Introduction

Trichothecene mycotoxins are produced by *Fusarium* species, especially *F. graminearum* and *F. culmorum*, which are known to be pathogenic fungi of wheat, rye, barley, corn and other cereal grains. The toxins are subdivided into four groups referred to as types A-D based on their structure. Type A (T-2 toxin, HT-2 toxin) and type B (deoxynivalenol (DON), nivalenol (NIV)) are considered, in the field of food safety, as important causative compounds of food poisoning. These toxins can be acutely toxic, leading to a refusal to feed or vomiting, and can cause chronic effects such as an increase in susceptibility to infectious diseases or hormone imbalance.

It is difficult to prevent the contamination of commodities with mycotoxins because their occurrence is affected by geographic and climatic factors. To reduce the level of exposure to these toxins, we have to evaluate their toxicity and to define regulatory levels in food such that they are unlikely to become a health concern.

In this paper, I introduce my research on toxicity and control of trichothecene mycotoxins, especially DON.

Effect of trichotecene mycotoxins on host resistance to infectious diseases

The trichothecenes have been reported to be potent immunosuppressive compounds and demonstrated to decrease host resistance to infectious diseases.

There are two major forms of resistance to bacterial infectious diseases. One is a non-specific
defense, in which neutrophils, NK cells and resident macrophages play an important role in the elimination of bacteria from infectious foci\(^5\). These cells are essential in the early stages of infection. The other is specific, in which humoral immunity and cellular immunity based on T cell-B cell interactions play an important role in defense in the late stages of infection\(^6-7\). It is generally accepted that non-specific defense is important for non-pathogenic bacterial infections and specific defense for pathogenic infections.

As for specific defense against pathogenic infections such as salmonellosis and listeriosis, some kinds of trichothecenes have been reported to decrease survival time\(^8\). However, in all these reports, animals were exposed to high doses of trichothecenes. The effect of lower dose exposure to trichothecenes on host resistance against bacterial infectious diseases is still unknown. Since there is no information for non-specific defense, we examined the effect of lower dose exposure to trichothecenes (DON, NIV, T-2 toxin, diacetoxyscirpenol (DAS) and fusarenone X (FX)) on host resistance to pathogenic and non-pathogenic infections.

As mice refused to drink more than 3 mg/kg feed of DON, FX and DAS, they were given 3 mg/kg feed of these trichothecene derivatives at a highest dose. NIV, T-2 toxin were given at 6 mg/kg feed as a highest dose. Mice in all treatment groups were given 2 mg/kg feed and 0.2 mg/kg feed as a moderate and a lowest dose, respectively. The survival ratio of each group given drinking water containing various concentrations of trichothecene derivatives was compared with that of the control group given toxin-free water. DON decreased the survival of salmonellosis when administered at 2 mg/kg feed. No change in resistance was seen in the groups given NIV and FX, even at a highest dose. The lowest dose treatment of T-2 enhanced host resistance against salmonella but a lowest dose of DAS reduced it. T-2 and DAS had no effect when given in a moderate or highest dose. The reason why the lowest dose affected the resistance against salmonella is unknown. These results indicated that dietary exposure to DON had the most likely chance of increasing the susceptibility to salmonellosis among five popular trichothecene derivatives.

Based on the results of host resistance to pathogenic infection, we examined the effect of DON and NIV on host resistance to non-pathogenic infection using a dose of 2 mg/kg. Exposure to DON or NIV did not affect the elimination of non-pathogenic bacteria from the peritoneal cavities and spleen. These results suggest that the relatively lower dose of DON and NIV do not affect the host resistance to non-pathogenic infection.

Taken together, these in \textit{vivo} findings have significance in the evaluation of the potential hazards associated with human exposure to these mycotoxins. Although DON is the predominant contaminant in foods world-wide, it is often accompanied by other 8-ketotrichothecenes, including FX, NIV, 15-AcDON, and 3-AcDON. The capacity for host resistance of these latter compounds in the human macrophage model described herein suggests that their presence in food poses a risk similar to that found for DON\(^9\).

