RESEARCH ARTICLE

Aflatoxin Contamination of Red Chili Pepper From Bolivia and Peru, Countries with High Gallbladder Cancer Incidence Rates

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Abstract

Chilean red chili peppers contaminated with aflatoxins were reported in a previous study. If the development of gallbladder cancer (GBC) in Chile is associated with a high level of consumption of aflatoxin-contaminated red chili peppers, such peppers from other countries having a high GBC incidence rate may also be contaminated with aflatoxins. We aimed to determine whether this might be the case for red chili peppers from Bolivia and Peru. A total of 7 samples (3 from Bolivia, 4 from Peru) and 3 controls (2 from China, 1 from Japan) were evaluated. Aflatoxins were extracted with acetonitrile:water (9:1, v/v) and eluted through an immuno-affinity column. The concentrations of aflatoxins B1, B2, G1, and G2 were measured using high-performance liquid chromatography (HPLC), and then the detected aflatoxins were identified using HPLC-mass spectrometry. In some but not all of the samples from Bolivia and Peru, aflatoxin B1 or aflatoxins B1 and B2 were detected. In particular, aflatoxin B1 or total aflatoxin concentrations in a Bolivian samples were above the maximum levels for aflatoxins in spices proposed by the European Commission. Red chili peppers from Bolivia and Peru consumed by populations having high GBC incidence rates would appear to be contaminated with aflatoxins. These data suggest the possibility that a high level of consumption of aflatoxin-contaminated red chili peppers is related to the development of GBC, and the association between the two should be confirmed by a case-control study.

Keywords: Gallbladder cancer - risk factor - consumption of food contaminated with aflatoxins - HPLC

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Introduction

A high level of consumption of red chili peppers was demonstrated to be an environmental risk factor for gallbladder cancer (GBC) in Chilean women who carry gallstones (GS) in a previous epidemiological study (Serra et al., 2002). However, the pathogenic mechanism by which GBC occurs through red chili pepper consumption in the presence of GS remains unclear. A recent study has shown that the highest incidence rates of GBC are found among populations living in the western parts of the Andes, and in North-American Indians, Mexican Americans and inhabitants of northern India (Wistuba and Gazdar, 2008). In South America, the highest rates were found among women in La Paz, Bolivia, and high incidence rates were found in Trujillo, Peru; Quito, Ecuador; and Cali, Colombia; (Lazcano-Ponce et al., 2001).

Bolivia is believed to be the place of origin of chili peppers known as “Andean peppers”. The consumption of red chili peppers is very popular not only among the Chilean people but also among the Bolivian and Peruvian people. These facts suggest that a high level of consumption of red chili peppers may be a risk factor for the development of GBC in Bolivian and Peruvian people.

Our previous study demonstrated that Chilean red chili peppers are contaminated with aflatoxins B1 and G1 (Tsuchiya el al., 2011). The relationship between exposure to aflatoxin B1 and the development of liver cancer has already been demonstrated by numerous studies (World Health Organization, 2002), and at least two studies have reported the association between exposure to aflatoxin B1 and the development of GBC (Sieber et al., 1979; Olsen et al., 1988). These findings suggest that aflatoxin-contaminated red chili pepper consumption may be associated with the development of GBC. However, to our knowledge, there has been no study on the concentration of aflatoxins in red chili peppers grown in countries having a high GBC incidence rate, in particular, Bolivia and Peru. We therefore hypothesized that Bolivian and Peruvian red chili peppers are contaminated with aflatoxins, as in the case of Chilean red chili peppers. The purpose of this

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study was to measure the concentration of aflatoxins in Bolivian and Peruvian red chili peppers to explain the relationship between aflatoxins-contaminated red chili pepper consumption and the development of GBC.

Materials and Methods

Materials

A total of 7 dry crushed or powdered red chili peppers (3 from Bolivia, 4 from Peru) were purchased at supermarkets in La Paz, Bolivia, and Lima, Peru. As controls, 3 kinds of red chili pepper (2 from China, 1 from Japan) purchased at supermarkets in each country, respectively, were used in this study. Bolivian and Peruvian red chili peppers were sent to our laboratory by international mail.

