RESEARCH ARTICLE

Ochratoxin A Contamination of Red Chili Peppers from Chile, Bolivia and Peru, Countries with a High Incidence of Gallbladder Cancer

Toshikazu Ikoma[1], Yasuo Tsuchiya[2]*, Takao Asai[3], Kiyoshi Okano[4], Naoko Ito[5], Kazuo Endoh[5], Masaharu Yamamoto[5], Kazutoshi Nakamura[2]

Abstract

Our previous study detected aflatoxins in red chili peppers from Chile, Bolivia, and Peru, each of which have a high incidence of gallbladder cancer (GBC). Since the aflatoxin B1 concentration was not so high in these peppers, it is important to clarify the presence of other mycotoxins. Here we attempted to determine any associations between the concentrations of aflatoxins and ochratoxin A (OTA) in red chili peppers, and the corresponding GBC incidences. We collected red chili peppers from three areas in Peru: Trujillo (a high GBC incidence area), Cusco (an intermediate GBC incidence area), and Lima (a low GBC incidence rate), and from Chile and Bolivia. Aflatoxins and OTA were extracted with organic solvents. The concentrations of aflatoxins B1, B2, G1, and G2, and OTA were measured by high-performance liquid chromatography. The values obtained were compared with the incidence of GBC in each area or country. All of the red chili peppers from the three areas showed contamination with aflatoxins below the Commission of the European Communities (EC) recommended limits (5 μg/kg), but the OTA contamination of two samples was above the EC recommended limit (15 μg/kg). The mean concentrations of OTA in the peppers from Chile (mean 355 μg/kg, range <5–1,059 μg/kg) and Bolivia (mean 207 μg/kg, range 0.8–628 μg/kg), which has a high incidence of GBC, were higher than that in Peru (14 μg/kg, range <5–47 μg/kg), which has an intermediate GBC incidence. The OTA contamination in the red chili peppers from Chile, Bolivia, and Peru was stronger than that of aflatoxins. Our data suggest that OTA in red chili peppers may be associated with the development of GBC.

Keywords: Gallbladder cancer - ochratoxin A - aflatoxin - incidence rate - HPLC - ecologic study

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Introduction

Aflatoxins B1 (AFB1), B2 (AFB2), and G1(AFG1) have been detected in red chili peppers from Chile, Bolivia, and Peru, each of which have a high incidence of gallbladder cancer (GBC) as shown in our previous studies (Tsuchiya el al., 2011; Asai et al., 2012). The association between exposure to AFB1 and the development of liver cancer had already been demonstrated by several studies (e.g., WHO 2002), especially when it is associated with hepatitis B or C virus infection (Kew, 2003), and some groups have reported an association between exposure to AFB1 and the risk of GBC (Sieber et al., 1979; Olsen et al., 1988). Since our earlier epidemiological study demonstrated that a high consumption of red chili peppers is a risk factor for GBC in Chilean women who had gallstones (Serra et al., 2002), we suspected that a high consumption of aflatoxin-contaminated red chili peppers may also be associated with the development of GBC. However, the aflatoxins in the red chili peppers from Chile, Bolivia, and Peru were detected at low concentrations, and the red chili peppers we used were not collected from areas with a high GBC incidence in each country. Areas showing the highest GBC mortality rate in these three countries were as follows: the south-inland region in Chile (Andia et al., 2008); near Lake Titicaca in Bolivia (Strom et al., 1995); and in the area of the city Trujillo in Peru (Lazcano-Ponce et al., 2001).

Although aflatoxins have not yet been established as an important risk factor for GBC, if the development of GBC is related to the high consumption of mycotoxin-contaminated red chili peppers, it is possible that mycotoxins other than AFB1 may be detected in red chili peppers, especially those from areas or countries with a high incidence of GBC. However, to our knowledge, no study has examined the concentrations of mycotoxins

[1]Hokuriku University, Kanazawa, [2]Division of Preventive Medicine, Niigata University Graduate School of Medical and Dental Sciences, [3]Department of Clinical Engineering and Medical Technology, Niigata University of Health and Welfare, Niigata, [4]Mycotoxin Research Association, Yokohama, [5]Department of Health and Nutrition, Niigata University of Health and Welfare, Niigata, Japan
**For correspondence: troof441@gmail.com*

other than aflatoxins in red chili peppers from Chile, Bolivia, or Peru.

