Toxicity of trichothecene mycotoxin nivalenol in human leukemia cell line HL60

Hitoshi Nagashima

1National Food Research Institute, National Agriculture and Food Research Organization, 2-1-12 Kannondai, Tsukuba, Ibaraki 305-8642, Japan

Abstract

The toxicity of nivalenol (NIV) to the human promyelocyte-derived cell line HL60 is reviewed. NIV cytotoxicity was examined after 24 h treatment, and the inhibitor studies were performed. Cells treated with 3 µg/mL or higher NIV were damaged, and more than half of the cells appeared dead. Regarding cell proliferation, the value of 50% inhibitory concentration of NIV was 0.16 µg/mL. Apparent DNA ladders were observed, showing that NIV induces apoptosis. Concentrations of NIV-caused morphologic damage are in accordance with DNA fragmentation, indicating that marked NIV-caused morphologic change is due to apoptosis. NIV increased interleukin-8 (IL-8/CXCL8) secretion. Conversely, NIV decreased the secretions of other cytokines monocyte chemotactic protein-1 (MCP-1/CCL2), macrophage inflammatory protein-1α (MIP-1α/CCL3), MIP-1β/CCL4, and regulated upon activation, normal T cell expressed and presumably secreted (RANTES/CCL5) concentration-dependently. That intracellular calcium ion chelator BAPTA-AM mitigated the cytotoxicity of NIV indicates that this effect is dependent on intracellular calcium ion. The results of an intracellular calcium ion modulator ryanodine receptor (RyR)1-specific inhibitor dantrolene treatment indicates that RyR1 contributes to NIV-induced toxicity. Stress-activated mitogen-activated protein kinases (SAPKs), c-Jun N-terminal kinases (JNKs) and p38s, occupy the crucial positions in NIV-associated retardation of cell proliferation and IL-8 secretion. Transcription factor nuclear factor-κB (NF-κB) inhibitors reduced NIV's effects, indicating that NF-κB is an important factor for exerting NIV toxicity. Regarding cell proliferation, no protective effect of geldanamycin, a molecular chaperone heat shock protein 90 (Hsp90)-specific inhibitor, was observed. Alternatively, Hsp90 appears to play a role in NIV-associated changes in cytokine secretions.

Introduction

Mycotoxins are secondary metabolites of various fungi and mycotoxin contamination of foodstuffs is a problem for many countries, particularly those developing. For example, Fusarium fungi are commonly found on cereals grown in the temperate regions of the Americas, Europe, and Asia. A variety of Fusarium fungi produce a number of different mycotoxins in the trichothecene class as well as certain other mycotoxins (zearalenone and fumonisins). At present, it is known that there are more than 100 trichothecene mycotoxins, and one of them is nivalenol (NIV, Fig. 1). In Japan, NIV contamination of wheat and barley is as prevalent as that of deoxynivalenol (DON), another trichothecene mycotoxin. The chemical structure of DON differs from NIV by only one oxygen atom, and NIV and DON are considered to share many aspects of toxicity. Although the acute toxicity of NIV has been reported to be equivalent to or more potent than that of DON, the paucity of reports suggests that NIV has garnered far less interest than DON. Under these circumstances, the study of NIV toxicity deserves more attention in Japan.

Trichothecene mycotoxins are extremely toxic to rapidly dividing cells, including leukocytes, and one of the leading signs of trichothecene toxicosis is the leukopenia known as alimentary toxic aleukia. International Agency for Research on Cancer (IARC, WHO) assessed carcinogenicity and classified them as “Group 3 (Not classified as to carcinogenicity to human).”
previous result that NIV did not exert mutagenicity\(^6\) is consistent with the conclusion of IARC\(^5\). In this review, I show NIV cytotoxicity to the human promyelocyte (one of the leukocytes) -derived cell line HL60 after 24 h treatment, and the results of inhibitor studies.

**Cytotoxicity Tests**

1. **Morphology**
   Change in morphology is the most fundamental adverse effect of toxins. At 1 µg/mL (3.2 µM) NIV (Fig. 2B), only slight morphologic damage was apparent, and most of the cells looked sound. On the other hand, cells treated with 3 or 10 µg/mL NIV were evidently damaged (Fig. 2C, D), and more than half of the cells looked dead under these experimental conditions\(^7\). Using morphologic damage as a criterion, the 50 % cytotoxic concentration (CC\(_{50}\)) of NIV is positioned between 1 and 3 µg/mL.

