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Impact of Fumonisin B1 on the Production of Inflammatory Cytokines by Gastric and Colon Cell Lines

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ABSTRACT

Fumonisins, a family of mycotoxins, are mainly toxic and carcinogenic. The present study was carried out to evaluate fumonisin B1 (FB1) effects on the production of inflammatory cytokines by gastric and colon cell lines.

The study was performed on two cell lines under *in vitro* condition, including gastric epithelial cell line (AGS) and human colon adenocarcinoma cell line (SW742). Lipopolysaccharide (LPS) was used for inflammatory cytokine induction. The culture medium was supplemented with 4.5–72 mg/l of FB1 for 72 h before cell induction. The supernatants were harvested 24 h after the induction and measured for cytokines by using enzyme-linked immunosorbent assay.

FB1 induced a dose-dependent increase in the production of tumor necrosis factor-α and interleukin-1β in both AGS and SW742 cell lines. This increase was statistically significant with concentration of FB1 between 9 and 72 mg/l ($P < 0.05$). FB1 also induced a dose-dependent decrease in interleukin-8 production. This decrease was seen in both cell lines and showed a statistical significance with FB1 concentration ($P < 0.05$).

The results show that FB1 increases inflammatory cytokines production by various gastric and intestinal cells. This effect in the long run can possibly be the basis for the occurrence or development of inflammation and subsequent atrophy in the above-mentioned tissues.

Keywords*:* Colon; Fumonisin B1; Gastric; IL-1β; IL-8; TNF-α

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INTRODUCTION

Fumonisins constitute a family of mycotoxins that mostly contaminate corn and other crops worldwide. Fumonisin B1 (FB1), a mycotoxin produced by *Fusarium verticillioides*, is the most abundant in this family.[1] Mycotoxin-contaminated food consumption is considered as an important health hazard for both humans and animals.[2] Following the ingestion of fumonisin-contaminated food, intestinal epithelial cells can be exposed to a high concentration of this compound.[3] The primary effect of fumonisin poisoning is ceramide synthase inhibition, which in turn leads to cellular sphingoid bases accumulation, ceramide and more complex sphingolipids depletion.[4] Frequent accumulation of free sphingoid bases can act as cancer promoters.[5]

The mechanism(s) of FB1 toxicity is complex; however, available data clearly show that FB1 can affect innate immunity as well as humoral and cellular responses.[6-8] These immunomodulatory effects of FB1 may be due to changes in various cytokines and chemokines of the immune system.[6,7,9] Immunoregulation in gastrointestinal cells has been described by the balance in the secretion of these mediators.[10, 11] Although anti-inflammatory cytokine expression such as interleukin-4 (IL-4) and interleukin-10 (IL-10) can protect intestinal or gastric tissues from inflammation, overexpression of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-8 (IL-8) may cause inflammatory responses.[12] Both TNF-α and IL-1β induce the macrophages and endothelial cells to secrete chemokines, increase the adherence of these cells to the endothelium, and subsequently, result in the recruitment of neutrophils to the site.[13] IL-8 is a potent chemoattractant of lymphocytes and neutrophils to the inflamed site, and thus has a key role in the abundant recruitment of neutrophils to the intestinal cells that are frequently observed in inflammatory bowel disease.[14] It has been reported that FB1 inhibits the proliferation of different lymphocyte subsets, decreases vaccination response in pigs, and reduces specific antibody synthesis.[7,9,15] Furthermore, it has been indicated that FB1 enhances the susceptibility to infectious diseases and affects the functions of monocytes and macrophages.[16-19] However, there is no report concerning the impact of FB1 on the production of proinflammatory cytokines by gastrointestinal cells. Therefore, this study was designed to assay the *in vitro* effects of FB1 on the lipopolysaccharide-induced production of gastrointestinal proinflammatory cytokines such as TNF-α, IL-1β, and IL-8.