Ochratoxin A (OTA) is a mycotoxin produced by Penicillium verrucosum, Penicillium nordicum, Aspergillus ochraceus, and Aspergillus carbonarius, and other Penicillium and Aspergillus species (Ringot et al., 2006). OTA is one of the most widespread and hazardous mycotoxins, and is found in foodstuffs such as coffee (Suarez-Quiroz et al., 2005), cocoa (Copetti et al., 2010), beer (Mateo et al., 2007), wine (Zimmerli and Dick, 1996), dried fruit (Bircan, 2009), cereals (Olsen et al., 2006), spices (Scheuer and Gareis, 2002), and nuts (Zaied, 2010). We therefore hypothesized that the red chili peppers from areas or countries with a high incidence of GBC are contaminated with aflatoxins, or ochratoxins, or both. The purposes of the present study were (1) to measure the concentrations of aflatoxins and OTA in red chili peppers consumed by Peruvian people living in three areas: Trujillo (a high GBC incidence area), Cusco (near Lake Titicaca, an intermediate GBC incidence area), and Lima (the capital of Peru, a low GBC incidence area), and (2) to compare the concentrations with their respective GBC incidence rates. We also measured the OTA concentration in red chili peppers from Chile, Bolivia, and Peru, and compared them with the GBC incidence rate in each country.

Materials and Methods

Materials

We purchased a total of 13 red chili peppers at central markets in Peru (six from Trujillo, two from Cusco, and five from Lima). Although the red chili peppers purchased in Cusco and Lima were dry forms, we were unable to get the dried forms in Trujillo; these six were fresh ones. In addition to the Peruvian red chili peppers, we purchased four types of red chili peppers at central markets in Santiago, Chile and La Paz, Bolivia. The fresh red chili peppers were dried by a vacuum freeze dryer (SSVD-13S, Isuzu Seisakusho Co., Sanjo, Japan) and then powdered by a dry mill (PM-2005, Osaka Chemical Co., Osaka, Japan). Before extraction, the dried or crushed red chili peppers were powdered by the dry mill (Osaka Chemical Co.).

Extraction of aflatoxins and OTA from the red chili peppers

Aflatoxins in the red chili peppers were extracted using our previously described method in reference to "Shoku-An No. 0728004" (Ministry of Health, Labour and Welfare, 2008). OTA was extracted with methanol/ aqueous and 1% NaHCO3 solution (70:30, v/v) and then eluted with methanol (Sugita-Konishi et al., 2006).

Measurement of aflatoxins

We used high-performance liquid chromatography (HPLC; D-2000, Hitachi, Tokyo) to measure the AFB1, AFB2, AFG1, and aflatoxin G2 (AFG2) concentrations in the red chili peppers by our previously described method (Asai et al., 2012). Briefly, we used the following operation conditions: column: Atlantis T3 C18 (5-µm particle size, 250 mm × 3.0 mm, Waters, Milford, MA, USA); column temperature: 40°C; mobile phase: acetonitrile : methanol : water (1 : 3 : 6, v/v/v); flow rate: 0.4 mL/min; detection wavelength: excitation wavelength 365 nm/emission wavelength 450 nm; injection volume: 20 µL.

Measurement of the OTA concentration

We measured the OTA concentration in the red chili peppers by HPLC as described previously (Sugita-Konishi et al., 2006). The HPLC conditions for OTA detection were as follows: column: ODS 250 mm × 4.6 mm, i.d. (3–5 µL); column temperature: 45°C; mobile phase: acetonitrile : water : acetic acid (55 : 43 : 2, v/v); flow rate: 1.0 mL/min; detection wavelength: excitation wavelength 333 nm/emission wavelength 460 nm; injection volume: 100 µL.

