Survey of cyclopiazonic acid contamination in corn from China and Southeast Asian countries

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Summary

Natural incidence of cyclopiazonic acid (CPA) in corn from the southern part of China and Southeast Asian countries including Indonesia, Thailand, and the Philippines, was surveyed using a combination of a clean-up procedure consisting of diethyl ether liquid-liquid extraction and HPLC-UV analysis. Out of 99 corn samples analyzed, 15 samples (15 %) were contaminated with CPA. The detected concentration of CPA ranged from 27 to 1510 µg/kg, with all CPA positive samples contaminated with aflatoxin B₁ (AFB₁). It was noteworthy that the high level of CPA together with AFB₁ over 1000 µg/kg was found in the samples intended for human consumption from China and the Philippines.

Key words : cyclopiazonic acid, aflatoxins, corn, China, Southeast Asia, Thailand, Indonesia, Philippines

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Introduction

Cyclopiazonic acid (CPA) is a toxic secondary metabolite produced by several species of *Penicillium* and *Aspergillus*, including *P. cyclopium*¹⁾, *P. camemberti*²⁾, *A. oryzae*³⁾, *A. tamarii*⁴⁾, and *A. flavus*⁵⁾. These fungi are widely distributed in nature and the natural occurrence of CPA has been reported in corn⁶⁾ and peanuts⁷⁾, often together with aflatoxins (AF).

Both CPA and AF have been shown to cause health problems in animals and humans. Toxicosis resulting from consumption of CPA-contaminated commodities has not been proven, but has been implicated as the causative agent in a human intoxication known as 'Koudua poisoning' involving consumption of contaminated millet⁸⁾. In addition, CPA was also suggested to be responsible for the outbreak termed 'Turkey X disease' in which AF were strongly implicated⁹⁾.

AF have been classified as Class I human carcinogens¹⁰⁾ and well recognized as serious food contaminants in the tropics more than in temperate zones of the world. Contamination levels of AF in corn as a staple food of many temperate countries and other corn-producing countries have been reported. Furthermore, natural co-occurrence of both AF and *Fusarium* toxins including fumonisins (FM), nivalenol and zearalenone has been reported in corn from China¹¹⁾ and Southeast Asian

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countries¹²⁻¹⁴). However, few surveys have been conducted to determine CPA contamination in agricultural commodities from Southeast Asian countries. Contamination level of CPA in corn as a staple food in these countries has not been well studied, and relevant data is very limited.

Reports on the natural occurrence of CPA in agricultural commodities are very limited. Gallagher *et al.* (1978) found CPA in AF-contaminated North American corn samples⁶). Urano *et al.* (1992) has studied the AF and CPA contamination in corn and peanuts from U.S.A.^{15, 16}). Only one report by Widiastuti *et al.* (1988) is available on tropical crops; this study analyzed CPA in Indonesian corn by thin layer chromatography¹⁷). In these reports, toxic solvents such as chloroform and dichloromethane were used as extraction and clean-up solvents.

Recently, we developed a method for the determination of CPA in corn using a clean-up procedure consisting of liquid-liquid extraction with diethyl ether coupled with HPLC-UV¹⁸). In the present study, we surveyed the CPA contamination levels in corn from the southern part of China and Southeast Asian countries using this method. Based on results, the simultaneous occurrence of CPA and AF was discussed. This is the first report of the natural occurrence of CPA in corn for human consumption from China and the Philippines.

Materials and Methods

Corn Samples Twenty samples of yellow corn kernel (more than 200 g each) intended for human consumption were collected in 1998 from Chongzuo County in Guanxi Province, in the southern part of China, an area with a high incidence of human primary hepatocellular carcinoma. Twelve samples of Indonesian corn (more than 100 g each) and 28 samples of Thai corn (more than 100 g each), intended for animal and human consumption, were collected in 1992 and 1993, respectively. Thirty-nine samples of Philippine corn (more than 100 g each) intended for human consumption were collected in 1997 and 1998; seven of these samples were white corn kernels and 32 samples were yellow corn kernels and corn grits. These samples were previously analyzed for AF in our laboratory^{11, 12, 19}) and were stored in zip-lock plastic bags at -20 °C until needed.