MATERIALS AND METHODS

Materials

Fumonisin B1 (Product Number, F 1147) and lipopolysaccharide (LPS) were purchased from Sigma-Aldrich Chemical Company. Gastric epithelial (AGS) and human colon adenocarcinoma cell line (SW742) were obtained from National Cell Bank of Iran, Pasteur Institute. Lymphocytes as normal cells were collected from the peripheral blood of healthy individuals.

Cell Viability Assay

To assess cell viability, a tetrazolium-based colorimetric assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-2H-tetrazolium bromide [MTT] assay) was used. To perform this bioassay, each cell line in growth log phase was harvested by trypsinization and resuspended in a complete growth medium to give a total cell count of 25×10^3 cells/ml. A 100-µl cell suspension was seeded into the wells of 96-well plates (Nunc, Denmark). The plates were incubated in a humidified air atmosphere at 37°C with 5% CO_2 overnight. Then, various dilutions of FB1 in 50-µl volume were added to give a total volume of 150 µl/well with final concentrations of 0, 4.5, 9, 18, 36 or 72 mg/l FB1. Three wells containing tumor cells cultured in 150 µl of complete medium were used as controls. After 48 h of incubation, 30 µl of 2.5 mg/ml MTT solution was added to each well, and the plates were incubated for another 1 h. The culture medium was then replaced with 100 µl of dimethyl sulfoxide (DMSO), and the absorbance of each well was measured using a microplate reader in a dual wavelength mode of 570 and 640 nm. Each set of experiments was independently performed 3 times, and the percentage of viable cells was determined using an established standard curve.

Cell Culture and *in Vitro* Experiments

The cells were grown in the complete culture medium consisting of RPMI-1640 medium supplemented with 10% fetal bovine serum (Biochrom, Berlin, Germany), 2 mM L-glutamine, 100 µg/ml streptomycin, and 100 U/ml penicillin (all from Gibco,

Paisley, UK) and were maintained in serial passage in 75-cm^2 flasks. The flasks were incubated in humidified air at 37°C with 5% CO_2. Each cell line in growth log phase was harvested using a solution containing 0.02% EDTA and 0.05% trypsin in PBS, and was then resuspended in complete growth medium. The cells were seeded in the wells of 24-well plates (Nunc, Denmark) in 2 ml of complete medium to give a final density of 2×10^6 cells/well. The plates were incubated in humidified air at 37 °C with 5% CO_2. After 48 h, the culture medium of the cells was changed with a completely fresh medium supplemented with different concentration of FB1 (final concentration of FB1 was 4.5–72 mg/l). The medium was changed every other day with a complete medium containing the same concentration of FB1. After 72 h of treatment with FB1, the cells were stimulated with LPS (10 ng/ml) for 24 h. The culture supernatants were then collected and stored at −20 °C for the measurement of cytokines. Three separate experiments were performed to get the mean level of the secretion of each cytokine. Cell viability was determined using MTT assay before *in vitro* culturing. All experimental procedures were done according to the guidelines of the Animal and Human Ethical Committee of Tehran University of Medical Sciences.

Cytokine Measurements

The cell culture supernatants were analyzed for the proinflammatory cytokine content, including TNF-α, IL-1β, and IL-8, using the commercial enzyme-linked immunosorbent assay (ELISA) kits (eBioscience, Bender Med Systems GmbH, Vienna, Austia). Cytokine production was measured according to the manufacturer's instructions. All assays were identically performed, except that different coating and detector antibodies phases. The absorbance of each well was read at 450 nm as the primary wavelength (620 nm as the reference wavelength), and the cytokines concentration of the samples were calculated using a standard curve generated from a purified recombinant cytokine versus the optical density. The lower detection limit of the measured cytokines was 2 pg/ml.

Statistical Analysis

Statistical analysis between groups was performed using Mann–Whitney U test. All data were expressed as mean±SD. Statistical significance was defined as $p < 0.05$.

