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Effect of Excessive Iodine on Immune Function of Lymphocytes and Intervention with Selenium*

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Summary: In order to study the effect of excessive iodine on immune function of lymphocytes and the role of selenium supplementation with excessive iodine intake, the changes of T lymphocyte number, ratio of subsets, activity of natural killer (NK) cells and lymphocytes proliferation response were investigated. 150 female BALB/C mice were randomly divided into 5 groups in terms of their body weight (n=30 in each group), and 10 of each group were taken as one batch for test. Mice in the 5 groups were orally administrated with iodine 0 (group Ⅰ), 1500 (group Ⅱ), 3000 (group Ⅲ), 6000 μg/L (group Ⅳ), iodine 6000 μg/L plus selenium 0.3 mg/L (group Ⅴ) respectively for 30 days. Lymphocyte proliferation response, $CD4^+/CD8^+$, Th1/Th2 and the activity of NK cells were measured. $CD4^+/CD8^+$ was significantly lower, while lymphocyte proliferation response stronger, and Th1/Th2 and the activity of NK cells significantly higher in group Ⅳ than in group Ⅰ (P<0.01). There was no significant difference in all indexes between group Ⅴ and group Ⅰ (P>0.05). It was suggested that excessive iodine as exogenous chemical materials can induce disorders of T lymphocyte immune function in mice. 0.3 mg/L selenium supplementation can protect mice against toxicity induced by 6000 μg/L iodine.
Key words: excessive iodine; lymphocyte; immune function; intervention

It is becoming increasingly clear that lymphocyte is crucial for autoimmunity disease in generation and development. Due to abnormal regulation of body's immune function, the changes in the T lymphocytic activity and T lymphocytic subset ratio are caused, which are responsible for initiating autoimmune responses. Epidemiological investigation indicates that high frequency of thyroid autoimmune disease on the area of excessive iodine intake is 2-3 times more than that of low iodine intake area. The data from laboratory and clinic identified that iodine can induce and aggravate thyroid autoimmune disease [1-4]. Thyroid autoimmune disease mainly includes Hashimoto's thyroiditis (HT) and Graves disease (Graves), which closely relates to lymphocyte immune function [1-4]. At present the aetiology of thyroid autoimmune disease caused by excessive iodine intake is still unknown. It is of great benefit to investigate the cytotoxic effects of iodine on lymphocyte immune function and how the lymph proliferation response and the ratio of lymphocyte subpopulations affected by excessive iodine. For this reason, mice were fed by different dosages of iodine for 30 days and lymph proliferation response, $CD4^+/CD8^+$, Th1/Th2 and NK cytoactive were detected, which would demonstrate the effect of excessive iodine on lymphocyte immune function. Meanwhile, to study how selenium prevent excessive iodine intake, 10 of each group in the 5 groups were orally administrated with 6000 μg/L iodine+0.3 mg/L selenium. This study would provide us with a basic theory about the aetiology of autoimmunity thyroid diseases with relation to lymphocyte immune function breakdown induced by excessive iodine intake and the effect on selenium supplementation against excessive iodine.

1 MATERIALS AND METHODS

1.1 Experimental Animals and Grouping

150 female BALB/C mice were randomly divided into 5 groups in terms of their body weight (n=30 in each group), and 10 of each group were taken as one batch for test. Mice in the 5 groups were orally administrated with iodine 0 (group Ⅰ), 1500 (group Ⅱ), 3000 (group Ⅲ), 6000 μg/L (group Ⅳ), iodine 6000 μg/L plus selenium 0.3 mg/L (group Ⅴ) respectively for 30 days. Lymph proliferation response, $CD4^+/CD8^+$, Th1/Th2, the activity of natural-killer cells (NK) were observed. The experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (DHEW Publication No. 85－23).

1.2 Determination of Lymphocytic Transformation Rate in Spleen by MTT Method

The spleens were taken out of mice in sterility, placed in a plat containing Hank's balanced salt solution and grinned softly to release the spleen cells. Then, cells

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were transferred to a 200 small hole-nylon mesh to get single cell suspension and centrifuged at 1000 r/min for 4 −5 min at 4°C, after which single cell was washed two more times with Hank's balanced salt solution. Then the cell pellet was suspended in 1 mL culture fluid, the number of cells counted and adjusted to 3×10^6 cells per mL. The cell suspension was added to 24-well culture plates, 1 mL for each, stimulated with ConA (7.5 μg/mL) and cultured for 72 h at 37°C in a humidified incubator with 5% CO_2. MTT was put into a well (5 mg/mL) 4 h before the end of the culture. Lastly, *OD* value was determined by a microplate reader in 570nm.