Extraction of Aflatoxins in Red Chili Peppers

The 10 red chili peppers were powdered using an ultra-centrifugal mill (ZM 200, Retsch Co., Ltd., Tokyo, Japan). Extraction of aflatoxins in red chili peppers was performed using the following process in reference to "Shoku-An No. 0728004" (Ministry of Health, Labour and Welfare, 2008): a total of 50 g of powdered red chili pepper was extracted with 400 ml of acetonitrile:water (9:1, v/v). The mixture of red chili pepper and extraction liquid was blended by a high-speed blender for 5 min and then was dissociated by a centrifuge. A 1-ml aliquot of the filtrate was diluted with 1% Tween 20 in phosphate-buffered saline to 10 ml. The diluted solution was transferred into an immuno-affinity column (AFLAKING, Horiba Co., Ltd., Tokyo, Japan) conditioned through purified water and then passed through the column at a flow rate of one drop per second. Then, the column was washed with 1% Tween 20 in phosphate-buffered saline. Aflatoxins were eluted with 3 ml of acetonitrile from the column. The elution was evaporated under reduced pressure at 40ºC using a rotary evaporator. A 0.1-ml portion of trifluoroacetic acid (TFA) was added to the residue, which was then stored at room temperature for 15 min in darkness. A 0.9-ml portion of acetonitrile:water (1:9, v/v) was added to the TFA mixture solution, and then 20 μl of the solution and aflatoxins standard solutions (each 10 μg/Kg) were subjected to high-performance liquid chromatography (HPLC) analysis.

Measurement of The Aflatoxins, and Their Identification

The concentrations of aflatoxins B1, B2, G1, and G2 in red chili peppers were measured by HPLC analysis using a Hitachi D-2000 Elite system (Hitachi High-Technologies Corporation, Tokyo, Japan). The operating conditions were as follows: column: Atlantis T3 C18 (5-μm particle size, 250 mm x 3.0 mm, Waters Corporation, Milford, Massachusetts, USA); column temperature: 40ºC; 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. Concentrations of aflatoxins were calculated by comparing with the aflatoxins standards. The recovery rates for each 10 μg/Kg of aflatoxins B1, B2, G1, and G2 were 102%, 102%, 104% and 109%, respectively. The detection limit for each aflatoxin was 0.5 μg/Kg.

The detected aflatoxins were identified by HPLC-mass spectrometry (Quattro Micro ESI, Waters Corporation, Milford, Massachusetts, USA) with a high-purity silica gel column (NaviC18, Wako Pure Chemicals, Osaka, Japan).

Results

Table 1 shows the concentrations and recovery rates of aflatoxins B1, B2, G1, and G2 in all of the samples we analyzed. The recovery rates for aflatoxins B1, B2, G1, and G2 were between 101.6% and 109.1%, and they were all in the acceptable range for the analysis (70-120%) defined by the Japanese Government. Aflatoxin B1 was detected in 4 samples, 1 from Bolivia, 2 from Peru, and 1 from China.

As shown in Figure 1, a Bolivian sample was contaminated with 11.3 μg/Kg of aflatoxin B1. Of 10 samples, 6 samples were not contaminated with aflatoxin B1. Aflatoxin B2 was detected only from 1 Bolivian sample, at a concentration of 0.6 μg/Kg. The concentrations of aflatoxin B2 in the remaining 9 samples were below the detection limit. None of the samples was contaminated with aflatoxins G1 or G2.