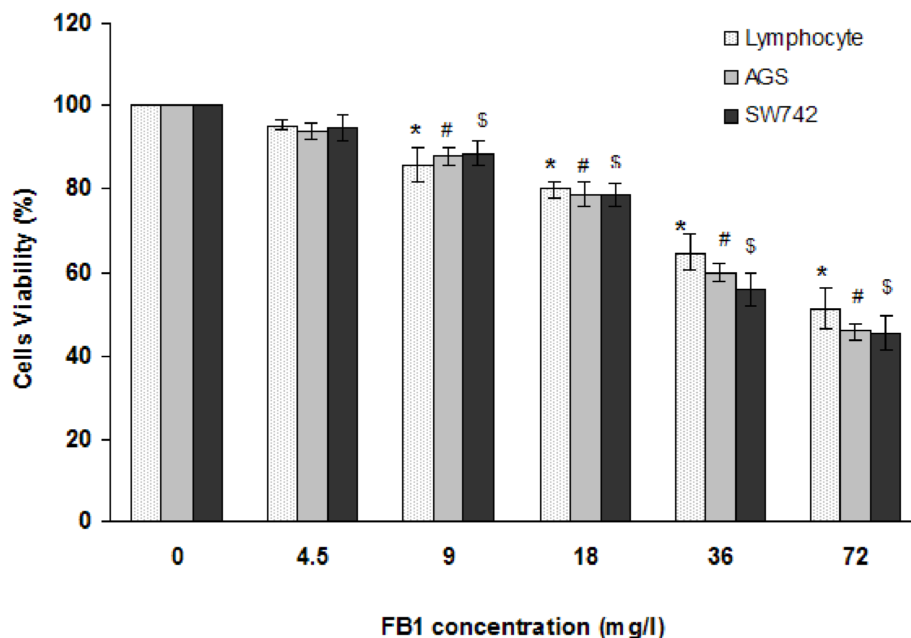

Figure 1. The effect of fumonisin B1 on the cell viability of lymphocytes, gastric and colon cell lines. All data were expressed as mean ± SD. $^{*,\#,\$}p < 0.05$ vs. Zero group. FB1, fumonisin B1; AGS, gastric epithelial cell line; SW742, colon adenocarcinoma cell line.

RESULTS

The effects of FB1 on cell viability were dose-dependent. FB1 caused a decrease in the viability of normal cells (lymphocytes), SW742 or AGS cell lines, and significantly (p<0.05) lowered the viability of these cells at 36 or 72 mg/l FB1 concentrations (Figure 1).

The cytokines profiles were greatly affected by the presence of FB1 in the culture media. Indeed, the toxin stimulated the synthesis of both TNF-α and IL-1β inflammatory cytokines by LPS in a dose-dependent manner. A serious increase in TNF-α and IL-1β concentrations was observed in LPS treated group for 72 h with 72 mg/l FB1. This increase was statistically significant with concentrations of FB1 between 36 and 72 mg/l for TNF-α in the AGS cell line (p< 0.05) and with concentrations between 4.5 and 72 mg/l in the SW742 cell line (p< 0.05, Figure 2). For IL-1β, this increase was also significant with concentrations of FB1 between 9 and 72 mg/l in the AGS cell line (p< 0.05) and with concentrations between 18 and 72 mg/l in SW742 cell line (p< 0.05, Figure 3).

