ASSESSING THE EFFECTS OF FUMONISIN IN THE PRODUCTION OF CYTOKINES AND THE ANALYSIS OF IL2 AND IL4 IN MICE

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ABSTRACT: The fumonisins are a group of toxic and carcinogenic mycotoxins. Fumonisin B1 is produced by Fusarium verticillioides (Moniliform) and it can usually infect maize and other crops. This toxin is neurotoxic, hepatotoxic and nephrotoxic for many animals and its carcinogenic effects in humans are well known. Some believe that the risk of Fumonisin toxicity is the result of sphingoid base accumulation. The present study examines the Fumonisin effects on levels of various cytokines in serum of mice. To do this, mice were randomly divided into two groups: Control Group and Experimental Group. Control Group was fed Fumonisin with a rate of 150 mg kg in the diet for 4 months. One day after the last treatment, the dead mice and their tissues were prepared for assessment of cytokines by ELISA test. According to studies on the levels of serum cytokines in the experimental group, the IL4 is significantly increased, while the IL2, shows a significant decrease, which means that Fumonisin can activate hT2 dependent cytokines and thus suppress hT2 dependent cytokines to the IL2.

Keywords: Fumonisin, Cytokines, IL2, IL4, Mice.

INTRODUCTION
Mycotoxins are low molecular weight substances that are chemically aromatic hydrocarbons and include a wide range of different compounds. In environmental factors such as: grinding temperature and agricultural products preparation for human and animal consumption, Mycotoxins are resilient. They have weak Antigenic and immunogenic, and therefore immune systems, are not activated against them. Some of well-known types of mycotoxins are Fusarium and Ochratoxin aflatoxins. (Wood GE, 1992).

Toxins are produced by most fungi. Till now Aspergillus, Penicillium and Fusarium have been considered the most important toxigenic molds (Kendra, 2007). Tricotoxocosis and Zearalenone and Moniliformin and Fumonisins are considered as Fusarium main toxins (Schaafsma & Hooker, 2007). B1, B2, B3 are the most important. FB1 is the most produced types of Fumonisin And it infects substances such as peanuts, cottonseed, rice, wheat and soybean in addition to maize (Schaafsma & Hooker, 2007). Apoptosis stimulation is one of the effects of Fumonisin and dead cells may not replace and they may atrophy, or the tissue may provide new cells, to replace the dead cells. Cell death and cell reconstruction is a method of chemical Carcinogen which targets a variety of tissues (Cohen, 1998).

Cytokines are proteins that are secreted from innate and adaptive cells and they control many actions of these cells. Cytokines are produced in response to microbes and other antigens. Effects of various cytokines on the cells involved in immune and inflammatory are different. by activation of the immune response, Cytokine stimulate the growth and differentiation of lymphocytes, and finally, the activate different cells eliminate microbes and other anti-pans (Gilman et al., 2001).

Cytokines divide into three groups of according to their biological functions: innate intermediaries and regulators, specific immune system and hematopoiesis stimulators specific (Vlahopoulos et al., 1999). IL2 is
growth factor for stimulated antigen T cells, and it is responsible for clonal expansion after antigen detection (Smith et al., 1983). It also increases production of other cytokines such as IL4 by T cells.

IL2 causes proliferation and differentiation in other immune cells and stimulates the growth of NK cells and increases the activity of cell death and produces Lymphokine-activated killer cells. (Thornton et al., 1998). IL4 is the major driver of IgE antibodies production and development of Th2 cells from T helper cells. (Sokol et al., 2008). The biological effects of IL4, are amplifying reactions involving IgE and Mast cells/Eosinophil and suppression of macrophages dependent reactions. Mice with no IL4 gene, have less than 10% of normal IgE levels.

According to the research, short-term poisoning with FB1 causes hepatotoxic effects. While, the long-term poisoning, causes chronic toxic hepatitis and fibrosis, which can sometimes lead to hepatocellular carcinoma (Jaskiewicz et al., 1987; Gelderblom et al., 1991, 2001).

It has also been shown that the induction of apoptosis by FB1 can be mediated by the accumulation of free sphingoid bases, but inhibition of ceramide synthase alone is, however, not responsible for the induction of apoptosis (Schmelz et al., 1998; Seefelder et al., 2003). Therefore, the purpose of this study is to examine the effects of cytokines, exposed to FB1, and studying two markers of IL2 and IL4 in FB1 toxicity.