The Japanese sample defined as the control was not

Table 1. Concentrations and Recovery Rates of Aflaoxins B1, B2, G1, and G2

Red chili peppers	Aflatoxins			
	B1	B2	G1	G2
Bolivia (1)	N.D.	N.D.	N.D.	N.D.
(2)	N.D.	N.D.	N.D.	N.D.
(3)	11.3	0.6	N.D.	N.D.
Peru (1)	0.9	N.D.	N.D.	N.D.
(2)	N.D.	N.D.	N.D.	N.D.
(3)	N.D.	N.D.	N.D.	N.D.
(4)	0.7	N.D.	N.D.	N.D.
China (1)	N.D.	N.D.	N.D.	N.D.
(2)	2.3	N.D.	N.D.	N.D.
Japan (1)	N.D.	N.D.	N.D.	N.D.
Values	10.2	10.2	10.4	10.9
Recovery rate (%)	102.1	101.6	103.6	109.1

*A total of 10 red chili peppers were measured by HPLC analysis using a Hitachi D-2000 Elite system. To calculate the recovery rates, each 10 μg/Kg portion of aflatoxin standard solution was subjected to HPLC analysis. Values are represented as μg/Kg.

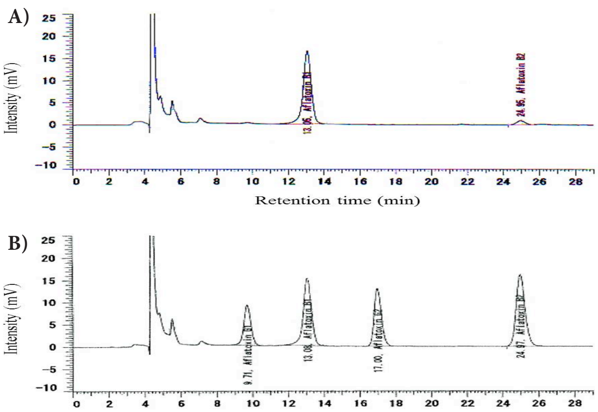

Figure 1. HPLC Chromatograms of Each 10 Mg/Kg Portion of Aflatoxin Standard (A) and Bolivian Red Chili Pepper (B). Aflatoxins B1 and B2 were Detected at Concentrations of 11.3 and 0.6 Mg/Kg, Respectively.

contaminated with any aflatoxins, while the Chinese sample was contaminated with aflatoxin B1.

Discussion

This study demonstrated that aflatoxin B1 or aflatoxins B1 and B2 were detected in red chili peppers from Bolivia and Peru, where the populations have a high incidence rate of GBC. In particular, a Bolivian red chili pepper had a high concentration of aflatoxin B1 (11.3 μg/Kg). However, aflatoxins were not detected in all of the chili peppers measured.

A previous hospital-based case-control study demonstrated that a high level of consumption of red chili peppers is associated with an increased risk of GBC in Chilean women who carry GS (Serra et al., 2002), but whether a pathogenic mechanism occurred in conjunction with a high level of consumption of red chili peppers remained as the issue to clarify. In the present study, we tested the hypothesis that red chili peppers grown in countries having high GBC incidence rates are contaminated with aflatoxins. The rationales underlying this hypothesis are as follows: (1) An association between exposure to aflatoxins and the development of GBC has been shown in previous studies (Sieber et al., 1979; Olsen et al., 1988). (2) Aflatoxins have been reported to be carcinogenic, mutagenic, teratogenic, and immunosuppressive in humans (World Health Organization, 2002). Among this group of aflatoxins, aflatoxin B1 shows the strongest toxicity to humans. (3) Aflatoxins B1 and G1 were detected in Chilean red chili peppers, though at low concentrations (Tsuchiya et al., 2010). All of this evidence suggests that low-level but protracted exposure to aflatoxins may be associated with an increased risk of GBC. If a high level of consumption of aflatoxin-contaminated red chili peppers is associated with the development of GBC, red chili peppers grown in countries having a high GBC incidence rate are possibly contaminated with aflatoxins.

To explain the pathogenic mechanism of the development of GBC by a high level of consumption of aflatoxin-contaminated red chili peppers, we must determine the concentrations of aflatoxins in red chili peppers from countries having a high GBC incidence rate. The data may provide clues to understanding the relationship between the two. However, to our knowledge, no study has determined the concentration of aflatoxins in red chili peppers from countries having a high incidence rate of GBC in South America. Such a study could prove useful, because a recent study has shown that the highest incidence rates of GBC are found among populations living in the western parts of the Andes (Wistuba and Gazdar, 2008).