2. **Cell Viability Tests**
   (1) **BrdU Incorporation**
   Because cell proliferation is the most essential biological phenomenon of living creatures, 5-bromo-2-deoxyuridine (BrdU) incorporation during DNA synthesis were measured to determine the rate of cell proliferation. The value of 50 % inhibitory concentration (IC\(_{50}\)) of NIV was 0.16 µg/mL (0.51 µM) (Table 1)\(^7\). This result is consistent with that of Minervini et al.\(^8\), who reported a value of 0.6 µM. In contrast, IC\(_{50}\)s reported by Thuvander et al.\(^9\) (0.24 to 0.36 µM) and Johannisson et al.\(^10\) (approximately 0.2 µM) were lower than 0.51 µM. Both of these other groups tested human lymphocytes, which might be more vulnerable to NIV than other cell types would be.

   The effects of NIV on cell proliferation in other cell lines were also investigated. Though the IC\(_{50}\) was slightly lower, the human lymphoblastic leukemia cell line MOLT-4 exhibited similar results to HL60 cells\(^11\). The rat aortic myoblast cell line A-10 showed the same trend as in the cases of HL60 and MOLT-4 cells\(^11\). The IC\(_{50}\) of NIV in the human hepatoblastoma cell line HepG2 was evidently higher than that in other three cell lines\(^11\).

   (2) **WST-8 Assay**
   Results of water-soluble tetrazolium (WST) -8 assay which measures mitochondrial succinic dehydrogenase activity indicated that the IC\(_{50}\) of NIV was 0.40 µg/ml (1.28 µM) (Table 1)\(^7\). The effect of NIV on mitochondrial succinic dehydrogenase activity has been examined using MTT assay\(^8,12\), which determines enzyme activities by the same principle as WST-8 assay, but the results of these previous studies vary. Minervini et al. reported that the IC\(_{50}\) of NIV in K562 cells was 0.5 µM\(^8\). According to Sugita-Konishi and Pestka, the IC\(_{50}\) of NIV in U937 cells was approximately 1 µg/mL (3.2 µM)\(^12\). It is likely that these discrepancies are due to differences in experimental conditions, including cell type.

   (3) **LDH Activity in Culture Media**
   Lactate dehydrogenase (LDH) is leaked to the culture media after cell death and subsequent rupture of the cell membrane. Cells in media containing either NIV or Tween 20 were cultured, and then media were subjected to LDH assay. The activities of the Tween 20-treated controls were designated to be 100 %. At 10 µg/mL NIV or lower, supernatant LDH activity was less than 50 % that in Tween 20-treated samples (CC\(_{50}\); Table 1)\(^7\). Although cells treated with 10 µg/mL NIV showed profound morphologic change (Fig. 2D), presumably the cells did not burst. Minervini et al. used trypan blue exclusion, another test that is based on breakage of cell membrane, to monitor cell viability.

---

**Table 1** Cell viability tests of HL60 cells

|          | BrdU (IC\(_{50}\)) | WST-8 (IC\(_{50}\)) | LDH (CC\(_{50}\)) (µg/mL) |
|----------|--------------------|---------------------|---------------------------|
| NIV      | 0.16 ± 0.03        | 0.40 ± 0.03         | >10                       |

BrdU: 5-bromo-2-deoxyuridine incorporation during DNA synthesis; WST-8: water-soluble tetrazolium-8 assay; LDH: lactate dehydrogenase activity in culture media; IC\(_{50}\): 50 % inhibitory concentration; CC\(_{50}\): 50 % cytotoxic concentration

---

**Fig. 1** Chemical structure of nivalenol (NIV).

**Fig. 2** Morphologic study of NIV-treated HL60 cells. A. Vehicle control. B-D. Cells treated with M(B) 1 µg/ml, (C) 3 µg/ml, or (D) 10 µg/ml NIV for 24 h.
They found that 80% of cells were viable even at 25.0 µg/mL NIV—a finding that is consistent with these results regarding cytoplasmic LDH activity.