**METHODOLOGY**

Site and Animals of research:
This study was conducted in Animals Experimental Lab. of Cancer Institute, Imam Khomeini Hospital in October 1389. In this study, 20 female Syrian mice aged 3 weeks old weighing 20gr were purchased from the Razi Vaccine and Serum Research Institute in Tehran. To avoid any risks during the research, all the mice were maintained in the same conditions with Temperature of +25°C and a relative Humidity Of 80% and 12:12h light:dark cycle. Mice were fed laboratory animals food without getting any substance for two weeks, in order to ensure their health. mice were randomly divided into two groups: Control Group(=10) and Experimental Group(=10).

Fusarium B1 in this research was from Fumonisin verticillioides isolated from Iranian maze strain and approved by a molecular method using specific Fumonisin verticillioides primers in Animals Experimental Lab. of Cancer Institute, Imam Khomeini Hospital in October 1389. In this study, 20 female Syrian mice aged 3 weeks old weighing 20gr were purchased from the Razi Vaccine and Serum Research Institute in Tehran. To avoid any risks during the research, all the mice were maintained in the same conditions with Temperature of +25°C and a relative Humidity Of 80% and 12:12h light:dark cycle. Mice were fed laboratory animals food without getting any substance for two weeks, in order to ensure their health. mice were randomly divided into two groups: Control Group(=10) and Experimental Group(=10).

And then Mice released for distribution. HPLC and GC methods were used. Then the pure toxins were form the Department of Mycology, Faculty of Veterinary Medicine. Then the toxin was mixed with the diets of mice. The mixture was prepared in Karaj Nuclear Medical Research Center. The food turned into powder and was mixed with 500 mg of white toxin powder. Then, by adding some water to the food, it turned into a paste and then turned into pellet by grinder pellet mill and then, in order to dry the pellet, we put it in the oven at a temperature of 75 °C for 24 72 hours, until it turned into commercial mouse pellets and is ready for experiment.

The experimental method:
Control group received commercial mouse foods during 4 months of the project period. The Control Group was fed Fumonisin with a rate of 150 mg kg in the diet during the experiment. Mice were weighed each week. After 4 months euthanasia was performed in both groups, and necropsy was performed on each lab mouse. The blood was centrifuged and the serum was isolated and stored in a freezer at -70 degrees celcius. the spleen stored in a freezer at -70 degrees celcius, immediately after isolation in order to keep the level of cytokines.

**CYTOKINE ASSAYS**

To assay cytokines and mice serums were measured using ELISA kit (ELISA, Bi65sciences, USA). In the plate there were 96 wells each coated with a specific antibody (for each cytokine). The wells were washed and 100 ml of serum from each mouse was added and then by using antibody conjugate, cytokines were identified, and then chromogen were added to each wells and by observing colors in wells, the plates were read at 450 nm by ELISA Plate Reader and to measure specific gene expression of IL2 and IL4, the RT-PCR was used.
RESULTS
No specific behavioral changes were observed. In addition, there were no differences in weight of the experimental group compared with the control group during the whole period of time.

Table 1: The results of the studied cytokines in mice treated with Fumonisin

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL2</td>
<td>01+28/3</td>
<td>23+91/1</td>
</tr>
<tr>
<td>IL4</td>
<td>11+31/2</td>
<td>30+14/5</td>
</tr>
</tbody>
</table>

According to the T student test between treatment and control groups, significant differences in IL4 were noticed. This differences were also significant in IL2.

DISCUSSION AND CONCLUSION
In this study, two important cytokines IL2 and IL4 were tested. The result shows that the IL4 is significantly increased in the treatment group as compared to the control group. However, it shows a significant reduction in the amount of IL2.

This means that Fumonisin activated hT2 dependent cytokines and thus suppress hT2 dependent cytokines to the LI2. What is important here is that if the immune response tend to hT2 dependent reactions, natural cellular immunity reaction which play a key role in many infections, will have disorders and hence prevalence of infection is expected in these animals. On the other hand, this situation provides immediate hypersensitivity reactions, which in turn will cause certain allergy to certain allergens. For example, patients with recurrent or chronic Vulvovaginal, are sensitive to certain allergens, and in these patients, the main types of cytokine responses are IL4, IL10 and IL13, and this causes no logical immune cellular response and it activates cytokines such as FNTα and IL2 and IL12 and other important cytokines.

Therefore, extensive research in the field Fumonisin performance based on a variety of pro-inflammatory and inflammatory cytokines is needed, in order to understand comprehensively the role of this toxin on the immune system and susceptibility of animals to infection, due to toxicity by this toxin.

REFERENCES