In South America, the countries with a high GBC incidence rate are Bolivia, Peru, Ecuador, and Colombia (Lazcano-Ponce et al., 2001). We focused on Bolivian and Peruvian red chili peppers. In the present study, of 7 red chili peppers from Bolivia and Peru, 3 samples were contaminated with aflatoxin B1 or aflatoxins B1 and B2. In particular, a Bolivian red chili pepper had a high concentration of aflatoxin B1 (11.3 μg/Kg), aflatoxin B2 was detected at a concentration of 0.6 μg/Kg in only this chili pepper, and the highest concentration of total aflatoxin (11.9 μg/Kg) was found in this chili pepper. The concentrations of aflatoxin B1 and total aflatoxin were above the maximum levels of aflatoxins in spices proposed by the European Commission, that is, 5.0 μg/Kg for aflatoxin B1, and 10.0 μg/Kg for the sum of aflatoxins B1, B2, G1, and G2 (Commission Regulation [EC] No 165, 2010). These findings were consistent with our previous finding that red chili peppers grown in Chile, where the population has a high GBC incidence rate, are contaminated with aflatoxins. The fact that aflatoxins were detected in Bolivian and Peruvian red chili peppers suggests that a high consumption of aflatoxin-contaminated red chili peppers is related to the development of GBC.

We had limitations in the present study. We were unable to collect red chili peppers from areas in Bolivia and Peru with high GBC incidence rates. A previous study found that being South American Indian and being a speaker of Aymara are extremely important risk factors for GBC in Bolivia (Strom et al., 1985). A large population of these people lives on the periphery of Lake Titicaca, located in Bolivia and Peru. Furthermore, the highest GBC incidence rate in Peru was shown in Trujillo (Lazcano-Ponce et al., 2001). Red chili peppers consumed by the people living in these areas may show higher concentrations of aflatoxins. Thus, further studies are needed to determine the concentrations of aflatoxins in red chili peppers from high GBC incidence areas in Bolivia and Peru.

We were also unable to clarify the pollutants in red chili peppers contaminated with aflatoxins. A recent study reported mold incidence such as *Aspergillus flavas* on chili peppers kept in cold storage for few years, and aflatoxin B1 was found in those samples (Kiran et al., 2005). We should clarify the pollutants of aflatoxins in red chili peppers to clarify the causative factors for GBC in Bolivian people.

To our knowledge, this was the first study to determine the concentration of aflatoxins in red chili peppers from Bolivia and Peru having high GBC incidence rates. Since some of the sample peppers were contaminated with aflatoxins just like the Chilean red chili peppers tested in a previous study, we cannot exclude the possibility that aflatoxin-contaminated red chili pepper consumption has the potential for causing GBC in Bolivia and Peru. However, not all of the chili peppers measured were contaminated with aflatoxins. Some of them had concentrations of aflatoxins below the detection limit of our analytical methods. Therefore, comprehensive investigations of the aflatoxin contamination source are needed to address this question.

In summary, the present study indicates that Bolivian and Peruvian red chili peppers are contaminated with aflatoxins. Our findings suggest that aflatoxin-contaminated red chili pepper consumption may play a role to the development of GBC in Bolivian and Peruvian people. However, since our findings showed no direct evidence of any relationship between the two, further investigations, such as a case-control study, are still needed.

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References

Commission Regulation (EC) (2010). Guidance document for competent authorities for the control of compliance with EU legislation on aflatoxins. http://ec.europa.eu/food/food/chemicalsafety/contaminants/guidance-22-03-2010.pdf

Kiran DR, Narayana KJP, Vijayalakshmi M (2005). Aflatoxin B1 production in chillies (Capsicum annuum L.) kept in cold stores. *African J Biotechnol*, **4**, 791-5.

Lazcano-Ponce EC, Miquel JF, Muñoz N, et al (2001). