Risk Assessment of Mycotoxins in Staple Foods from the High-Risk Area for Human Esophageal Cancer in China

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Abstract

We attempted to estimate whether mycotoxins might account for the increased risk of human esophageal cancer (HEC) in Henan province, China. Corn and wheat as staple foods were collected in 1989, 1995 and 1997 from Linxian and Shangqiu counties, the high- and low-risk areas of HEC in Henan, respectively, and analyzed for the occurrence of trichothecenes, fumonisins, zearalenone and aflatoxin B₁. Among these mycotoxins examined, fumonisin B₁ (FB₁) and deoxynivalenol (DON) were major toxins exposing populations in both areas through corn consumption.

According to the risk assessment of FB₁ and DON in both areas based on their levels in the staples, the estimated probable daily intake (PDI) values of FB₁ and DON for the high-risk area were 1.6 to 1.9 and 4.9 to 9.2 times higher, respectively, than those for the low-risk area. As compared with the tolerable daily intake (TDI) values of FB₁, which is estimated 800 ng/kg bw per day based on the published toxicological data, PDI values of FB₁ especially in 1995 and 1997 for both areas were 3.0 to 6.8 times of the TDI value. The PDI value of DON for the high-risk area was above its TDI (1,000 ng/kg bw per day) in 1989 and 1995, whereas the PDI for the low-risk area was below the TDI in three years. The results obtained in the present study hardly support the hypothesis that FB₁ contamination increases the risk of HEC in Henan province, China. Considering HEC as a multifactorial disease, however, FB₁ as well as DON may be considered to be one of factors involved in HEC.

Key words : risk assessment, fumonisin, deoxynivalenol, esophageal cancer, China.

Introduction

The highest incidence areas of human esophageal cancer (HEC) in the world are reported in parts of China, southern Africa and northeastern Iran¹⁾. In China, HEC is prevalent in certain provinces that border on the Taihang Mountains in northern China, notably, Henan, Hebei, and Shanxi provinces²⁾. In particular, Linxian county in Henan is well-known as the highest risk area, where the average age-adjusted mortality rates per 100,000 were reported as 161 for males and 103 for females.

Etiological studies have indicated that HEC is a multistage and multifactorial disease. Fungal contamination in foods and exposure to mycotoxins, together with poor nutritional status, deficiency of certain vitamins and trace elements, nitrate and nitrite in drinking water

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and nitrosamine in foods, and *Candida* esophagitis have been considered as possible contributory factors in the development of HEC²⁾. Investigations on the contamination of toxigenic fungi and mycotoxins in cereals from HEC risk areas have been in progress in China and South Africa^{1,3,4)}. However, so far the correlation between mycotoxins and the incidence of HEC has not yet been clarified.

In regard to mycotoxin contamination in grains from HEC areas, comparative studies on the natural occurrence of *Fusarium* mycotoxins (fumonisins, trichothecenes and zearalenone) in corn and wheat from Linxian, the high-risk area and Shangqiu, the low-risk area were carried out in our laboratory⁵⁻⁷⁾. In Transkei, southern Africa, the relationship of *Fusarium* mycotoxins and HEC has been extensively studied. High concentrations of various *Fusarium* mycotoxins were found in moldy home-grown corn collected from the high HEC area in Transkei⁸⁾. Furthermore, fumonisins were found in corn samples from the high-risk area at significantly higher level than those from the low-risk area^{8,9)}, suggesting an association between the contamination of corn with fumonisins and the incidence of HEC. However, this is contrary to the results previously obtained in China⁵⁻⁷⁾.

The present study focused on the occurrence of mycotoxins in staple foods from Linxian and Shangqiu, which lies about 250 km southeast of Linxian in Henan. By chemical studies on the natural occurrence of mycotoxins in corn and wheat of the subsequent year's harvest from both areas, it might be possible to have a relatively comprehensive knowledge of mycotoxin status in staple foods and to assess the possible association of mycotoxins with the incidence of HEC in China.