1.3 Detection of $CD4^+/CD8^+$ Ratio by Flow Cytometry

The spleen cell suspension was incubated with FITC-conjugated CD4 and PE-conjugated CD8 antibodies and double stained at room temperature in dark for 30 min. After having been washed three times with 0.1% BSA-PBS, the cells were resuspended in 500 μL 0.1% BSA-PBS and immediately submitted to measurement on a FACS Calibur flow cytometer (Becton Dickinson, USA). The ratio of CD4/CD8 was calculated.

1.4 Detection of Intracellular Cytokines IFN-γ/IL-4 Ratio by Flow Cytometry

One mL spleen cell suspension was stimulated in a container and then cultured with ConA (7.5 μg/mL) in 24-well culture plates for 72 h at 37°C in a humidified incubator with 5% CO_2 in the presence of 10 μg/mL of brefeldin A. Brefeldin A might activate intracellular cytokines and made the cytokine be detected easily. At the end of the culture, activated lymphocytes supernatants were harvested in tubes and fixed by 100 μL 4% paraformaldehyde solution and incubated at room temperature in dark for 20 min, then washed with 0.1% BSA-PBS. Each tube was added with 100 μL buffer containing 0.05% Triton-X-100 and 0.3% BSA, incubated for 10 min at room temperature in dark, then washed two times and restored in 100 μL 0.1% BSA-PBS. The samples were incubated with FITC-conjugated IFN-γ, and PE-conjugated IL-4 antibodies for 30 min at room temperature in dark respectively, washed two times and analyzed by flow cytometer. Then, the ratio of IFN-γ/IL-4 was calculated.

1.5 Analysis of NK Cytoactive

The spleens were taken out of mice in sterility. Spleen cells suspension were considered as effector cells. YAC-1 cells were considered as target cells and adjusted to 4×10^5 cells per mL. 100 μL of the effector cells and target cells respectively (effector cell:target cell=50:1) were mixed and added into 96-well culture plates. 100 μL of the effector cells and basic medium respectively were mixed and served as the natural releasing well. 100 μL of target cells and 1% NP40 respectively were mixed and served as the maximum releasing well. The culture plate was incubated for 4 h at 37°C in a humidified incubator with 5% CO_2 and centrifuged at 1500 r/min for 5 min at 4°C. 100 μL of the supernatant was drown off and dropped into another 96-well culture plates. Meanwhile, 100 μL of the substrate of lactic acid dehydrogenase was added. After the reaction at room temperature for 3-5 min, 1 mol/L HCl (30 μL) was added to each well and *OD* value was determined by a microplate reader in 490 nm.

1.6 Statistics Analysis

All the data were expressed $\bar{x}\pm s$. The intergroup difference was evaluated by the *F*-test of one-way analysis of variance (ANOVA). The difference was considered to be significant at $P<0.05$.

2 RESULTS

2.1 Effect of Excessive Iodine on Lymphocytic Reproductive Activity and Intervention with Selenium in Mice

From fig. 1, it was found that the OD value in group Ⅳ was increased significantly as compared with that in group Ⅰ ($P<0.01$), but there was no significant difference between group Ⅰ and group Ⅱ or group Ⅲ. There was no change in the lymphocytic reproductive activity in group Ⅴ in comparison to group Ⅰ.

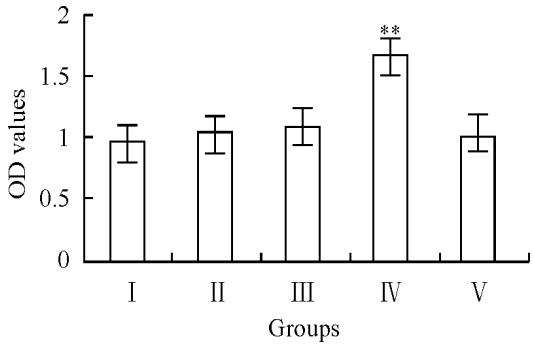

Fig. 1 Effect of excessive iodine on T lymphocytic reproductive activity and intervention with selenium in mice ($^{**}P<0.01$, as compared with group Ⅰ)

2.2 Effect of Excessive Iodine on $CD4^+/CD8^+$ Ratio Variation and Intervention with Selenium in Mice

As shown in fig. 2, the ratio of $CD4^+/CD8^+$ in group Ⅳ was significantly lower than in group Ⅰ ($P<0.01$), but there was no significant difference between group Ⅱ, group Ⅲ, group Ⅴ and group Ⅰ.