3. Apoptosis

When cells are exposed to certain chemicals, they commit suicide (apoptosis) by activating an innate intracellular death program. Internucleosomal DNA fragmentation (DNA ladder) is one of the most common hallmarks of apoptosis, therefore, NIV-induced DNA fragmentation was investigated (Fig. 3). At 3 and 10 µg/mL NIV, apparent DNA ladders were observed (Fig. 3; lanes 5, 6), showing that NIV induces apoptosis. Faint DNA ladder was observed at 1 µg/mL (Fig. 3; lane 4). Concentrations of NIV-caused morphologic damage (Fig. 2; marked at 3 and 10 µg/mL NIV) are in accordance with DNA fragmentation (Fig. 3), indicating that marked NIV-caused morphologic change is due to apoptosis. Ueno et al. reported that DNA fragmentation was observed at 0.01 µg/mL NIV in HL60 cells, however, because of some differences in experimental conditions, we did not detect DNA fragmentation even at 0.3 µg/mL (Fig. 3; lane 3).

Cytokine Secretion

Cytokines are proteins, each of which exerts wide-ranging immune and inflammatory responses, particularly the proinflammatory cytokines are crucial to the development of diverse pathologic phenomena. Therefore, to illustrate the mechanism underlying the toxicity, NIV-caused changes in the secretions of cytokines interleukin-8 (IL-8/CXCL8), monocyte chemotactic protein-1 (MCP-1/CCL2), macrophage inflammatory protein-1α (MIP-1α/CCL3), MIP-1β/CCL4, and regulated upon activation, normal T cell expressed and presumably secreted (RANTES/CCL5) were investigated.

1. IL-8

Cells were cultured in the media containing various concentrations of NIV, and then the levels of IL-8 in the culture media were quantified. NIV increased IL-8 secretion (Table 2). The values from samples treated with 3 or 10 µg/mL NIV were smaller than that from cells exposed to 1 µg/mL, because of the damage to the cells (Fig. 2C, D). Sugita-Konishi and Pestka also found that NIV induced IL-8 secretion, consistent with the possibility that IL-8 is responsible for NIV-induced pathologic phenomena.

2. MCP-1

Next, MCP-1, a proinflammatory cytokine, was addressed. NIV clearly decreased MCP-1 secretion (Table 2) in a concentration-dependent manner. Since the values of IL-8 secretion from samples treated with NIV were greater than that from cells exposed to vehicle (Table 2), reduction of MCP-1 secretion is not accounted for the damage to the cells. Kinser et al. reported that DON up-regulated the MCP-1 mRNA in mouse spleen cells, however, they did not document the secretion of MCP-1.

![Fig. 3 DNA ladders from NIV-treated HL60 cells. Lane 1 contains molecular weight marker (100 bp DNA ladder). Lane 2 corresponds to vehicle-treated negative control. Lanes 3-6 correspond to HL60 cells treated with 0.3, 1, 3, and 10 µg/ml NIV for 24 h, respectively.](image)

| Table 2 | The effects of NIV on cytokine secretion |
|---------|----------------------------------------|
| NIV     | 0 µg/mL | 0.3 µg/mL | 1 µg/mL | 3 µg/mL | 10 µg/mL |
| IL-8    | 100 ± 18.4 | 184.3 ± 13.3* | 946.1 ± 38.9* | 391.9 ± 55.7* | 126.8 ± 8.1 |
| MCP-1   | 100 ± 0.6 | 74.9 ± 1.6* | 69.8 ± 0.5* | 64.8 ± 0.3* | 63.9 ± 1.9* |
| MIP-1α  | 100 ± 8.7 | 78.0 ± 6.6* | 63.6 ± 5.7* | 40.1 ± 1.2* | 39.8 ± 3.9* |
| MIP-1β  | 100 ± 7.5 | 87.7 ± 2.3* | 54.1 ± 5.6* | 17.7 ± 0.2* | 15.9 ± 0.5* |
| RANTES  | 100 ± 6.9 | 55.4 ± 2.0* | 51.5 ± 0.3* | 58.5 ± 0.7* | 58.0 ± 0.3* |

Vehicle-treated controls were designated as 100 %. *P < 0.05 versus control (0 µg/mL). IL-8: Interleukin-8, MCP-1: Monocyte Chemotactic Protein-1, MIP-1: Macrophage Inflammatory Protein-1, RANTES: Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted.
3. MIP-1α, MIP-1β, and RANTES