**Toxicity of NIV**

NIV is considered to be an important trichothecene mycotoxin produced by \textit{Fusarium} species because of its frequent co-contamination in wheat and barley with DON in Japan\(^10\). However, there
have been scarce data regarding the toxicity profile of this toxin. We studied the subchronic toxicity of NIV in male and female F344 rats by exposure through diets at doses of 0, 6.25, 25, and 100 mg/kg feed for 90 days). Since immunotoxicity and metabolism are the special concerns of NIV, immune function and activities of detoxifying enzymes in the liver were also examined using male rats). During the experimental period, suppression of body weight gain as well as loose stool was observed at 100 mg/kg feed from the 1st week until the end of the experiment in both sexes. Suppression of body weight gain was also observed at 25 mg/kg feed from week 6 in males and at week 4 in females. At necropsy, an increase of relative testicular weight and a decrease of relative thymus weight in females were detected at 100 mg/kg feed. Hematologically, a decrease in white blood cell counts was noted at 100 mg/kg feed in males and from 6.25 mg/kg feed in females. In addition, a decrease of platelet counts in both sexes, a decrease of red blood cell counts in males, and a decrease of hemoglobin in female occurred at 100 mg/kg feed. Histopathologically, treatment-related changes were predominantly observed in the hematopoietic and immune systems in both sexes and in the female reproductive system at 100 mg/kg feed, such as atrophy of the thymus, reduction of hematopoietic cells in the bone marrow, atrophy of the uterine corpus, thinning of the vaginal mucosa (diestrus), increase of the ovarian atretic follicles, and increase of castration cells in the anterior pituitary. Examination of serum immunoglobulin levels revealed a slight increase of IgM at 100 mg/kg feed, while IgM at lower doses and IgG or IgA at any dose did not fluctuate. Flow cytometric analysis of splenic cells revealed increases of the B-cell population from 25 mg/kg feed and a decrease of the T-cell population at 100 mg/kg feed associated with an elevation in the ratio of helper/cytotoxic T lymphocytes at 100 mg/kg feed. On the other hand, an increase of splenic NK activity against Yac-1 cells was observed in all treatment groups, while the level at 100 mg/kg feed was lower than that observed at 25 mg/kg feed. At 100 mg/kg, a reduction of the NKR-P1A+ splenic cell counts was apparent. With regard to the activities of detoxifying enzymes, i.e., cytochrome P450 3A2 and 1A2, and glutathione-S-transferase isozymes, in the liver, NIV did not induce any dose-related change. Furthermore, the de-epoxy form of NIV, a bacterially metabolized NIV, could not be detected in the feces or sera of administered animals. Taken together, NIV targets the female reproductive system as well as hematopoietic and immune systems in rats after 90-day exposure through the diet. With regard to immunotoxicity, an effect was apparent at 25 mg/kg feed judging from an increase of the splenic B-cell population, while an increase of NK activity was apparent from 6.25 mg/kg feed as a signature of beneficial host-defense responses. NIV-induced toxicity was not accompanied with a xenobiotic reaction in the liver nor by enterobacterial metabolism. Based on hematological data, the low level of observed effect of NIV was determined to be 6.25 mg/kg feed (0.4 mg/kg body weight/day).

Setting provisional standard for DON in unpolished wheat

Among these toxins, DON, T-2 and HT-2 toxins were evaluated at the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2001. For DON, the provisional maximum tolerable daily intake (PMTDI) was established as 1 µg/kg of body weight per day. With the determination of PMTDI for DON, the Ministry of Health, Labor and Welfare of Japan (MHLW) has conducted
surveillance of DON in wheat. Surveillance results revealed that the average DON concentration in imported wheat (n=178) and domestic wheat (n=199) was 60 ng/g and 160 ng/g, respectively. Taking into consideration the weighted average of the supply flow, the average level of contamination was calculated as 71 ng/kg. In domestic rice (n=124), the average was 2.6 ng/g. As DON levels in rice were low, their contribution to the extent of exposure to DON can be neglected. Based on the results, MHLW recommended a level of 1.1 mg/kg in unpolished wheat as a provisional standard in 2002.

The rationale of the provisional standard was followings. By assuming that the reduction rate of DON is 50 % from unpolished wheat to the final product, the consumption of wheat is 89.8 g/person/day as an average of all Japanese, the average body weight is 50 kg, the allowance limit of DON contamination level in unpolished wheat so as not to exceed 1 µg/kg of body weight/day in Japan was estimated at 1.1 mg/kg. At the same time, to establish the analytical method for determination of DON in unpolished wheat, an HPLC analytical method coupled to a multifunctional clean-up column has been validated in a collaborative study using naturally contaminated wheat and spiked wheat by 12 laboratories. From the result of this collaborative study, the relative standard deviations for repeatability (RSDr) and reproducibility (RSDR) of naturally contaminated wheat were in the range 5.8–20.7 %. The HorRat was less than 1.0. From the spiking test, the recovery rate, RSDr, RSDR and HorRat values were 100.0 %, 11.2 %, 10.3 % and 0.5, respectively. The limit of quantification is 0.10 mg/kg from the range obtained in a linear calibration13).