Extraction and clean-up Extraction and clean-up were conducted following the procedure reported by Hayashi and Yoshizawa.¹⁸) Briefly, ground samples (15 g each) were extracted with acetonitrile - 1 % sodium bicarbonate (7:3, v/v, 50 ml) and centrifuged at 1,400 x g for 5 min. The supernatant extract (30 ml) was mixed with 0.05 mol/L lead acetate (60 ml) and filtered using an Advantec GA-55 glass filter (Advantec Toyo Kaisya, Ltd., Tokyo, Japan). The filtrate (40 ml) was adjusted to pH 2 by adding 0.5 mol/L HCl (ca. 5.5 ml), and partitioned twice with diethyl ether (25 ml). As an additional clean-up step, the combined diethyl ether extract (ca. 50 ml) was extracted twice with 5 % sodium bicarbonate (20 ml). The combined aqueous layer (ca. 40 ml) was acidified to pH 2 with 6 mol/L HCl, which was partitioned twice with diethyl ether (25 ml). Finally, the combined diethyl ether was evaporated in vacuo and dissolved in methanol - 1 m mol/L zinc sulfate (85:15, v/v, 0.5 ml). An aliquot (10 µl) of the sample extract was injected into the HPLC column.

HPLC analysis The quantification of CPA in corn samples was performed by HPLC with UV detection under the following conditions: Shimadzu SCL-6A system connected to a Shimadzu SPD-M10AVP photodiode array detector interfaced with a Shimadzu CBM-10A communications bus

module and a Shimadzu CLASS-LC10 model FMV-6300 DX 2c computer; reversed phase column - TSK-gel ODS-80TM CTR, 5 μ m, 100 x 4.6 mm, TOSOH Co., Tokyo, Japan); oven temperature - 40 °C; wavelength - 279 nm; methanol - water (85:15, v/v) as mobile phase A ; methanol - 4 m mol/L zinc sulfate (85:15, v/v) as mobile phase B; a linear gradient from mobile phase A to mobile phase B in 10 min at a flow rate of 1.2 ml/min. The limit of quantification in corn was 25 ng CPA/g. The average recoveries from triplicate corn samples spiked with 25, 50, 100 and 200 ng CPA/g were 64.7, 68.5, 74.6 and 75.4 %, respectively.

Results and Discussion

A typical HPLC chromatogram of CPA in a naturally contaminated corn sample is shown in Fig. 1. The presence of CPA in corn samples was confirmed by HPLC-photodiode array detection as shown in Fig. 2. Incidence and concentration of CPA in corn from China and Southeast Asian countries including Thailand, Indonesia, and the Philippines are summarized in Table 1. Out of 99 corn samples surveyed, 15 samples (15 %) were contaminated with CPA: China (7), Thailand (1), Indonesia (1), and the Philippines (6). The detected concentration of CPA ranged from 27 to 1510 μ g/kg (mean, 53 μ g/kg).

Occurrence of CPA in corn from China and the Philippines Natural co-occurrence of CPA and AF in corn samples intended for human consumption from China¹¹⁾ and the Philippines¹⁹⁾ are summarized in Table 2. Data of AFB₁ contamination were cited from our previous publications^{11, 12, 19)}.

CPA was detected in 7 samples (35 %) of Chinese corn with concentration ranging from 42 to 1220 μ g/kg (mean, 129 μ g/kg). The highest level of CPA (1220 μ g/kg) was found in the sample (CZ-98-C10) contaminated with the highest level of AFB₁ (2496 μ g/kg). Li *et al.*¹¹⁾ reported on the high incidence of simultaneous contamination with AF and *Fusarium* toxins such as FM in these corn samples. In the case of the Philippine samples, 6 (15 %) were found to be naturally contaminated with

Fig. 1. HPLC chromatograms of CPA in Chinese corn.