Trichothecene mycotoxins induce leukopenia, presumably due to, in part, the inhibition of hematopoiesis. NIV's effects on the secretions of anti-hematopoietic cytokines MIP-1α and MIP-1β were addressed. Since these cytokines bind to cysteine–cysteine motif chemokine receptor 5, and RANTES is known to exert its biological activities via the identical receptor, NIV-induced effect on RANTES secretion were also documented. Treatment with NIV tapered MIP-1α secretion in a concentration-dependent manner (Table 2). In comparison, low concentrations of NIV had modest inhibitory effects on MIP-1β secretion, whereas high concentrations considerably inhibited (Table 2). Because treatment with 10 µg/mL NIV was shown to cause widespread damage to HL60 cells (Fig. 2D), NIV concentrations exceeding 10 µg/mL are unlikely to induce the secretion of cytokines, indicating that NIV does not appear to induce either MIP-1α or MIP-1β secretions. DON induces the secretions of these cytokines, indicating that the mechanisms underlying the toxicities of DON and NIV differ. Although NIV treatment lowered RANTES secretion, the effect was moderate compared with the effects on MIP-1α and MIP-1β (Table 2).

Inhibitor Studies

1. Intracellular Calcium Ion

Many extracellular signals are known to increase intracellular calcium ion concentration, therefore, the effects of the intracellular calcium ion chelator 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid tetraacetoxymethyl ester (BAPTA-AM) on NIV toxicity were investigated. BrdU incorporation was chosen for this purpose because this test was the most sensitive measure of cell viability (Fig. 2, Table 1). That BAPTA-AM decreased endozepine triakontatetraneuropeptide and histamine-induced IL-8 secretion, respectively, indicates that this case is not an exceptional phenomenon. Since intracellular calcium ion contributes to the NIV cytotoxicity, and extracellular calcium ion chelator EGTA did not affect NIV-caused retardation of cell proliferation (unpublished data), the hypothesis that calcium ion is discharged from the intracellular calcium ion stores into the cytoplasm by the action of NIV was tested. Ryanodine receptors (RyRs) are localized in the endoplasmic reticulum (ER) membrane and are specialized intracellular calcium ion channels for the rapid and extensive release of calcium ion. Upon external stimulation, RyRs mediate the release of calcium ion from the ER calcium store to the cytoplasm. The effect of an RyR1-specific inhibitor dantrolene on cell proliferation was investigated. Our results (Table 3) suggest that RyR1 may play a role in the NIV-associated retardation of cell proliferation. It is reported that dantrolene antagonized morphine-caused inhibition of cell proliferation.

It is likely that RyR1 is crucial for NIV-induced IL-8 secretion (Table 3). Goyal et al. reported that dantrolene inhibited IL-8 secretion induced by an adhesion produced by enterogreggative Escherichia coli. Since these results are consistent with other reports, NIV might share signal transduction pathway(s) with these proteins. It appears that dantrolene alleviates the effect of NIV on MCP-1 secretion (Table 3).

2. SAPKs

Mitogen-activated protein (MAP) kinases have drawn the attention of scientists, because they are important signal-transducing enzymes that are involved in numerous facets of cellular regulation. In mammals, four groups of MAP kinases have been identified. Two of these (c-Jun N-terminal kinases (JNKs) and p38s) are categorized as stress-activated MAP kinases (SAPKs). In response to external stressors, SAPKs are converted into their active, phosphorylated forms and mediate numerous biological phenomena. Firstly, phosphorylated SAPKs after NIV treatment were quantified. NIV increased the quantities of active forms of SAPKs; the quantities peaked at a NIV concentration of around 1 µg/mL (Fig. 4). These results led to hypothesize that NIV exerts its toxicity through SAPK signal-transduction pathways.

Table 3 Summary of inhibitor studies

|             | ICI       | RyR1     | JNK       | p38       | NF-κB     | Hsp90     |
|-------------|-----------|----------|-----------|-----------|-----------|-----------|
|             | BAPTA-AM  | Dantrolene| SP600125  | SB203580  | Dexamethasone| PDTC     | Geldanamycin|
| Cell Proliferation | o        | o        | o         | o         | o         | o         | -         |
| IL-8        | o         | o        | o         | o         | o         | -         | x         |
| MCP-1       | -         | o        | -         | o         | o         | o         | o         |

ICI: Intracellular Calcium Ion, RyR1: Ryanodine Receptor 1, JNK: c-Jun N-Terminal Kinase, NF-κB: Nuclear Factor-κB, Hsp90: 90-kDa Heat Shock Protein, O: Inhibited the effect of NIV, x: Not Inhibited, -: Not Determined.
The effects of the JNK-specific inhibitor SP600125 and the p38-specific inhibitor SB203580 were investigated. JNKs occupy one of the important positions in NIV-associated retardation of cell proliferation (Table 3)\(^\text{24}\). SB203580 attenuated the effect of NIV. This result indicates that p38s also play considerable roles in NIV-associated retardation of cell proliferation (Table 3)\(^\text{24}\).

