

SHORT PAPER

Adverse Effect of T-2 Toxin and the Protective Role of Selenium and Vitamin E on Peripheral Blood B lymphocytes

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ABSTRACT

Background: T-2 mycotoxin belongs to the Trichothecene family and has damaging effects on the immune system. **Objective:** To investigate the toxic effect of T-2 toxin on the percentage of peripheral blood B lymphocytes and the potential protective role of selenium and vitamin E. **Method:** Frequencies of B lymphocytes (CD19⁺) were analyzed after injection of sublethal doses of T-2 toxin into Balb/c mice at different time points, using flowcytometry. Additionally, the effects of selenium and vitamin E on B lymphocyte, as either prophylaxis or simultaneously administered with T-2 toxin, were investigated. **Results:** After injection of a sublethal dose of T-2 toxin, the number of B cells (CD19⁺) significantly decreased at 12 h and became normal at 72 h. When selenium was injected both 24 h before and simultaneously with T-2 toxin, it was able to inhibit B lymphocyte (CD19⁺) reduction. In contrast, injecting vitamin E, 24 h before or simultaneously with T-2 toxin did not regulate B lymphocyte alteration. **Conclusion:** Selenium plays pivotal role on altered B lymphocyte subset induced by T-2 toxin comparing to vitamin E.

Ahmadi A, et al. *Iran J Immunol.* 2015; 12(1):64-69

Keywords: B Lymphocytes, Flowcytometry, Selenium, T-2 Toxin, Vitamin E

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INTRODUCTION

T-2 toxin, a secondary metabolite produced by *Fusarium* Spp. and other fungi, is structurally classified in the Trichothecenes family. This family is mostly recognized through their inhibitory effects on protein synthesis and also the induction of apoptosis in eukaryotic cells. Among this family, T-2 toxin is well known due to its potent toxic activity, and consequently, several mycotoxicose outbreaks (1-3). Furthermore, T-2 toxin post exposure can cause increased susceptibility to infectious and autoimmune diseases as well as cancer (4). It was shown that acute doses of T-2 toxin can affect both the innate and adaptive immune systems through the suppression of Th1 and Th2 responses (5). Moreover, it was demonstrated that T-2 toxin has some adverse effects on B lymphocytes as the main effector cell in the humoral immune response (6). For example, some studies have confirmed apoptosis and a significant reduction in B lymphocytes and their precursors induced by T-2 toxin (7,8). The oxidative stress, one of the most important mechanisms of T-2 toxin activity, occurs either by the overproduction of reactive oxygen species (ROS) or by depletion of cell antioxidant levels leading to apoptosis (9,10). Hence, antioxidant agents such as selenium and vitamin E are greatly involved in the prevention and treatment of T-2 toxicity (11). Accordingly, we have previously studied the protective effect of selenium and vitamin E against T-2 toxin induced alteration on T lymphocytes (12). Herein, the present study aims to examine T-2 toxin effects on peripheral B lymphocytes, and investigate the protective role of selenium and vitamin E against oxidative stress induced by T-2 Toxin.

MATERIALS AND METHODS

Assessment of B Lymphocyte Frequency after Injection of T-2 Toxin. A Sublethal dose of T-2 toxin, (2mg/kg) (Sigma, St. Louis, MO), was intra-peritoneally injected to 4 groups of BALB/C mice (male, 6-8-wk-old, 20-22 g) as previously described (12). The control group only received phosphate buffered saline (pH 7.4). Peripheral blood cells were obtained intermittently after 12, 24, 48, and 72 hours of injection by puncturing of eye vein and collected in a heparinized tube. To count the B lymphocyte and their precursor in blood samples, one tube was added with FITC-conjugated rat anti-mouse CD19 (Serotech, UK) (5 µl antibody [1:10 dilution of stock from supplier]). As isotype controls, rat IgG2a FITC-conjugate was used. All samples were incubated for 30 min at 4°C and after washing the cells were re-suspended in flowcytometric buffer (BD, Franklin Lakes, NJ) and evaluated using a BD FACS Calibur flowcytometer. A minimum of 10,000 events were acquired for each sample. All records were analyzed using Flowjo software (V 7.6.1, Ashland, OR).

Evaluation of Selenium and Vitamin E Effect. Selenium (aka. Sodium Biselenite) and vitamin E were prepared in PBS and olive oil, respectively (at 1mg/ml) (12). In one group of mice, 500 µl of selenium, at 3 mg/kg i.p., was injected 24 hours before T-2 toxin injection (prophylactic effect) and other groups were simultaneously injected with the same dose of selenium and a sublethal dose of T-2 toxin. Also, 500 µl of the vitamin E was administrated, i.p., into the other 2 groups of mice [14.3 mg/kg] 24 h both before and concurrent with the T-2 toxin. Then, 24 hours after T-2 toxin the T-2 toxin injection, blood samples were collected and the B lymphocyte levels were determined

by flowcytometry. Each control group separately received PBS, T-2, Se, and vitamin E alone.

