmosphere containing 50 mL/L CO_2. IL-2 activation expressed in 1×10^3 U/L was measured.

Measurement of IL-2R expression

The cells of each group, after induction and production of IL-2, were collected to measure IL-2R expression by indirect immune fluorescence and the positive rate was expressed as percentage of positive cells.

Measurement of lipid peroxidation

The supernatant from each group or patients' blood plasma was subjected to the measurement of MDA or LPO content by using microflorescence technique.

Measurement of membrane fluidity

The cells of each group were suspended in the RPMI-1640 culture medium to a cell density of 1×10^6/L. The same amount of 2×10^{-6} mol/L fluorescence probing agents DPH (sigma product) was added and cultured at 25 ℃ for 30 min. The polarization deviation degree (P) was measured under the condition of 432 nm (transmitting light) /362 nm (irritating light) on MPF-4 polarization meter and the particle adhesive degree (R) was calculated. The value of R and membrane fluidity were in inverse proportion.

Statistical analysis

All the data were expressed as mean±SD, and analyzed by *t*-test and ANOVA analysis. $P<0.05$ was considered statistically significant.

RESULTS

Effects of selenium on IL-2 activation and IL-2R expression in human PBMCs

As shown in Table 1, after PBMCs in normal group were treated with tBHP, both IL-2R expression and IL-2 secreting activity were significantly declined, which was similar to those of the patient group. Six hours after addition of selenium, the above-mentioned changes in the cells of these two groups were obviously inhibited.

Table 1 Influence of selenium on IL-2 and IL-2R expression (mean±SD)

Groups	Cases	IL-2 activation (1×10^3 U/L)	IL-2R expression (%)
Normal control group	10	72.96±11.36	60.58±10.56
+tBHP	10	42.12±12.06[b]	37.05±8.06[b]
+Selenium+tBHP	10	53.26±18.15[a]	52.12±9.68[a]
Patient control group	22	40.26±9.55[b]	31.05±5.09[b]
Patients+selenium	23	60.32±15.24[c]	54.06±5.22[c]

[b]$P<0.01$ *vs* normal control group; [a]$P<0.05$ *vs* tBHP inducing damage group; [c]$P<0.05$ *vs* patient control group.

Influence of selenium on PBMC membrane fluidity

As shown in Table 2, after PBMCs in normal group were treated with tBHP, the amount of MDA increased and the membrane fluidity obviously lowered. The PBMC membrane fluidity of the patient group was also significantly lower than that of the normal control group. Six hours after addition of selenium, the above-mentioned changes could be obviously inhibited.

Changes in IL-2R expression and IL-2 secreting activity of PBMCs of patients before and after selenium treatment

As shown in Table 3, after treatment with selenium, both the IL-2R expression and IL-2 secreting activity of PBMCs were significantly increased, while the content of MDA in the culture media was obviously decreased compared with the groups without selenium treatment ($P<0.05$).

Table 2 Influence of selenium on PBMC membrane fluidity (mean±SD)

Groups	Cases	R	MDA (μmol/L)
Normal control group	10	0.14±0.01	0.93±0.08
+tBHP	10	0.19±0.02[b]	2.32±0.25[b]
+Selenium+tBHP	10	0.16±0.02[a]	1.36±0.09[a]
Patient control group	22	0.17±0.01[b]	1.44±0.08[b]
Patients+selenium	23	0.15±0.01[c]	1.21±0.09[c]

[b]$P<0.01$ *vs* normal control group; [a]$P<0.05$ *vs* tBHP inducing damage group; [c]$P<0.05$ *vs* patient control group.

Table 3 IL-2 and IL-2R expression in PBMCs of patients before and after selenium treatment (mean±SD)

Groups	Cases	IL-2 activation (1×10^3U/L)	IL-2R expression (%)
Before treatment			
Normal control group	22	43.22±9.25	31.24±5.20
Selenium treated group	23	42.26±9.55	31.05±5.09
After treatment			
Normal control group	18	49.45±15.25	35.12±6.49
Selenium treated group	17	60.32±13.28[a]	46.05±4.46[b]

[b]$P<0.01$ *vs* before treatment.

Changes in PBMC membrane fluidity of patients before and after selenium treatment

As shown in Table 4, the PBMC membrane fluidity recovered remarkably and the content of MDA in blood plasma decreased strikingly, whereas no significant changes were observed in the control group. The result was similar to that *in vitro* experiment.

Table 4 Effect on PBMC membrane fluidity of patients before and after selenium treatment (mean±SD)

Groups	Cases	R	Lipid peroxide (nmol/L)
Before treatment			
Normal control group	22	0.17±0.01	7.36±4.15
Selenium treated group	23	0.17±0.01	7.36±4.08
After treatment			
Normal control group	18	0.16±0.01	6.15±3.85
Selenium treated group	17	0.15±0.01[b]	5.02±2.50[b]

[b]$P<0.01$ *vs* before treatment.