JNKs appear important for NIV-induced IL-8 secretion (Table 3)\(^\text{24}\). It is conceivable that p38s contribute to NIV-induced IL-8 secretion (Table 3)\(^\text{24}\). The involvement of SAPKs in the signal transduction pathway associated with the toxicity of DON has been reported\(^\text{25,26}\). According to Pestka et al., DON elicits IL-8 secretion in Jurkat human T cells, but NIV does not\(^\text{27}\). Although NIV and DON are structurally very much alike, these results indicate that NIV toxicity is different from DON toxicity. As reported by Hobbie et al.\(^\text{27}\) and Nagashima et al.\(^\text{28}\), it is common that SAPKs contribute to IL-8 secretion. Islam et al. reported that DON-elicited IL-8 secretion is p38-dependent but not JNK-dependent in human U937 monocyte cells and human primary blood mononuclear cells\(^\text{27}\). The discrepancy of the results suggests that NIV and DON induce IL-8 secretion differently.

3. NF-κB

Nuclear factor-κB (NF-κB) was originally detected as a transcription-enhancing complex governing the immunoglobulin light chain gene enhancer. In unstimulated cells, NF-κB complexes are retained in cytoplasmic form through binding to an inhibitor protein (IκB). A diverse spectrum of stimuli can liberate NF-κB-IκB complexes for nuclear translocation and activate this transcription factor that is responsible for diverse biological phenomena. An NF-κB inhibitor dexamethasone abated the inhibitory effect of NIV on cell proliferation (Table 3)\(^\text{29}\). The results that dexamethasone mitigated the effect of NIV indicate that NF-κB is important for NIV-caused changes in cytokine secretion (Table 3)\(^\text{29}\). To validate the above results, the effects of another NF-κB inhibitor, pyrrolidinedithiocarbamate (PDTC) were investigated. PDTC substantially alleviated the effect of NIV on IL-8 and MCP-1 secretion (Table 3)\(^\text{29}\).

Copious groups have reported that stimuli-induced IL-8 gene expression is NF-κB dependent\(^\text{30,31}\). Gray and Pestka showed that DON-induced IL-8 gene expression is regulated by NF-κB in human monocytes\(^\text{32}\). Furthermore, PDTC lessened DON-induced IL-8 secretion drastically in human intestinal epithelium\(^\text{33}\).

4. Hsp90

The 90-kDa heat shock protein (Hsp90), originally identified as a heat- and stress-induced protein, is a molecular chaperone involved in the folding, stabilization, activation, and assembly of its client proteins. Numerous client signaling proteins in a wide range of biological processes have been found to be regulated by Hsp90\(^\text{34}\).

The effect of the Hsp90-specific inhibitor geldanamycin was documented. With regard to cell proliferation, since no protective effect of geldanamycin was observed (Table 3)\(^\text{35}\), it is unclear whether Hsp90 is concerned with NIV-caused retardation of cell proliferation. NIV-caused retardation of cell proliferation was antagonized by an NF-κB inhibitor\(^\text{29}\). Since geldanamycin impairs NF-κB activity\(^\text{36,37}\), geldanamycin was anticipated to alleviate NIV-caused retardation of cell proliferation, but did not (Table 3). Other than NF-κB, geldanamycin down-regulates cell proliferation-promoting cellular factors such as c-Src\(^\text{38}\) and c-Raf\(^\text{39}\) mediated by Hsp90 inactivation. Above results indicate that with respect to the NIV-caused retardation of cell proliferation, the effects of inactivation of these crucial factors would be more potent than the protective effect of NF-κB inhibition.