A) CPA standard (10 ng)

B) CPA-free corn sample (CZ-98-12)

C) CPA-contaminated corn (CZ-98-16, 335 μ g/kg)

Fig. 2. UV spectra of CPA obtained from HPLC-photodiode array analysis.
 ——— CPA in a contaminated Chinese corn sample (CZ-98-16)
 - - - - - CPA standard

Table 1. Incidence and concentration of CPA in corn intended for human consumption from China and the Philippines

Country	No. of samples	CPA		
		Detected	Concentration, $\mu\text{g/kg}$	
		No. (%)	Mean	Range
China	20	7 (35)	129	42-1220
Thailand	28	1 (4)	42	42
Indonesia	12	1 (8)	63	63
Philippines	39	6 (15)	69	27-1510
Total	99	15 (15)	53	27-1510

Table 2. Natural co-occurrence of CPA and AF in corn samples intended for human consumption from China and the Philippines^{*1}

Country	Sample code	Location	Concentration, $\mu\text{g/kg}$		
			CPA	AFB ₁	AFB ₂
China	CZ-98-C6	Tiaiping	72	698	111
	CZ-98-C8	Tiaiping	129	103	11
	CZ-98-C10	Tiaiping	1220	2496	320
	CZ-98-C11	Tiaiping	42	185	29
	CZ-98-C13	Madalu	288	2121	281
	CZ-98-C16	Nalong	335	150	21
	CZ-98-C20	Zuozhou	52	290	55
Philippines	CW 6f	Iloilo	27	10	9
	CW Ba	Metro Manila	29	369	— ^{*2}
	CW 6b	Iloilo	63	2	—
	CW 6d	Iloilo	76	44	—
	CW 6a	Iloilo	210	319	—
	CW 6c	Iloilo	1510	1215	10

^{*1} Data of AFB₁ and AFB₂ contamination were cited from previous publications: China⁽¹¹⁾ and the Philippines⁽¹⁹⁾.

^{*2} Not detected

CPA, with levels ranging from 27 to 1510 µg/kg (mean, 69 µg/kg). The highest level of CPA (1510 µg/kg) was also found in the sample (CW 6c) contaminated with the highest level of AFB₁ (1220 µg/kg)¹⁹. The co-incidence of CPA and AF in yellow corn was higher than that in white corn from both China and the Philippines (data not shown).

It is noteworthy that the co-occurrence of CPA and AFB₁ at levels over 1000 µg/kg was found in the samples intended for human consumption from China and the Philippines. All CPA positive samples were contaminated with AFB₁; CPA was not found in non-AF contaminated samples. There was no quantitative correlation between CPA and AFB₁ concentrations.

Occurrence of CPA in corn from Thailand and Indonesia Among 28 Thai corn and 12 Indonesian corn samples analyzed, one sample each was contaminated with CPA at a level of 42 µg/kg (Thai corn) and 63 µg/kg (Indonesian corn); these samples were co-contaminated with AFB₁ at levels of 84 µg/kg and 65 µg/kg¹², respectively. Because the occurrence of CPA in Thai and Indonesian corn samples was lower than those from China and the Philippines, it is necessary to conduct further studies in the former countries.

Widiastuti *et al.*¹⁷ reported heavy contamination of Indonesian corn with CPA at levels ranging from 30 to 9220 µg/kg together with AFB₁. However, as the quantification of CPA was carried out by thin layer chromatography, those data should be interpreted with caution. Goto *et al.*²⁰ reported that 21 out of 22 isolates of *A. flavus* from agricultural commodities and soil from Indonesia produced CPA, and 7 of these isolates produced AFB₁. There are well-defined species: *A. flavus* produces only B group AF (AFB₁, AFB₂) and sometimes CPA, while *A. parasiticus* produces both B and G group AF, but never CPA¹⁰. However, this definition has been complicated by more recent taxonomic finding¹⁰. Therefore, in order to solve this problem, further continuous survey of mycotoxin contamination in agricultural commodities and mycological study on Asian tropical crops are important steps towards eliminating the risk of mycotoxin contamination.

It has long been recognized that mycotoxin contamination is a serious problem with detrimental effects on human and animal health and productivity, and serious trade and economic repercussions. However, apart from the survey of AF, there have been very few reports on the co-occurrence of *Aspergillus* toxins such as CPA. This is the first report of the natural occurrence of CPA in corn for human consumption from China and the Philippines.

In conclusion, the co-occurrence of CPA and AF in corn from the southern part of China and Southeast Asian countries was ascertained. Taking into consideration that high levels of CPA, i.e. over 1000 µg/kg, was found together with high levels of AFB₁ in the samples intended for human consumption from the southern part of China and the Philippines, further continuous survey of mycotoxin contamination in agricultural commodities in these areas needs to be addressed in order to eliminate the risk of mycotoxin contamination.