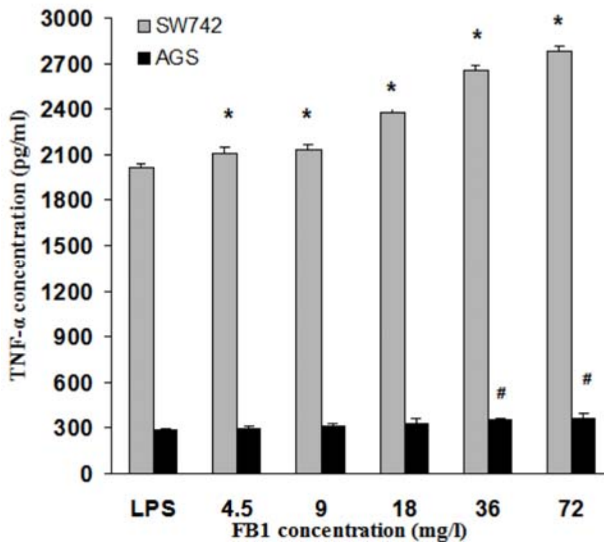

Figure 2. The effect of fumonisin B1 on the *in vitro* production of TNF-α by LPS in gastric and colon cell lines. The culture medium was supplemented with 4.5–72 mg/l of FB1 for 72 h. The culture supernatants were collected and the concentrations of tumor necrosis factor-α (TNF-α) cytokine were measured by ELISA. All the data were expressed as mean ± SD. $^{*,\#}p$ < 0.05 vs. LPS group. FB1, fumonisin B1; LPS, lipopolysaccharide; AGS, gastric epithelial cell line; SW742, colon adenocarcinoma cell line.

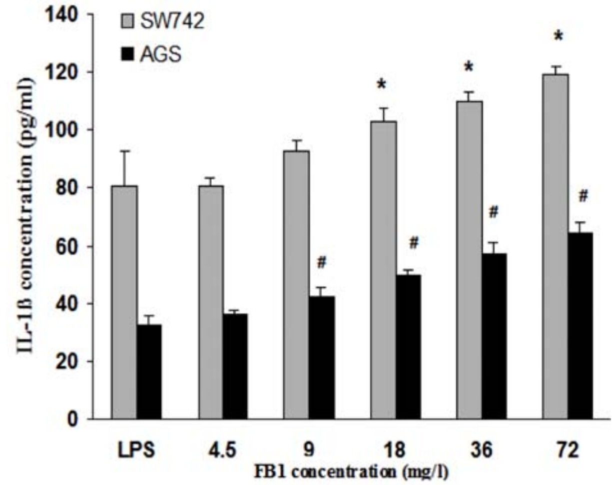

Figure 3. The effect of fumonisin FB1 on the *in vitro* production of IL-1β by LPS in gastric and colon cell lines. The culture medium was supplemented with 4.5–72 mg/l of fumonisin B1 for 72 h. The culture supernatants were collected and the concentrations of interleukin-1 beta (IL-1β) were measured by ELISA. All the data were expressed as mean ± SD. $^{*,\#}p$< 0.05 vs. LPS group. FB1, fumonisin B1; LPS, lipopolysaccharide; AGS, gastric epithelial cell line; SW742, colon adenocarcinoma cell line.

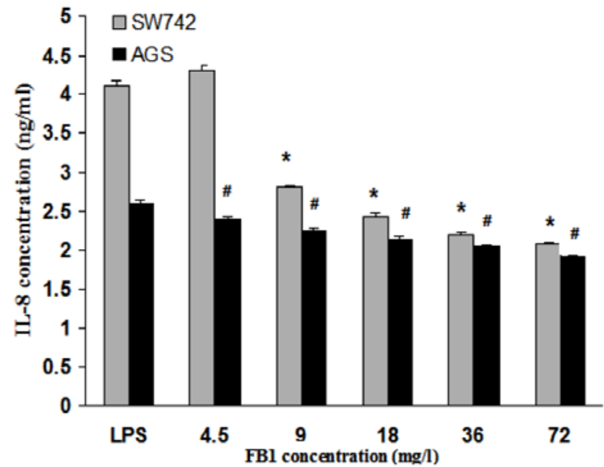

Figure 4. The effect of fumonisin B1 on the *in vitro* production of IL-8 by LPS in gastric and colon cell lines. The culture medium was supplemented with 4.5–72 mg/l of fumonisin B1 for 72 h. The culture supernatants were collected and the concentrations of interleukin-8 (IL-8) were measured by ELISA. All the data were expressed as mean ± SD. $^{*,\#}p$ < 0.05 vs. LPS group. FB1, fumonisin B1; LPS, lipopolysaccharide; AGS, gastric epithelial cell line; SW742, colon adenocarcinoma cell line.