Materials and Methods

Cereal Samples

A total of 131 corn and 100 wheat samples were obtained by visiting different peasant families in Linxian and Shangqiu counties in 1989, 1995 and 1997. Among these, 76 corn and 65 wheat samples were collected from different HEC-patient families randomly selected in Linxian, whereas 55 corn and 45 wheat samples were from different peasant families with HEC-patient free in Shangqiu. All samples were home-grown and intended for human consumption. Wheat comprises 70 to 80% of the staple food in both areas, while corn comprises 20% and 5 to 20% in Linxian and Shangqiu, respectively. There is not so much difference in recipes as well as means of food processing in both areas.

Analysis of Mycotoxins

Corn and wheat samples for analyses of *Fusarium* mycotoxins were extracted according to the procedure described previously⁵⁻⁷⁾. Briefly, a ground sample (20 g) was extracted with acetonitrile-water (3 : 1). The filtrate was defatted, and the aqueous acetonitrile layer was divided into two portions chromatographed either on a Florisil column for analysis of trichothecenes and zearalenone or on a strong anion-exchange cartridge (Sep-Pak Accell Plus QMA) for fumonisin analysis. Fumonisins in extracts were derivatized with o-phthalaldehyde and analyzed by HPLC for FB₁, FB₂ and FB₃ according to the method of Shephard *et al*¹⁰⁾, with a slight modification. Trichothecenes were reacted with a trimethylsilylating reagent and determined by GC-MS with a selected ion-monitoring mode. Zearalenone was also analyzed by HPLC.

Results and Discussion

Exposure to Fumonisin

Table 1 shows the incidences and contamination levels of FB₁, FB₂ and FB₃ in corn samples from the two areas. In three year's surveys, the incidence of total fumonisins in Linxian corn was 1.6 to 1.9 times higher than those in Shangqiu corn. However, similar mean levels of FB₁ in positive samples from Linxian and Shangqiu were obtained: 872 and 890 ng/g in 1989, 3,458 and 3,391 ng/g in 1995 and 2,744 and 2,751 ng/kg in 1997, respectively. The levels of individual fumonisins in corn from both areas were insignificantly different in three year's surveys ($P > 0.05$).

The distribution of fumonisin contamination in 1995 and 1997 is illustrated in Figure 1. Fumonisin was detected at over 1,000 ng/g in 44 to 47% and 30 to 40% of samples from the two areas in 1995 and 1997, respectively. In addition, heavily contaminated corn samples at over 5,000 ng/g, the level to be suspected of involving in animal intoxications^{11,12}, were found in 16% of Linxian corn and 11% of Shangqiu corn. The results is a matter of concern in relation to food safety and human health risk, although nothing has been confirmed regarding effects of fumonisins on human population.

Exposure to Trichothecenes

The natural occurrence of trichothecenes in corn and wheat from the two areas in 1995 and 1997 is shown in Table 2. DON, 15-ADON and NIV were determined in Linxian corn, whereas only DON and NIV were in Shangqiu corn. DON and 15-ADON were major trichothecenes in corn, and their incidences in Linxian were 2.1 to 3.7 times of those in Shangqiu. Their levels were also significantly higher in Linxian corn than those in Shangqiu corn ($P < 0.01$). Thus, the trichothecenes, especially DON, frequently contaminated corn

Table 1 Natural occurrence of fumonisins in corn from the high- and low-risk areas of HEC in China.

Year	Area	% positive	Mean concn. (max.), ng/g			
			FB ₁	FB ₂	FB ₃	Total
1989*	High	48.1	872 (2,960)	300 (550)	—**	—
	Low	25.0	890 (1,730)	447 (450)	—	—
1995	High	79.4	2,730 (21,000)	696 (4,350)	384 (1,660)	3,458 (27,010)
	Low	50.0	2,702 (8,470)	591 (1,220)	310 (576)	3,391 (10,270)
1997	High	73.3	2,028 (8,290)	595 (2,190)	276 (1,030)	2,744 (11,510)
	Low	46.7	2,082 (5,330)	263 (1,210)	239 (622)	2,751 (7,160)

* From Yoshizawa *et al.*: Appl. Environ. Microbiol.(1994).

** Not examined.

Insignificant difference ($P > 0.05$) of fumonisin levels between the two areas was found.