Statistical Analysis. All data were analyzed by In Stata software. We used the two way analysis of variance (ANOVA) for the statistical analysis. Statistical significance was defined as $p < 0.05$.

RESULTS and DISCUSSION

B Lymphocytes Frequencies after Injection of T-2 Toxin. The results showed that injection of a sublethal dose of T-2 toxin could extremely influence the peripheral B lymphocyte levels in comparison with the control group. A great reduction in the frequencies of B lymphocytes ($CD19^+$ cells) was noted at the 12 h post-injection time point and remained depressed until 72 h post-injection (Figure 1). The results showed that B ($CD19^+$) cells were very sensitive to cytotoxic and apoptotic effects of T-2 toxin and highly reduced in their population levels.

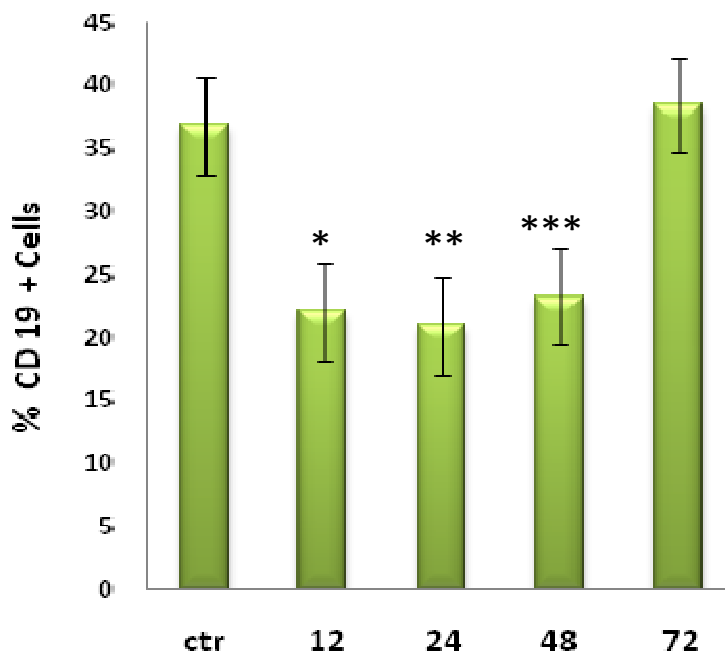


Figure1. T-2 toxin induced changes in mouse peripheral B lymphocyte. Blood levels of $CD19^+$ cells. Data expressed as mean % in 10^6 WBC analyzed (\pm SD)/sample/mouse; $n = 8$ /time point. *, **, ***P value significantly different from control (ctr) at $p < 0.001$.

Effects of Selenium and Vitamin E on T-2 Toxin Mediated B lymphocyte Alteration. Selenium (injected both prophylactically and concurrently with T-2 toxin) could significantly protect B lymphocytes ($CD19^+$) against the cytotoxic effect of T-2 toxin (Figure 2). Neither prophylactic nor simultaneous administration of vitamin E could protect lymphocytes against the cytotoxicity of T-2 toxin (Figure 3).

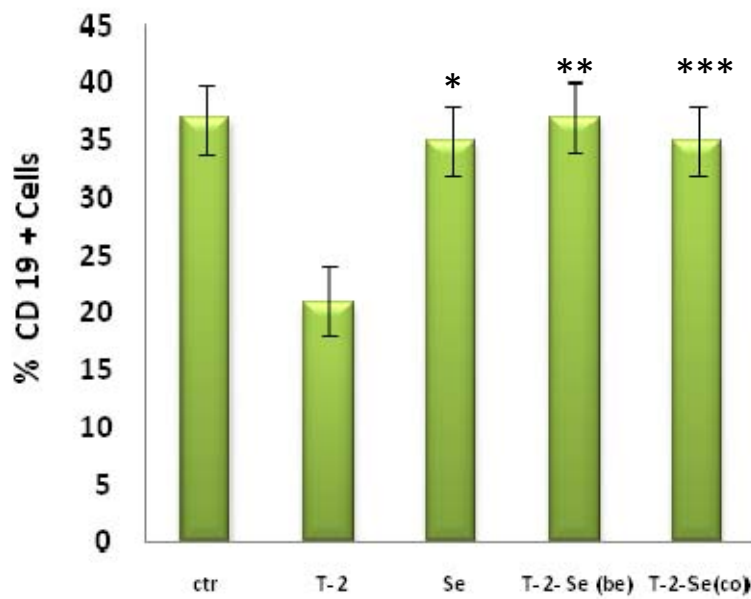


Figure2. Protective role of selenium on T-2 toxin induced changes in peripheral CD19+B lymphocyte. T-2 = T-2 toxin alone, Se= Selenium alone, T-2-Se (Se 24 hr prior to toxin), and T-2-Se (co)= Se concurrently administered with toxin. Data expressed as mean % in 10^6 white blood cells analyzed (\pm SD)/sample/mouse; n = 8/time point. Value significantly different from 'T-2 toxin' only at *, **p < 0.01 or ***p < 0.05.