Table 1. The effect of fumonisin B1 (72 mg/l) on the production of inflammatory cytokines in peripheral blood lymphocytes

Cytokines \ Parameters	LPS	LPS+FB1
TNF-α (pg/ml)	763 ± 40	*983 ± 45
IL-1β (pg/ml)	69 ± 14	*109 ± 6
IL-8 (pg/ml)	3326 ± 151	*2930 ± 205

All the data were expressed as mean ± SD. *$p < 0.05$ vs. LPS group. FB1, fumonisin B1; LPS, lipopolysaccharide; IL-8, interleukin-8; IL-1β, interleukin-1 beta; TNF-α, tumor necrosis factor-α

In contrast, the synthesis of IL-8 dose dependently reduced in the presence of FB1 at concentrations higher than 4.5 mg/l in the AGS cell line ($p<0.05$) and 18 mg/l in the SW742 cell line ($p<0.05$, Figure 4). However, the increase/decrease in the cytokine production in SW742 cell line was more than observed in AGS cell line.

In peripheral blood lymphocytes, FB1 increased the production of both TNF-α and IL-1β inflammatory cytokines and decreased the synthesis of IL-8 cytokine ($P < 0.05$) (Table 1).

DISCUSSION

The results of the present study showed that the FB1 can increase proinflammatory cytokine levels, such as IL-1β and TNF-α, and decrease the amount of IL-8 in the cell lines of the stomach and colon. This increase/decrease in cytokines production was concentration-dependent in such a way that we noticed a higher amount of cytokines when more concentrated FB1 was used. The results also showed that the increase/decrease in cytokine production in SW742 cell line was more than AGS cell line.

Several studies have shown the stimulating and inhibitory roles of FB1 in cytokine production with contradictory results. In some of these studies, FB1 was considered as a stimulating factor in the production of inflammatory cytokines, while in others, it was introduced as an inhibitory agent.[7,20] There are few reports on FB1 immunotoxicity. The toxicity mechanism of FB1 is complex and might involve several cellular tissues;[21] however, the existing information shows that FB1 can affect the inherent humoral and cellular immunity, and hence, it can be said that FB1 somehow plays a role in the occurrence of inflammation.[6,22] In the present study, we showed that this mycotoxin dose-dependently causes an increase in the production of TNF-α and IL-1β and also decreases IL-8 production in the cell lines of stomach and colon. Therefore, this study can underpin the probable role of FB1 in the occurrence or development of inflammation in the stomach or colon. Previous reports have shown that FB1 decreases the reproduction of lymphocytes and causes a reduction in specific antibody synthesis.[15] FB1 also decreases the immune responses by affecting immune system centers and disturbs the functions of macrophages and lymphocytes.[23] Chiba et al. (2007) reported that FB1 could increase the production of TNF-α and IL-6 by inhibiting the synthesis of ceramide.[24] Odhav et al. (2008) performed a study on lymphocytes and neutrophils obtained from the peripheral blood of healthy people and patients afflicted with breast and gastrointestinal cancers.[20] They came to a conclusion that FB1 not only decreases the number of lymphocytes and neutrophils but also inhibits the production of TNF-α and G-CSF cytokines by these cells. One can conclude that FB1 has an inhibitory effect on the human immune system, especially in patients suffering from cancer.[20]

In another study (performed both in vivo and in vitro) by Bouhet et al. (2006) on ileal samples, it was concluded that FB1 could decrease IL-8 expression at the mRNA and protein level in a dose-dependent manner.[25] Their study is in complete agreement with the present research on the effect of FB1 on the production of IL-8. Sharma et al. (2006) also showed that FB1 could increase the mRNA expression of TNF-α and IL-1α in mice peripheral blood.[26] Bhandari et al. (2002) demonstrated that subcutaneous injection of FB1 in mice could increase TNF-α and IL-1β in the tissues of kidney and liver.[27] These findings again correspond with those of the present study.