It appears that Hsp90 plays a role in NIV-associated changes in cytokine secretion (Table 3)\(^\text{35}\). Malhotra et al. and Teruya et al. reported that geldanamycin abated tumor necrosis factor-α\(^\text{40}\) and Legionella pneumophila\(^\text{37}\) -induced IL-8 secretion, respectively. In these reports, the authors indicated that the inhibition of IL-8 secretion was achieved via Hsp90-mediated NF-κB inactivation\(^\text{36,37}\). Since NF-κB specific inhibitors decrease NIV-induced IL-8 secretion\(^\text{39}\), geldanamycin may exert its effect through NF-κB inactivation. Because copious client proteins are known to be regulated by Hsp90\(^\text{34}\), further studies are required to identify which Hsp90 client protein(s) contribute to NIV-caused changes in cytokine secretion in order to elucidate the detailed mechanism of NIV-induced cytotoxicity. The effects of radicicol, another Hsp90-specific inhibitor, on cytokine secretion were investigated. The results showed that Hsp90 is unlikely to be involved in the NIV-caused reduction in MIP-1α and MIP-1β secretions\(^\text{40}\). It is suggested the presence of at least two signal transduction pathways to regulate cytokine secretion. In other words, Hsp90-dependent (IL-8 and MCP-1) and -independent (MIP-1α and MIP-1β) pathways\(^\text{35,40}\). Further studies are required to identify which factor(s) is the pivotal trigger point to elucidate

**Fig. 4** NIV triggers phosphorylation of SAPKs. HL60 cells were treated with NIV at the concentrations indicated for 24 h. Western blots of whole-cell proteins were reacted with (A) anti-phospho-JNK and (B) anti-phospho-p38 antibodies.
detailed difference(s) in the mechanisms of NIV-caused toxicities.

References

1) Creppy, E. E.: Update of survey, regulation and toxic effects of mycotoxins in Europe. Toxicol Lett, 127, 19-28 (2002)

2) Nakajima, T., Yoshida, M.: Mycotoxin productivity and virulence of Fusarium graminearum species complex causing Fusarium head blight on wheat and barley in the western part of Japan. Jpn J Phytopathol, 73, 106-111 (2007)

3) Yoshizawa, T.: Thirty-five years of research on deoxynivalenol, a trichothece mycotoxicin: with special reference to its discovery and co-occurrence with nivalenol in Japan. Food Safety, 1, 12-31 (2013)

4) Joffe, A. Z.: "Microbial Toxins VII" (eds. Kadis, S. Ciegler, A., Ajl, S.J.), pp.139-189 (1971) Academic Press Inc, New York, USA

5) IARC: IARC monographs on the evaluation of carcinogenic risks to humans, Volume 56, pp.445-466 (1993), IARC, Lyon, France

6) Nagashima, H., Nakagawa, H., Iwashita, K.: "Animal Cell Technology: Basic & Applied Aspects, Volume 15" (eds. Ikura, K., Nakao, M., Ichikawa, A., Teruya, K., Shirahata, S.), pp.301-306 (2009), Springer-Verlag GmbH, Berlin, Germany

7) Nagashima, H., Nakagawa, H., Iwashita, K.: Cytotoxic effects of nivalenol on HL60 cells. Mycotoxins, 56, 65-70 (2006)

8) Minervini, F., Fornelli, F., Flynn, K.M.: Toxicity and apoptosis induced by the mycotoxins nivalenol, deoxynivalenol and fumonisin B1 in a human erythroleukemia cell line. Toxicol in Vitro, 18, 21-28 (2004)

9) Thuander, A., Wikman, C., Gadhasson, I.: In vitro exposure of human lymphocytes to trichotheccenes: Individual variation in sensitivity and effects of combined exposure on lymphocyte function. Food Chem Toxicol, 37, 639-648 (1999)

10) Johannisson, A., Bjökhag, B., Hansson, W., Gadhasson, I.L., Thuander, A.: Effects of four trichotheccene mycotoxins on activation marker expression and cell proliferation of human lymphocytes in culture. Cell Biol Toxicol, 15, 203-215 (1999)

11) Nagashima, H., Kushiro, M., Nakagawa, H., Iwashita, K.: Comparison of antiproliferative effects of trichotheccene mycotoxins, nivalenol and deoxynivalenol, in cultured cells. Rep Nat Food Res Inst, 76, 29-32 (2012)