Recovery rates and detection limits

The recovery rates for each 2.5 µg/kg were 85% in AFB1, 86% in AFB2, 90% in AFG1, and 90% in AFG2. The detection limits of the aflatoxins assay were each 0.5 µg/kg. The recovery rate for 10 µg/kg of OTA was 87%, and the detection limits of the OTA assay was 0.5 µg/kg.

Results

OTA in Peruvian red chili peppers. AFB1 was detected

Table 1. Concentration of Aflatoxins B1, B2, G1, and G2, and Ochratoxin A in Red Chili Peppers from Peru

Areas	Aflatoxin				Ochratoxin
	B1	B2	G1	G2	A
Trujillo 1	n.d.	n.d.	n.d.	n.d.	n.d.
Trujillo 2	n.d.	n.d.	n.d.	n.d.	n.d.
Trujillo 3	n.d.	n.d.	n.d.	n.d.	n.d.
Trujillo 4	n.d.	n.d.	n.d.	n.d.	n.d.
Trujillo 5	n.d.	n.d.	n.d.	n.d.	n.d.
Trujillo 6	n.d.	n.d.	n.d.	n.d.	n.d.
Cusco 1	0.9	n.d.	n.d.	n.d.	11.5
Cusco 2	1.6	n.d.	n.d.	n.d.	47.1
Lima 1	n.d.	n.d.	n.d.	n.d.	n.d.
Lima 2	n.d.	n.d.	n.d.	n.d.	n.d.
Lima 3	n.d.	n.d.	n.d.	n.d.	2.8
Lima 4	n.d.	n.d.	n.d.	n.d.	0.6
Lima 5	1.5	n.d.	n.d.	n.d.	32.3

Aflatoxin concentrations in red chili peppers were measured by HPLC using a Hitachi D-2000 Elite system; Values are presented as µg/kg; n.d.: Not detected (i.e., below the detection limit 0.5 µg/kg)

Table 2. Concentrations of Ochratoxin A in dried red chili peppers from Chile, Bolivia, and Peru

Samples	Countries		
	Chile	Bolivia	Peru
1	198.8	1.9	11.5
2	1059.1	0.8	47.1
3	163.4	196.3	n.d.
4	n.d.	628.3	n.d.
5			2.8
6			0.6
7			32.3
Mean	355.4	206.8	13.5

Values are represented as µg/kg; n.d.: Not detected (i.e., below the detection limit 0.5 µg/kg) from 3 red chili peppers, 2 from Cusco (concentrations: 0.9 and 1.6 μg/kg), and 1 from Lima (concentration: 1.5 μg/kg). The concentrations of AFB1 detected from 3 samples were below 5 μg/kg of the limitation set by the Commission Regulation (EC), and no differences in the AFB1 concentration were found among 3 areas. Furthermore AFB2, AFG1, and AFG2 were not detected in the red chili peppers.

OTA was detected in 5 of 13 red chili peppers (2 from Cusco and 3 from Lima), and the concentrations of those from Cusco were 11.5 and 47.1 μg/kg, or those from Lima were 0.6, 2.8, and 32.3 μg/kg. The concentration of OTA in the red chili peppers from Trujillo was all below 0.5 μg/kg of our detection limit for OTA. The mean concentration (29.3 μg/kg) of OTA in the red chili peppers from Cusco was higher compared with that (7.1 μg/kg) in Lima, though OTA was not detected in 2 samples from Lima. The OTA concentrations detected in the red chili peppers were compared with the OTA limitation set by the EC, and 1 from Cusco (1/2, 50%) and 1 from Lima (1/5, 20%) were higher than 15 μg/kg.