Fig. 1 Distribution of fumonisin levels in corn from the high-and low-risk areas of HEC.

in the high-risk area, which was in good agreement with the previous results⁵⁾.

DON and NIV were detected in wheat samples from both areas, and none of other trichothecenes monitored were found. Incidences of DON and NIV in Linxian were 1.6 and 1.8 times higher, respectively, than those in Shangqiu. Mean levels of DON and NIV were 83 ng/kg and 29 ng/g in Linxian, and 40 ng/g and 12 ng/g in Shangqiu, respectively. Although concentrations of DON and NIV in wheat from Linxian were significantly higher than those from Shangqiu ($P < 0.01$ for DON ; $P < 0.05$ for NIV), their levels in wheat were below 220 ng/g and much lower than those in corn. These results suggest that corn is a major source of DON exposure to local populations in the high-risk area.

Although no definitive evidence indicating the involvement of DON and NIV in carcinogenesis is available, there are several reports suggesting this possibility of trichothecenes. Mixtures of trichothecenes were shown to promote esophageal tumors initiated in animals by nitrosamine¹³⁾. DON was suggested to be a clastogenic tumor formation toxin¹⁴⁾. Recently, genotoxicity of NIV was found in cultured cells and multiple mouse organs including the gastrointestinal tract by the alkaline single-cell gel electrophoresis assay¹⁵⁾. The differences in the incidence and levels of trichothecenes between the two areas confirmed in the present study together with the previous results⁵⁾ may provide an additional

Table 2 Occurrence of trichothecenes in corn and wheat from the high- and low-risk areas of HEC in China.

Year	Area	% positive	Mean concn. (max.), ng/g			
			DON	15-ADON	NIV	Total
Corn						
1995	High	73.5	400	237	86	517
			(1,160)	(620)	(153)	(1,780)
	Low	20.0	51	nd	59	55
1997	High	93.3	(87)	(—)	(107)	(107)
			129	59	59	176
	Low	46.7	(393)	(168)	(75)	(606)
			85	45	45	124
			(171)	(80)	(45)	(235)
	Wheat					
1995	High	92.0	83	nd	29	87
			(193)	(—)	(50)	(218)
	Low	60.0	40	nd	12	40
1997	High	66.7	(125)	(—)	(22)	(125)
			28	nd	95	38
	Low	0.0	(138)	(—)	(95)	(138)
			nd	nd	nd	—
			(—)	(—)	(—)	

Significant difference ($P < 0.01$) of trichothecene levels between the two areas was found in two years.

support for the possible involvement of these mycotoxins in HEC in China.

Exposure to Zearalenone and Aflatoxins

The incidences of zearalenone in corn from Linxian were 3.5 to 11 times of those in Shangqiu. Its levels in Linxian corn, even below 170 ng/g, were significantly higher than those in Shangqiu corn in both 1995 ($P < 0.01$) and 1997 ($P < 0.05$), which was in line with that found in 1989's samples⁵. As for wheat, zearalenone was detected at low incidences and levels (below 25 ng/g) in Linxian samples but free in Shangqiu samples (data not shown).

As reported by others^{16,17}, aflatoxin B1 was detected at very low levels in both areas in 1995 and 1997 surveys, except one Shangqiu corn (129 ng/g).

Risk Assessment of Fusarium Mycotoxins

According to the present study, the major mycotoxins contaminated staple foods in the two areas were fumonisins and trichothecenes, especially FB₁ and DON. In order to evaluate the potential health risk of the mycotoxins to the local populations posed by staple consumption, the risk assessment of FB₁ and DON was conducted on the basis of both estimated probable daily intakes (PDI) and tolerable daily intakes (TDI) of these toxins. For this purpose, it was assumed that the daily food intake of an adult of 60 kg bw is 150 g for corn and 600 g for wheat.

Risk Assessment of FB₁

On the basis of the carcinogenicity study of FB₁ in rats, its no-observed effect level

Table 3 Probable daily intake (PDI) of fumonisin B₁ in the high- and low-risk areas of HEC in China.