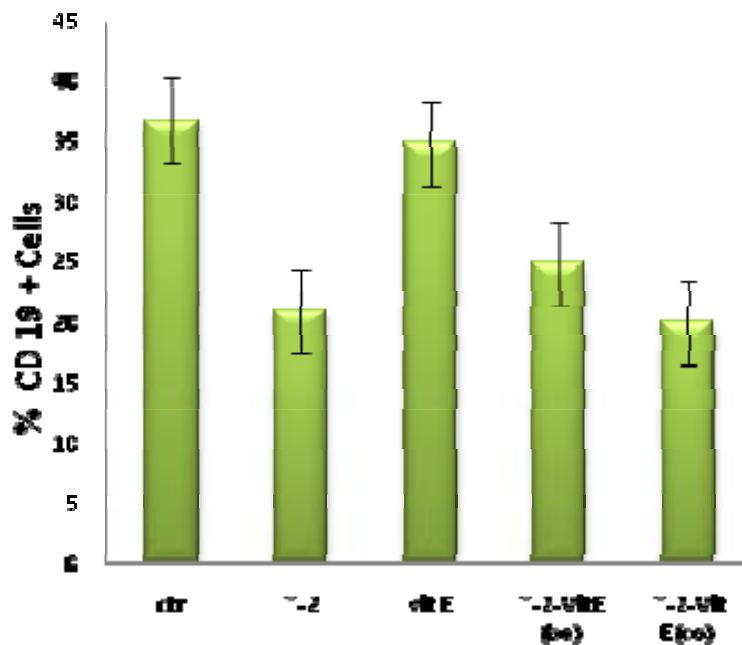


Figure3. Protective role of Vitamin E on alterations in peripheral CD19+B lymphocyte levels due to T-2 toxin. T-2 = T-2 toxin alone, Vit E= Vitamin E alone, T-2-Vit E (Vit E 24 hr prior to toxin), and T-2-Vit E (co)= Vit E in concurrently with toxin. Data expressed as mean % in 10^6 white blood cells analyzed (\pm SD)/sample/mouse; n = 8/time point.

T-2 toxin intoxication can lead to diarrhea, hemorrhage, leucopenia, bone marrow damage, radiomimetic injury to tissues, and immunosuppression. T-2 toxin can cause apoptosis in eukaryotic cell via oxidative stress mainly through ROS induced intrinsic (mitochondrial) apoptotic pathway. Also, other mechanisms such as Ribotoxic stress response, lipid peroxidation, and DNA damage are involved in T-2 toxin's toxicity (9,13).

T-2 toxin had adverse effects on peripheral blood B (CD19⁺) lymphocyte. In agreement with our data, an earlier study showed that T-2 toxin had an adverse effect on B cells in secondary lymphoid (such as lymph node and spleen) and generative organs (such as bone marrow) (7). Another study reported that T-2 toxin intoxicated ducks experienced atrophy in both their thymus and Bursa of Fabricius due to lymphocyte depletion (14). Moreover, the CD21⁺ B cells decreased steadily in the ileal Peyer's patches of pigs treated with a chronic dose of T-2 toxin (15). Finally, a study showed that T-2 toxin could affect the mice thymus, mesenteric lymph nodes, and Peyer's patches and cause a decline in CD19⁺ B-lymphocyte population levels (16).

Our data showed that the maximum change in peripheral CD 19⁺ B lymphocytes occurred between 12-48 h after exposure. Then, perhaps due to the fast metabolism of the toxin in the blood, B lymphocytes tend to return to normal states (10). We previously showed that maximum alteration of T lymphocytes is seen at 24 h after T-2 toxin exposure. Similarly, change in hematological parameters, blood lymphocytes, granulocytes, and RBC was reported at 24 h post injection (17). Our results showed that a single dose of selenium had a protective role on B lymphocytes against T-2 toxin. In line with our study, previous surveys showed that selenium can have protective effects against T-2 toxin cytotoxicity, both in vitro and in vivo, and could potentially be applied as feed additive against intoxications caused by T-2 toxin (5-7,12).

Although vitamin E is a well known natural antioxidant widely used against T-2 toxin (11), in this study, it did not protect lymphocytes against T-2 toxin cytotoxicity, either in the format of prophylactic or simultaneous administration. There could be several possible theories behind this. First, some studies have demonstrated that the water-soluble form of vitamin E is more effective than the fat-soluble one for protection against T-2 toxin (18). Second, multi-dose administration may be another important factor affecting the outcomes. Third, it is known that T-2 toxin itself can affect vitamin E transportation/absorption by the cells (19). However, there may exist other causes to help clarify our findings, which should be determined through further studies.

In conclusion, this research supports pervious data about the effects of an acute dose of T-2 toxin on peripheral B cells. Additionally, in contrast to vitamin E, selenium has a better immunomodulatory effect on the alteration of peripheral B lymphocyte levels induced by T-2 toxin and could provide protection against this potent mycotoxin.

ACKNOWLEDGEMENTS

We would like to thank Mrs. Haghighi for her technical help in the flowcytometry experiments of this study. The authors declare that this study has received no financial support, from either public or private funds.

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