12) Sugita-Konishi, Y., Pestka, J.J.: Differential upregulation of TNF-α, IL-6, and IL-8 production by deoxynivalenol (vomitoxin) and other 8-ketotrichothecenes in a human macrophage model. J Toxicol Environ Health A, 64, 619-636 (2001)

13) Ueno, Y., Umemori, K., Niimi, E., Tanuma, S., Nagata, S., Sugamata, M., Ihara, T., Sekijima, M., Kawai, K., Ueno, I., Tashiro, F.: Induction of apoptosis by T-2 toxin and other natural toxins in HL-60 human promyelocytic leukemia cells. Nat Toxins, 3, 129-137 (1995)

14) Nagashima, H., Nakagawa, H., Kushiro, M., Iwashita, K.: The in vitro approach to the cytotoxicity of a trichothece mycotoxin nivalenol. Jpn Agr Res Quart, 43, 7-11 (2009)

15) Kinser, S., Jia, Q., Li, M., Laughter, A., Cornwell, P., Corton, J.C., Pestka, J.J.: Gene expression profiling in spleens of deoxynivalenol-exposed mice: immediate early genes as primary targets. J Toxicol Environ Health A, 67, 1423-1441 (2004)

16) Graham, G.J., Zhou, L., Weatherbee, J.A., Tsang, M.L., Napolitano, M., Leonard, W.J., Pragnell, I.B.: Characterization of a receptor for macrophage inflammatory protein 1α and related proteins on human and murine cells. Cell Growth Differ, 4, 137-146 (1993)

17) Nagashima, H., Nakagawa, H., Kushiro, M.: Opposite effects of two trichothece mycotoxins, deoxynivalenol and nivalenol, on the levels of macrophage inflammatory protein (MIP)-1α and MIP-1β in HL60 cells. Environ Toxicol Pharmacol, 34, 1014-1017 (2012)

18) Marino, F., Cosentino, M., Fietta, A.M., Ferrari, M., Cattaneo, S., Frigo, G., Leccini, S., Frigo, G.M.: interleukin-8 production induced by the endoepine triakontatetra-neuropeptide in human neutrophils: role of calcium and pharmacological investigation of signal transduction pathway, Cell Signal, 15, 511-517 (2003)

19) Matsubara, M., Tamura, T., Ohmori, K., Hasegawa, K.: Histamine H1 receptor antagonist blocks histamine-induced proinflammatory cytokine production through inhibition of Ca2+-dependent protein kinase C, Raf/MEK/ERK and IKK/IKβ/NF-κB signal cascades. Biochem Pharmacol, 69, 433-449 (2005)

20) Petegon, F.V.: Ryanodine receptor: Structure and function. J Biol Chem, 287, 31324-31632 (2012)

21) Nagashima, H., Nakagawa, H., Kushiro, M.: Ryanodine receptor inhibitor dantrolene alleviates nivalenol-induced cytotoxicity in HL60 cells. Rep Nat Food Res Inst, 74, 1-5 (2010)

22) Hauser, K. F., Stiene-Martin, A., Mattson, M. P., Elde, R. P., Ryan, S. E., Godleske, C.C.: µ-opioid receptor-induced Ca2+ mobilization and astroglial development: MORphine inhibits DNA synthesis and stimulates cellular hypertrophy through a Ca2+-dependent mechanism, Brain Res, 720, 191-203 (1996)

23) Goyal, A., Bhattacharyya, S., Majumdar, S., Narang, A., Ghosh, S.: Cellular response induced by a galactose-specific adhesin of enterogaegregative Escherichia coli in INT-407 cells, FEMS Immunol Med Microbiol, 55, 378-387 (2009)

24) Nagashima, H., Nakagawa, H., Kushiro, M., Iwashita, K.: Contribution of stress-activated MAP kinases to nivalenol-caused cytotoxicity and interleukin-8 secretion in HL60 cells. Mycotoxins, 59, 67-73 (2009)

25) Pestka, J.J., Uzarski, R.L., Islam, Z.: Induction of apoptosis and cytokine production in the Jurkat human T cells by deoxynivalenol: role of mitogen-activated protein kinases and comparison to other 8-ketotrichothecenes. Toxicology, 206, 207-219 (2005)

26) Islam, Z., Gray, J.S., Pestka, J.J.: p38 Mitogen-activated protein kinase mediates IL-8 induction by the ribotoxin deoxynivalenol in human monocytes. Toxicol Appl Pharmacol, 213, 235-244 (2006)