When an analytical method is used to detect mycotoxins, the recovery has to be recognized as an official standard for the purpose of enforcement, recovery and RSDr and RSDR values were required to be in the range of 70-110 %, < 20 % and < 30 %, respectively. The HorRat value is the ratio of RSDr to RSDR predicted from the estimated mean concentration. A value between 0.5 and 1.5 is considered to be acceptable14). Thus this method could be adopted as a validated method to enforce the regulation of DON in wheat intended for use in food and feed15).

Reduction of DON concentrations in processing and cooking

To evaluate the exposure assessment of DON more accurately, we examined the reduction of DON level in processing and cooking, procedures that had been performed in Japan.

As there is the possibility that food processing such as heating and boiling produces new toxic compounds, we examined the DON residue in final products such as noodles and bread made from naturally contaminated wheat flour by using a chemical analytical method (HPLC) and bioassay with Swiss 3T3 cells.

The results of chemical and biological analysis of DON residues in noodles demonstrate that boiling reduced both the DON concentration and its cytotoxicity, and that the residue of DON leaches into the boiling water. Although it is possible that a new compound having metabolic cytotoxicity was generated in the boiling water solids this appears not to be a serious problem to health because the boiling water is commonly discarded.

In contrast, analyses of the DON level in bread suggest that DON was not reduced as a chemical compound but that rather its biological toxicity was significantly reduced. This fact indicates the possibility that a new complex is produced in bread during cooking, such as a DON-binding protein...
or a DON-binding carbohydrate, which were less cytotoxic than DON itself. Many studies on the reduction of DON in the baking process demonstrated that baking could not reduce the level of DON, and that the level of DON level was simply measured by chemical analytical methods. Our study was the first report to show the possibility that the baking process reduces the cytotoxicity of DON.

Using these results, we attempted to estimate the risk of exposure to DON based on the consumption of wheat products in Japan. A national nutritional survey showed that 50 % of wheat was consumed as bread and other 50 % as noodles. When the DON retention level in each final product was assayed by a chemical or biological analysis, its exposure level from the final wheat products consumed was calculated as 69.5 % by chemical analysis, 59.3 % and 60.6 % by bioassay of the DON level in raw flour. The risk of exposure to DON was estimated at 69.5 % or less of the DON level in raw flour if people ate noodles and bread (50:50) as the final wheat products, which reflects the current consumption of final wheat products in Japan.

**Conclusion**

Japan is the world’s biggest importer of food with the ratio of imported food accounting for more than 60 % of all foodstuffs. To prevent this food from exposure to mycotoxins, we need to establish regulations for major mycotoxins in food based on a scientific rationale. In this paper I introduced my research on mycotoxins in the field of toxicology, food safety and regulatory science. To achieve optimal regulations to ensure human safety, it is my hope that mycotoxin research progress.

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**References**

トリコテセン系カビ毒の毒性と制御に関する研究

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カビ毒は食品を汚染する自然毒の1つであるが、慢性毒性として発ガン性や免疫毒性を引き起こす。カビ毒の健康被害を防止するためには、食品からのカビ汚染量を最小限に抑えることが重要であるが、そのためには適切な基準値設定が求められる。我々のおこなった基準値設定に基づく科学的根拠に関する研究を、特にトリコテセン系カビ毒を中心にまとめた。毒性関係では、トリコテセン系カビ毒（DON, NIV, DAS, T-2トキシン, FX）の摂取の、サルモネラ等の感染性細菌および大腸菌等の非感染性細菌への感染抵抗性に対する影響を明らかにした。また、NIVの90日反復投与試験結果から、新たな毒性を明らかにした。2002年に小麦のDONの暫定基準値が設定されたが、その根拠とともに、分析法のパリデーションのための妥当性試験も行った。暴露評価で重要な位置を占める調理、食品加工中の減衰試験においては、従来の理化学分析法だけでなく、内在毒性を検知するバイオアセッサイ法を確立し、総合的な暴露評価の方法を提示した。

キ・ワ・ド：DON, NIV, トリコテセン系カビ毒、毒性、リスク評価、制御