Year	Area	No. sample	Fumonisin B ₁ Mean*	(ng/g) (max.)	PDI (ng/kg bw)**
1989 ***	High	27	420	(2,960)	1,050
	Low	20	223	(1,730)	560
1995	High	34	2,170	(21,000)	5,430
	Low	20	1,350	(8,470)	3,380
1997	High	15	1,490	(8,290)	3,730
	Low	15	970	(5,330)	2,430

* Mean of all samples analyzed.

** PDI was based on the daily food intake (150 g corn and 600 g wheat) per person (60 kg bw).

*** Calculated from the data of Yoshizawa *et al.* (1994).

Table 4 Probable daily intake (PDI) of deoxynivalenol (DON) in the high- and low-risk areas of HEC in China.

Year	Area	Food	No. sample	DON level Mean*	(ng/kg) (max.)	PDI (ng/kg bw)**
1989***	High	corn	27	553	(3,505)	1,380
		wheat	15	28	(309)	280
						1,660
	Low	corn	20	40	(612)	100
		wheat	15	8	(36)	80
						180
1995	High	corn	34	259	(1,160)	650
		wheat	25	70	(193)	700
						1,350
	Low	corn	20	5	(87)	10
		wheat	15	21	(125)	210
						220
1997	High	corn	15	120	(393)	300
		wheat	15	19	(138)	190
						490
	Low	corn	15	40	(171)	100
		wheat	15	—	(—)	0
						100

*** See the footnote of Table 3.

*** Calculated from the data of Luo *et al.* (1990).

(NOEL) was estimated to be 0.8 mg/kg bw per day¹⁸⁾. If a safety factor of 1,000 is selected arbitrarily because it is the borderline value for differentiating between toxic and carcinogenic effects¹⁸⁾, the calculated TDI (NOEL/safety factor) of FB₁ is 800 ng/kg bw per day.

The estimated PDI values for Linxian and Shangqiu populations are shown in Table 3. The PDI value for FB₁ in Linxian was 1.5 to 1.9 times of those in Shangqiu. Especially, the PDI values of FB₁ in 1995 and 1997 for both populations were 3.0 to 6.8 times of the TDI value (NOEL/1,000). Moreover, for people in the high-risk area who consumed corn contaminat-

ed with FB₁ at a maximum level of 21 mg/kg, the calculated PDI value is 52,500 ng/kg bw per day, as high as 65.6 times of the TDI.

Thus, the back ground level of FB₁ exposure is generally high in Linxian as well as Shangqiu, implying the potential health risk of the toxin to populations of both areas. In addition, because of not so much difference in the PDI values of FB₁ between two areas, it is difficult to support the hypothesis that fumonisin contamination increases the risk of HEC in Henan province, China.

Risk Assessment of DON

In spite of being one of the least acutely toxic trichothecene, frequent contamination of grains with DON is an important human and animal safety issue. Kuiper-Goodman¹⁹⁾ estimated the TDI of DON to be 3,000 and 1,500 ng/kg bw per day for adults and infants, respectively. Animal experiments revealed monogastric animals, especially swine and dogs show the greatest sensitivity to DON with the NOEL of 0.1 mg/kg bw per day²⁰⁾. Evaluation of the hazard potential indicated that DON is unlikely to exhibit specific effects in the absence of general toxicity such as DNA-interaction, reproductive toxicity, neurotoxicity or immunotoxicity. Hence, an uncertainly factor of 100 as a minimum is recommended resulting in the TDI proposal for DON of 1,000 ng/kg bw per day.

The estimated PDI values of DON for Linxian and Shangqiu populations are shown in Table 4. The PDI of DON for Linxian was 4.9 to 9.2 times of those for Shangqiu in three years' survey. In Linxian, the PDI value for DON was above the TDI in 1989 and 1995, whereas the PDI value for Shangqiu was below the TDI in three years. The results implicated that the human health risk by consuming corn and wheat contaminated with DON is possible for Linxian.

In conclusion, the results obtained in the present study hardly support the hypothesis that FB₁ contamination increases the risk of HEC in Henan province, China. Considering HEC as a multifactorial disease, however, FB₁ as well as DON may be considered to be one of factors involved in HEC.

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