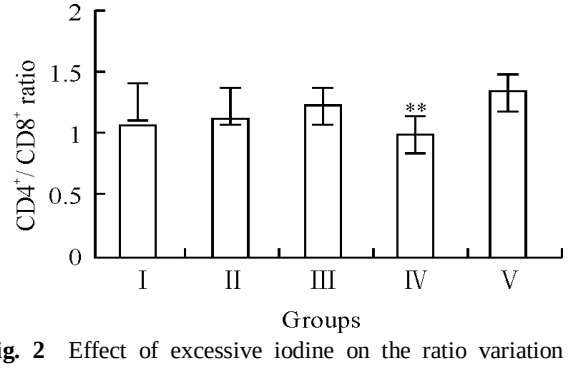

Fig. 2 Effect of excessive iodine on the ratio variation of $CD4^+/CD8^+$ and intervention with selenium in mice ($^{**}P<0.01$ as compared with group Ⅰ)

2.3 Effect of Excessive Iodine on Ratio of Th1/Th2 and Intervention with Selenium in Mice

As shown in fig. 3, as compared with group Ⅰ, the ratio of IFN-γ/IL-4 was increased obviously in group Ⅳ, but there was no significant changes in the ration of

IFN-γ/IL-4 in groups Ⅱ, Ⅲ and Ⅴ.

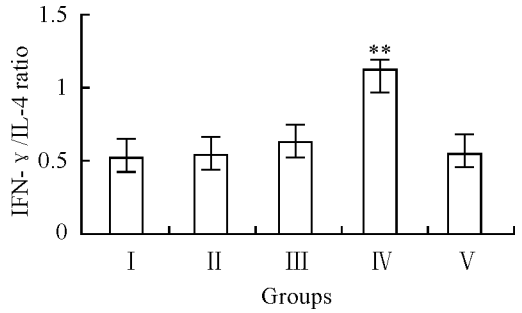

Fig. 3 Effect of excessive iodine on the ratio of IFN-γ/IL-4 and intervention with selenium in mice ($^{**}P$<0.01 as compared with group Ⅰ)

2.4 Effect of Excessive Iodine on NK Cytoactive and Intervention with Selenium in Mice

From fig. 4, it was discovered that excessive iodine intake could induce the change of NK cytoactive. The percentage of NK cytoactive in group Ⅳ was markedly increased in comparison with group Ⅰ (P<0.01), but there was no significant changes in groups Ⅱ, Ⅲ and Ⅴ.

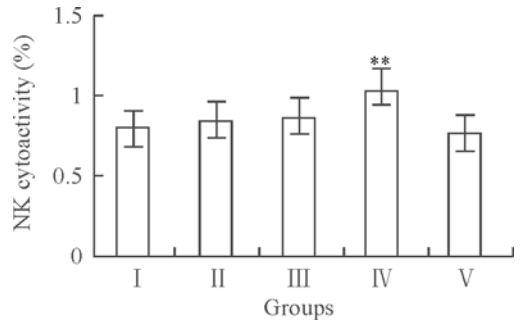

Fig. 4 Effect of excessive iodine on NK cytoactivity and intervention with selenium in mice ($^{**}P$<0.01 as compared with group Ⅰ)

3 DISCUSSION

In the earlier studies, it has been evident that goiter is easily accessible in mice orally administrated with 3000 μg/L iodine (0.75 mg/kg body weight), 10 times of that of the normal iodine intake, for three months[5]. Accordingly, in this study, BALB/C mice used for studying effect of excessive Iodine on immune function of lymphocytes were assigned into four groups based on the iodine 3000 μg/L according to immune toxicologic rule, 1500, 3000, 6000 μg/L respectively and tap water, geometric progression relation of 3000 μg/L, which would be of great benefit to determine how the iodine affects lymphocyte immune function. In addition, to study the selenium protective mechanisms under the circumstance of excessive iodine intake, 6000 μg/L iodine+0.3 mg/L selinium group (group V) was set up to reveal the effects of the selenium intervention.

Pathogenesis of autoimmune disease is believed to begin with the activation of autoaggression T cell (allergized). Normally there are relatively stable population of T cells and their subgroups in tissue till immune function is in disorder. From this study, it was found that lymph proliferation response was activated abnormally in group Ⅳ (6000 μg/L iodine), suggesting that the lymphocyte immune function is disordered.

The most accurate method to study exogenous chemical immunotoxicity is to measure cell surface molecules with FACS[6]. The marked changes in the ratio of $CD4^+/CD8^+$ identified the generation of immunotoxicity. $CD4^+$ T cells are divided into two-functional subpopulations–Th1 and Th2. The excursion of their ratio is responsible for initiating and aggravating some diseases, eg, thyroid autoimmune disease[7, 8]. The intensive excursion of INF-γ/IL4 in 6000 μg/L iodine group (group Ⅳ) has demonstrated the risk of thyroid autoimmune disease caused by excessive iodine.