27) Hobbie, S., Chen, L.M., Davis, R.J., Galán, J.E.: Involvement of mitogen-activated protein kinase pathways in the nuclear responses and cytokine production induced by Salmonella typhimurium in cultured intestinal epithelial cells. J Immunol, 159, 5550-5559 (1997)

28) Nagashima, H., Nakamura, K., Goto, T.: Stress-activated MAP kinases regulate rubratoxin B-caused cytotoxicity and cytokine secretion in hepatocyte-derived HepG2 cells. Toxicol Lett, 155, 259-267 (2005)
29) Nagashima, H., Kushiro, M., Nakagawa, H.: Nuclear factor-κB inhibitors alleviate nivalenol-induced cytotoxicity in HL60 cells. Environ Toxicol Pharmacol, 31, 258-261 (2011)

30) Mukaida, N., Morita, M., Ishikawa, Y., Rice, N., Okamoto, S., Kasahara, T., Matsushima, K.: Novel mechanism of glucocorticoid-mediated gene expression. Nuclear factor-κB is target for glucocorticoid-mediated interleukin 8 gene expression. Proc Natl Acad Sci USA, 269, 13289-13295 (1994)

31) Muñoz, C., Pascual-Salcedo, D., Castellanos, M.C., Alfranca, A., Aragonés, J., Vara, A., Redondo, J.M., de Landázuri, M.O.: Pyrrolidine dithiocarbamate inhibits the production of interleukin-6, interleukin-8, and granulocyte-macrophage colony-stimulating factor by human endothelial cells in response to inflammatory mediators: Modulation of NF-κB and AP-1 transcription factors activity. Blood, 88, 3482-3490 (1996)

32) Gray, J.S., Pestka, JJ.: Transcriptional regulation of deoxynivalenol-induced IL-8 expression in human monocytes. Toxicol Sci, 99, 502-511 (2007)

33) Maresca, M., Yahi, N., Younès-Sakr, L., Boyrom, M., Caporiccio, B., Fantini, J.: Both direct and indirect effects account for the pro-inflammatory activity of enteropathogenic mycotoxins on the human intestinal epithelium: Stimulation of interleukin-8 secretion, potentiation of interleukin-1β effect and increase in the transepithelial passage of commensal bacteria. Toxicol Appl Pharmacol, 228, 84-92 (2008)

34) Li, J., Buchner, J.: Structure, function and regulation of the Hsp90 machinery. Biomed J, 36, 106-117 (2013)

35) Nagashima, H., Nakagawa, H., Kushiro, M.: Geldanamycin, an inhibitor of heat shock protein 90, mitigates nivalenol-caused changes in cytokine secretion in HL60 cells. Jpn Agr Res Quart, 45, 441-444 (2011)

36) Malhotra, V., Shanley, T.P., Pittet, J.F., Welch, W.J., Wong, H.R.: Geldanamycin inhibits NF-κB activation and interleukin-8 gene expression in cultured human respiratory epithelium. Am J Respir Cell Mol Biol, 25, 92-97 (2001)

37) Teruya, H., Higa, F., Akamine, M., Ishikawa, C., Okudaira, T., Tomimori, K., Mukaida, N., Tateyama, M., Heuner, K., Fujita, J., Mori, N.: Mechanisms of Legionella pneumophila-induced interleukin-8 expression in human lung epithelial cells. BMC Microbiology, 7, 102 (2007)

38) Nagashima, H., Matsumura, F.: 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)-induced down-regulation of glucose transporting activities in mouse 3T3-L1 preadipocyte. J Environ Sci Health, B37, 1-14 (2002)

39) Schulte, T. W., Blagosklonny, M.V., Romanova, L., Mushinski, J.F., Monia, B.P., Johnston, J.F., Nguyen, P., Trepel, J., Neckers, L.M.: Destabilization of Raf-1 by geldanamycin leads to disruption of the Raf-1-MEK-mitogen-activated protein kinase signaling pathway. Mol Cell Biol, 16, 5839-5845 (1996)

40) Nagashima, H., Nakagawa, H.: Differences in the toxicities of trichothecene mycotoxins, deoxynivalenol and nivalenol, in cultured cells. Jpn Agr Res Quart, 48, 393-397 (2014)