Table 2 shows the concentrations in dried red chili peppers from Chile, Bolivia, and Peru. In the red chili peppers from Chile, OTA was detected in 3 of 4 samples (75%), showing the range of 163.4-1059.2 μg/kg. In those from Bolivia, OTA was detected in all 4 samples, showing the range of 0.8-628.3 μg/kg. In the dried red chili peppers from Peru, OTA was detected in 5 of 13 samples (38%), and the range was 0.6-47.1 μg/kg. In 3 of 4 from Chile, 2 of 4 from Bolivia, and 3 of 13 from Peru showed higher concentrations than the limitation for OTA by the EC. The mean OTA concentration in the dried red chili peppers among the 3 countries was higher in the following order: Chile> Bolivia>Peru.

Discussion

In this study, we observed that the dried red chili peppers from Peru are more greatly contaminated with OTA rather than aflatoxins. OTA was observed at high concentrations in the red chili peppers from Chile, Bolivia and Peru, each of which has a high incidence of GBC.

Several environmental and genetic risk factors for the development of GBC have been demonstrated (Wistuba and Gazdar, 2004; Baez et al., 2010). Although as noted earlier, Serra et al. (2002) showed that a high consumption of red chili pepper increases the risk of GBC in Chilean women who carry gallstones, the pathogenic mechanism has not yet been clarified. We hypothesized that GBC would occur in relation to the high consumption of aflatoxin-contaminated red chili peppers, and we measured the concentrations of aflatoxins in red chili peppers from Chile, Bolivia and Peru. Our findings were as follows: AFB1 and AFG1 were detected in Chilean red chili peppers (Tsuchiya et al., 2011), AFB1 and AFB2 were detected in those from Bolivia (Asai et al., 2012), and AFB1 was detected in those from Peru (Asai et al., 2012).

These findings suggest that a high consumption of aflatoxin-contaminated red chili pepper may be related to the development of GBC in these countries. However, our results lack the power to explain the association between a high consumption of aflatoxin-contaminated red chili peppers and the development of GBC, because (1) the aflatoxins were detected at low concentrations and (2) the red chili peppers we used were not collected from the area in each country with the highest GBC incidence.

The incidence of GBC shows significant geographic and ethnic variations (Hariharan et al., 2008). The highest incidence of GBC has been reported for populations living in the western parts of the Andes, and in North-American Indians, Mexican Americans, and inhabitants of northern India (Lazcano-Ponce et al., 2001). The highest mortality rates have been also reported among Chilean Mapuche Indians, Bolivians, and Chilean Hispanics living in South America (Wistuba and Gazdar, 2004). In Chile, Bolivia, and Peru, the highest incidence rates of GBC have been reported among the populations living in the southern part of the country, especially the south-inland region in Chile (Andia et al., 2008), near Lake Titicaca in Bolivia (Strom et al., 1995), and in Trujillo in Peru (Lazcano-Ponce et al., 2001).

Here we conducted an ecological study to clarify the associations between a high consumption of aflatoxin-contaminated red chili peppers and the incidence of GBC. The red chili peppers were collected from Trujillo (showing the highest GBC mortality rate), from Cusco (showing the intermediate GBC incidence rate), and from Lima (showing a low GBC incidence rate). Contrary to our expectation, AFB1 was detected in only three red chili peppers (two from Cusco and one from Lima), and all of the concentrations were below 1.6 μg/kg, and were mostly consistent with our previous reported value, 0.7 μg/kg (Tsuchiya et al., 2010). We were unable to determine the association between the AFB1 concentration of red chili peppers and the GBC incidence rate among the three areas in Peru. Our findings suggest that one or more mycotoxins other than AFB1 may be associated with GBC development, if the mycotoxin contamination of red chili peppers is related to the development of GBC among the people living in Peru, Bolivia and Chile.