DISCUSSION

Previous studies have shown that the activating oxidation or the organic peroxidation of the external chemical system could influence various functions of immune cells[15,17-19]. Our previous study demonstrated that PBMCs from the patients with chronic hepatitis showed significantly increased production of IL-2 and expression of IL-2R after warmed with selenium for 6 h[16]. In this experiment, our results showed that human PBMC producing IL-2 activation and IL-2R expression percentage obviously decreased after being induced by tertiary butyl peroxides. The content of lipoperoxide MDA in the culture liquid greatly increased, which was similar to the changes in PBMCs from chronic hepatitis B patients cultured *in vitro*. It was further reported that free particles and lipoperoxide receptor system probably associated with lipoperoxide reaction of

tertiary butyl peroxide could induce PBMC membranes, and thus resulting in changes of the normal structure and nature of PBMC membranes. Besides, some abnormal changes took place in receptors, enzymes and particle passages associated with the membranes, cell energy metabolism, signal transmission, proliferation, differentiation, *etc*, thereby finally leading to the organic disorder of PBMCs[20,21].

Fluidity, one of the basic characteristics of the membranes, is the basic precondition of cells showing various functions. In many pathologic cases, excessive free particles and initiating lipoperoxide could affect membrane fluidity and lead to disorder of cell immune functions[22-25]. Our results showed that PBMC membrane fluidity in normal group obviously decreased after treated with peroxide, and was accompanied by MDA increase in the culture supernatant. Moreover, PBMC membrane fluidity decreased and its IL-2-producing activity as well as IL-2R expression percentage changed unanimously, which was identical with the result of the cultured PBMCs from patients with chronic hepatitis. Abnormally strengthened lipoperoxide reactions could lead to decrease of PBMC membrane fluidity, which might be one of the causes of immune function disorder of patients with chronic hepatitis.

Studies shown that selenium influences immune cell functions through two ways[13-16,26]. On the one hand, selenium can affect the mRNA expression of some immune cell surface receptors, such as IL-2R, iron transporter protein receptor (TfR). On the other hand, it is associated with the functions of selenium anti-oxidization. Free particles can directly damage lymphocyte membranes and destroy their completeness and fluidity, thus restraining the expressions of their surface markers and immune functions. Selenium and enzymes in combination with selenium can inhibit cell membrane peroxidization damage and defend membrane fluidity as well as functional expression. In our experiment, the degree of membrane fluidity, IL-2 secretion and IL-2R expression of PBMCs caused by selenium still remained high. Meanwhile, the content of MDA in the culture supernatant was greatly decreased, suggesting that selenium might defend human PBMC membrane fluidity and its normal functional expression through lipoperoxidization damage induced by anti-tertiary butyl peroxide.

PBMCs and IL-2 system play an essential role in cell immune system of patients with chronic hepatitis, maintaining PBMC functions and correcting IL-2 system disorder, which is of great significance in treatment of chronic hepatitis[1-3]. It has been found that internal or external selenium can improve PBMC functions and increase Il-2 secretion and IL-2R expression percentage in patients with chronic hepatitis. It has been initially proved that selenium treatment is helpful to correct dysfunction of PBMCs in patients with chronic hepatitis, which is of great significance in the complete recovery of hepatic functions of patients.

REFERENCES

1 **Reshetnyak VI**, Sharafanova TI, Ilchenko LU, Golovanova EV, Poroshenko GG. Peripheral blood lymphocytes DNA in patients with chronic liver diseases. *World J Gastroenterol* 2001; **7**: 235-237

2 **Reshetnyak VI**, Sharafanova TI, Ilchenko LY, Poroshenko GG. DNA structure in peripheral blood lymphocytes from patients with chronic viral liver damages. *Bull Exp Biol Med* 2002; **133**: 399-400

3 **Wang JP**, Li XH, Zhu Y, Wang AL, Lian JQ, Jia ZS, Xie YM. Detection of serum sIL-2R, IL-6, IL-8, TNF-α and lymphocytes subsets, mIL-2R in patients with chronic hepatitis B. *Shijie Huaren Xiaohua Zazhi* 2000; **8**: 763-766

4 **Tulek N**, Saglam SK, Saglam M, Turkyilmaz R, Yildiz M. Soluble interleukin-2 receptor and interleukin-10 levels in patients with chronic hepatitis B infection. *Hepatogastroenterology* 2000; **47**: 828-831

5 **Yu MW**, Horng IS, Hsu KH, Chiang YC, Liaw YF, Chen CJ. Plasma selenium levels and risk of hepatocellular carcinoma among men with chronic hepatitis virus infection. *Am J Epidemol* 1999; **150**: 367-374

6 **Loguercio C**, De Girolamo V, Federico A, Feng SL, Crafa E, Cataldi V, Gialanella G, Moro R, Del Vecchio Blanco C. Relationship of blood trace elements to liver damage, nutritional status, and oxidative stress in chronic nonalcoholic liver disease. *Biol Trace Elem Res* 2001; **81**: 245-254