References

- 1) Holzapfel, C. W.: Tetrahedron, **24**, 2101-2119 (1968)
- 2) Still, P. E., Eckardt, C., Leistner, L.: Fleischwirtschaft, **58**, 876-877 (1978)
- 3) Orth, R.: Ann. Nutr. Aliment, **31**, 617-624 (1977)

- 4) Dorner, J. W.: Appl. Environ. Microbiol., **46**, 1435-1437 (1983)
- 5) Luk, K. C., Kobbe, B., Townsend, J. M.: Appl. Environ. Microbiol., **33**, 211-212 (1987)
- 6) Gallagher, R. T., Richard, J., L., Stahr, H. M., Cole, R. J.: Mycopathologia, **66**, 31-36 (1978)
- 7) Lansden, J. A.: Appl. Environ. Microbiol., **45**, 766-769 (1983)
- 8) Rao, B. L., Husain, A.: Mycopathologia, **89**, 177-180 (1985)
- 9) Cole, R. J.: Mycotoxin Res., **2**, 3-7 (1986)
- 10) International Agency for Research on Cancer: IARC Monographs on the Evaluation of Carcinogenic Risk to Humans, Vol. 82, Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene. pp. 171-300 (2002), IARC, Lyon
- 11) Li, F. Q., Yoshizawa, T., Kawamura, O., Luo, X. Y., Li, Y. W.: J. Agric. Food Chem., **49**, 4122-4126 (2001)
- 12) Yamashita, A., Yoshizawa, T., Aiura, Y., Sanchez, P. C., Dizon, E. I., Arim, R., H., Sardjono: Biosci. Biotech. Biochem., **59**, 1804-1807 (1995)
- 13) Yoshizawa, T., Yamashita, A., Chokethaworn, N.: Food Addit. Contam., **13**, 163-168 (1996)
- 14) Ali, N., Sardjono, Yamashita, A., Yoshizawa, T.: Food Addit. Contam., **15**, 377-384 (1998)
- 15) Urano, T., Trucksess, M. W., Matusik, J., Dorner, J. W.: J. AOAC Int., **75**, 319-322 (1992)
- 16) Urano, T., Trucksess, M. W., Beaver, R. W., Wilson, D. M., Dorner, J. W., Dowell, F. E.: J. AOAC Int., **75**, 838-841 (1992)
- 17) Widiastuti, R., Maryam, R., Blaney, B. J., Stoltz, S., Stoltz, D. R.: Mycopathologia, **104**, 153-156 (1988)
- 18) Hayashi, Y., Yoshizawa, T.: "New horizon of mycotoxicology for assuring food safety" (eds. Yoshizawa, T., Kumagai, S., Goto, T.), pp. 309-313 (2004), Jpn. Assoc. Mycotoxicol., Tokyo.
- 19) Arim, R.: Ph.D. Thesis, Ehime university, pp. 74-77 (2000)
- 20) Goto, T., Ginting, E., Antarlina, S. S., Utomo, J. S., Ito, Y., Nikkuni, S.: "Mycotoxin contamination: health risk and prevention project" (eds. Kumagai, S., Goto, T., Kawai, K., Takahashi, H., Yabe, K., Yoshizawa, T., Koga, H., Kamimura, H., Akao, M.), pp. 211-215 (1999), Jpn. Assoc. Mycotoxicol., Tokyo.

中国及び東南アジア産トウモロコシのシクロピアゾン酸汚染

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中国南部(広西省)の肝癌高発地域及び東南アジア(タイ, インドネシア, フィリピン)産トウモロコシ 99 検体のシクロピアゾン酸(CPA)汚染を, 新規に考案した分析法により検討したところ, 15 検体に(15%)に CPA が検出され, その汚染濃度は 27-1510 $\mu\text{g}/\text{kg}$ であった. CPA が検出されたすべての検体からアフラトキシン B₁(AFB₁) が検出された. 特に, 中国産及びフィリピン産の食用トウモロコシの汚染頻度が高く, 1000 $\mu\text{g}/\text{kg}$ を超える濃度の CPA と AFB₁ の同時汚染であることが注目された.

キーワード: シクロピアゾン酸, アフラトキシン, トウモロコシ, 中国, 東南アジア, タイ, インドネシア, フィリピン