IL-8 was reported to play a key role in lymphocyte and neutrophil infiltration to the regional inflammation.[28] It seems that FB1 in the host intestine can reduce lymphocyte and polymorph cell migration to the inflammatory regions by inhibiting the synthesis of IL-8. Therefore, it would be a logical explanation for their malfunction in bacterial intestinal distortion that may lead to bacterial accumulation, intestinal inflammation, or infection. This theory was confirmed in a study conducted by Sansonetti et al. (1999) in which they showed that experimentally induced rabbit bacterial shigellosis was 3 times more in the intestinal

tissue compared to the control group when the secretion of IL-8 was omitted.[29] Studies have also shown that IL-8 can affect other immune cells such as T cells and dendritic cells, accelerate the proliferation of the intestinal epithelial cells, and interfere with the healing process of a cellular injury.[30, 31] Thus, FB1 inhibitory effects on IL-8 synthesis can disturb epithelial cell repair and normal cell structure. On the other hand, experimental studies on the role of TNF-α and IL-8 have shown that these cytokines are involved in regulating the proliferation of intestinal epithelial cells.[30] Therefore, unparalleled changes in TNF-α and IL-8, as mentioned here, on FB1 exposure of the intestinal epithelial cells can reduce or interfere with the proliferation of these cells.

As pointed out earlier, the increase/decrease in the cytokine production in the SW742 cell line was more than that in the AGS cell line. A plausible explanation for this difference in cytokine production may be the higher FB1 production in the colon and its inhibitory effects on ceramide synthesis compared to that in the gastric tissue.[4] Thus, the intestinal cells are more sensitive to FB1 than the stomach cells. A confirmatory study of this hypothesis has also been done by Kouadio et al. (2005) in which they demonstrated that Caco-2 (a human colonic cell line) was more sensitive to FB1 than other cell lines.[32] Other studies have shown that 24 h after the addition of a dose of FB1 in monkey food, much of this toxin is found in the intestinal cells.[33] On the other hand, FB1 is excreted through the bile ducts into the intestine, which exposes these cells to FB1. These findings again correspond with those of the present study.

Previous studies have shown a strong relationship between the expression of inflammatory cytokines like TNF-α and IL-1β in the epithelial cells of stomach afflicted by inflammation, atrophy, and onset of gastric cancer.[34-39] Reports have also indicated a strong correlation between inflammation of the stomach and occurrence of gullet and gastric cancer. Stomach body atrophy has also shown to be a provocateur in gastric cancer occurrence[40,41], and its inflammatory form is a risk factor for non-cardia gastric cancer.[42] The existence of chronic inflammation can lead to atrophy, metaplasia, dysplasia, and finally to gastric cancer.[43] Chronic atrophic gastritis and the resulting intestinal metaplasia are considered as the preconditions for intestinal-type gastric cancer. Infectious agents and environmental and host-dependent factors are involved in gastric cancer etiology. It has also been shown in other studies that gastric cancer in humans is caused by the atrophy of oxyntic glands, gastric acid-producing epithelial cells, and linear changes through hyperplasia and metaplasia in the gastric mucosa.[44-46]

Epidemiological studies in some parts of South Africa, Japan, and China have shown that occurrence of gullet and stomach cancers in high-risk regions compared with low-risk regions is along with high levels of FB1 in maize crops. Moreover, individual cases of gullet cancer in people consuming more maize are higher. Such a pattern has been seen in regions with a high incidence of gullet cancer, like Iran, China, southern Africa, and Japan.[47-50] In all, we may conclude that indirect consumption of fumonisin in the long run can cause inflammation and atrophy, which, in turn, may have a significant contribution in stomach and gullet cancer occurrence. Therefore, fungi and mycotoxin production control is of great importance. Supplementary and experimental studies are, however, necessary to demonstrate this claim.

CONCLUSION

The results of the present study show that FB1 increases the production of inflammatory cytokines by various gastric and intestinal cells. This effect in the long term can possibly be a basis for the occurrence or development of inflammation and consequent atrophy and cancer in the aforementioned tissues.

Conflict of Interest

The author(s) declare(s) that they have no conflict of interest to disclose.

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