Moreover, NK cells can help T lymphocyte to proliferate. In this study, it was found that excessive iodine intake could result in the abnormal activation of NK cells. It was might be contributed to the fact that the dosage led to the exposure of concealed antigen and the enhancement of cell toxic action, finally resulting in organism self-recognition.

In summary, iodine is related closely to lymphocyte immune response, and excessive iodine intake can result in lymphocyte immune dysfunction, even cause thyroid autoimmune diseases.

Iodine is a strong oxidant. Investigation discovered that excessive iodine intake for a long time could decrease the activity of anti-oxidation enzyme–SOD and GSH-Px of blood, brain, and thyroid, and increase the level of MDA[9]. Hence, it might lead to oxidative damage to some cells, which might result in proliferation and differentiation of immune cells or alter the immune response.

GSH-Px, which depends on selenium, is a very important antioxidase to the body. Selenium can clean oxygen free radical and inhibit the production of free radical[10]. Accordingly, favorable nutritional status of selenium is very useful and important to prevent the human body from the injury of free radicals.

At present, it is becoming increasingly clear that excessive iodine intake requires higher concentration of selenium in the liver and kidney of mice, as the activity of GSH-PX can be suppressed obviously by excessive iodine[11]. These demonstrate that selenium metabolism has much to do with iodine, for excessive iodine will desire more selenium supplement. For this reason, it was assumed that certain concentration of selenium could be supplemented to enhance the activity of selenoenzyme GSH-Px and the anti-oxidation ability of organism to protect cells against oxygen free radicals and toxic compounds induced by excessive iodine intake.

The selenium concentration in feedstuff is 0.1 μg/L. In this study, a group of 6000 μg/L iodine+0.3 mg/L selenium (group V) was set up and selenium (0.3 mg/L) was utilized to suppress the damage caused by 6000 μg/L iodine. The results revealed that there was no significant difference in the immunotoxicity between interventional group (group V) and control group (group I), indicating that adequate selenium has a favorable interventional

effect on excessive iodine intake.

In addition, it is known that goiter is easily accessible in mice orally administrated with 3000 μg/L iodine for three months[5]. This study discovered that all indexes of T lymphocyte immune function in the mice were altered significantly in one month after the mice had been orally administrated with 6000 μg/L iodine (0.75 mg/kg body weight). It was inferred that goiter, the disorder of lymphocyte immune function, and excessive iodine intake are related to one another. Therefore, the presumable result is that goiter is related to the disorder of lymphocyte immune function caused by excessive iodine intake.

REFERENCES

1 Allen E M, Appel M C, Braveman L E. The effect of iodine ingestion on the development of spontaneous lymphocytic thyroiditis in the diabetes-prone BB/W rat. Endocrinology, 1986,118:1977-1981
2 Li M, Eastman C J, Boyages S C. Iodine induced lymphocytic thyroiditis in the BB/W rat: early and late immune phenomena. Autoimmunity, 1993,14(3): 181-187
3 Many M C, Maniratunga S, Varis I *et al*. Two-step development of hashimoto-like thyroiditis in genetically autoimmune prone non-obese diabetic mice: effects of iodine-induced cell necrosis. Endocrinology, 1995,147: 311-320
4 Laurberg P. Iodine intake−what are we aiming at?. Clin Endocrinol Metab, 1994,79: 17-19
5 Gao Q J, Liu Y, Xu C L *et al*. Study on dose-reaction relationship between different iodine dose and morphology and function of thyroid. Chin Public Health (Chinese), 2002,18:423-424
6 Zh X, Liu Y G, Toxicology. Bejing: Beijing Medical University & Chinese Xiehe Medical University Union Publishing House, 1997.5
7 Guo J, Rapoport B, Mclachlan S M. Balance of Th1/Th2 cytokines in thyroid autoantibody synthesis in vitro. Autoimmunity, 1999,30:1-9
8 O'Shea J J, Ma A, Lipsky P. Cytokines and autoimmunity. Nat Rev Immunol, 2002,2:37-45
9 Xiang J M, Chen Z P, Di H J. Effects of excessive iodine on antioxidizing ability of mice. Chin J Endemiol (Chinese), 1999,18:245-249
10 Zeng J, Luo H J. Progress in trace elements and health selenic research. Studies Trace Elements Health (Chinese), 2003,2(2):52-56
11 Yang X F, Hou X H. Effects of excessive iodine on antioxidizing ability of mice and intervention with selenium. Chin Public Health (Chinese), 2004,20(9): 1081-1082

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