7 **Hatano R**, Ebara M, Fukuda H, Yoshikawa M, Sugiura N, Kondo F, Yukawa M, Saisho H. Accumulation of copper in the liver and hepatic injury in chronic hepatitis C. *J Gastroenterol Hepatol* 2000; **15**: 786-791

8 **Loguercio C**, Federico A. Oxidative stress in viral and alcoholic hepatitis. *Free Radic Biol Med* 2003; **34**1: 1-10

9 **Hoek JB**, Pastorino JG. Ethanol, oxidative stress, and cytokine-induced liver cell injury. *Alcohol* 2002; **27**: 63-68

10 **Naghii MR**. Sulfur mustard intoxication, oxidative stress, and antioxidants. *Mil Med* 2002; **167**: 573-575

11 **Marotta F**, Safran P, Tajiri H, Princess G, Anzulovic H, Ideo GM, Rouge A, Seal MG, Ideo G. Improvement of hemorheological abnormalities in alcoholics by an oral antioxidant. *Hepatogastroenterology* 2001; **48**: 511-517

12 **Cui YH**, Wang XL, Liu Q, Liu P. Influences of anti-oxidation of salvianolic acid B on proliferation of rat cultured hepatic stellate cells. *Shijie Huaren Xiaohua Zazhi* 2002; **10**: 317-319

13 **Kiremidjian-Schumacher L**, Roy M. Effect of selenium on the immunocompetence of patients with head and neck cancer and on adoptive immunotherapy of early and established lesions. *Biofactors* 2001; **14**: 161-168

14 **Jahnova E**, Horvathova M, Gazdik F, Weissova S. Effects of selenium supplementation on expression of adhesion molecules in corticoid-dependent asthmatics. *Bratisl Lek Listy* 2002; **103**: 12-16

15 **Lee CY**, Wan JM. Immunoregulatory and antioxidant performance of alpha-ocopherol and selenium on human lymphocytes. *Biol Trace Elem Res* 2002; **86**: 123-136

16 **He SX**, Chang XM, Zhou QS, Li HX, Wu YE. Effects of selenium on the IL-2 production and IL-2R expression of PBMC from patients with chronic active hepatitis B. *Shijie Huaren Xiaohua Zazhi* 1999; **7:** 658

17 **Lushpaeva IuA**, Medvedeva IV, Fadienko GR. Changes in clinical and laboratory findings on oxidation metabolism in lymphocyte membranes of rheumatoid arthritis patients on synchronous programmed intensive therapy. *Klin Med* 2002; **80**: 39-42

18 **Dey A**, Parmar D, Dhawan A, Dash D, Seth PK. Cytochrome P450 2E1 dependent catalytic activity and lipid peroxidation in rat blood lymphocytes. *Life Sci* 2002; **71**: 2509-2519

19 **Jobin N**, Garrel DR, Champoux J, Bernier J. Improved immune functions with administration of a low-fat diet in a burn animal model. *Cell Immunol* 2000; **206**: 71-84

20 **Maccarrone M**, Manca-di-Villahermosa S, Meloni C, Massoud R, Mascali A, Guarina R, Finazzi-Agro A, Taccone-Gallucci M. Arachidonate cascade, apoptosis, and vitamin E in peripheral blood mononuclear cells from hemodialysis patients. *Am J Kidney Dis* 2002; **40**: 600-610

21 **Azenabor AA**, Hoffman-Goetz L. Effect of exhaustive exercise on membrane estradiol concentration, intracellular calcium, and oxidative damage in mouse thymic lymphocytes. *Free Radic Biol Med* 2000; **1**: 84-90

22 **Gornicki A**, Gutsze A. Erythrocyte membrane fluidity changes in psoriasis: an EPR study. *J Dermatol Sci* 2001; **27**: 27-30

23 **Ferrante MC**, Meli R, Mattace Raso G, Esposito E, Severino L, Di Carlo G, Lucisano A. Effect of fumonisin B1 on structure and function of macrophage plasma membrane. *Toxicol Lett* 2002; **129**: 181-187

24 **Garcia JJ**, Reiter RJ, Karbownik M, Calvo JR, Ortiz GG, Tan DX, Martinez-Ballarin E, Acuna-Castroviejo D. N-acetylserotonin suppresses hepatic microsomal membrane rigidity associated with lipid peroxidation. *Eur J Pharmacol* 2001; **428**: 169-175

25 **Gornicki A**, Gutsze A. *In vivo* and *in vitro* influence of etretinate on erythrocyte membrane fluidity. *Eur J Pharmacol* 2001; **423**: 127-134

26 **Zhao R**, Zhang GC, Yu BM, Zheng MH, Li DH, Zhu YM, Hu BY. Effect of selenium on T lymphocyte against colonic cancer cells. *Shijie Huaren Xiaohua Zazhi* 2000; **8**: 80-83

Edited by Kumar M and Wang XL **Proofread by** Xu FM