OTA is a mycotoxin produced by Aspergillus and Penicillium fungus, and is one of the most widespread and hazardous mycotoxins. OTA has been classified as a possible human carcinogen (Group 2B) by the International Agency for Research on Cancer (IARC, 1993). OTA has been shown to be a nephrotoxic (Sauvant et al., 2005), hepatotoxic (Ayed-Boussema et al., 2012), teratogenic (Wangikar et al., 2005) and immunotoxic (Mechoud et al., 2012) mycotoxin to several species of animals, and it has been reported to cause kidney and liver tumors in mice and rats (Huff, 1991). OTA can be metabolized in the kidney, liver and intestines (Wu et al., 2011). OTA is hydrolyzed in the digestive tract to the less toxic substance, ochratoxin alfa (OT alpha, OTα). OTA is oxidized by cytochromes P450 (CYPs) in the liver and kidney, where small amounts of oxidized OTA metabolite are produced. This metabolite is excreted in bile, and then the bile is stored in the gallbladder (Ringot et al., 2006). In light of this process, the possibility of the development of GBC due to a high consumption of OTA-contaminated red chili pepper could not be excluded. We therefore measured the concentrations of OTA in dried red chili peppers from

Chile, Bolivia and Peru and compared them with the incidence of GBC in each country. OTA was detected in three samples from Chile (3/4, 75%), four from Bolivia (4/4, 100%), and five from Peru (5/7, 71.4%).

Although a specific maximum limit for OTA in foodstuffs is not set in all countries, in the European Union (EU), maximum levels for certain contaminants in foodstuffs have been set by the Commission Regulation (EC), and that for chili peppers is 15 µg/kg, issued in 2015 (EC No. 1881, 2006). An OTA limit of 5 µg/kg in foodstuffs was set by the Codex Alimentarius Standard (CODEX STAN 193-1995, Rev. 3-2007). In the present study, OTA concentrations >15 µg/kg of were detected in three red chili peppers from Chile (75%), two from Bolivia (50%), and two from Peru (29%). Lazcano-Ponce et al. (2001) reported that Chile and Bolivia have the highest incidence of GBC and that Peru has an intermediate incidence. Since in the present study the mean OTA concentrations in the dried red chili peppers from Chile and Bolivia were higher compared to those from Peru, we suspect that the OTA contamination of red chili peppers may be associated with the development of GBC.

The present study has some limitations. We were unable to collect dried red chili peppers in Trujillo, which has the highest GBC incidence in Peru, and fresh red chili peppers in Cusco and Lima. We were thus unable to compare the differences in the aflatoxins and OTA concentrations in dried red chili peppers among the three areas, Trujillo, Cusco, and Lima. It is necessary to determine the concentrations of these mycotoxins in dried red chili peppers from Trujillo for the ecological study of the association between the aflatoxins and OTA concentrations in red chili peppers and the incidence of GBC. However, our findings indicated that the red chili peppers from Chile, Bolivia, and Peru are more contaminated with OTA than with aflatoxins. Our findings suggest that OTA rather than aflatoxins may be related to the development of GBC.

The fresh red chili peppers from Trujillo were not contaminated with aflatoxins or OTA, suggesting that postharvest handling is the most important to prevent aflatoxin and OTA contaminations in red chili peppers. The causes of aflatoxin or OTA contamination described in a previous study (Organization of American States in cooperation with the Mayan Research Foundation) were as follows: drought stress, postharvest handling, diseased fruits, drying temperature, product moisture content, temperature, and hygienic aspects. As described, aflatoxins and OTA are produced before and after harvest, but our present findings demonstrate that postharvest handling is more important than conditions before harvest in these countries.

To our knowledge, this was the first study to examine the association between exposure to OTA and the risk of GBC. Additional studies are required. Nonetheless, the consistency in the association between OTA contamination in red chili peppers and the incidence of GBC in Chile, Bolivia, and Peru argues strongly against the role of chance as an explanation for our findings.

In summary, the red chili peppers from Peru had a higher degree of OTA contamination than aflatoxin contamination. The concentrations of OTA in dried red chili peppers from Chile and Bolivia (which each have a high incidence of GBC) were higher than that in Peru, with an intermediate GBC incidence. Although our findings require confirmation by further studies, OTA may be an important factor in the development of GBC in these countries.

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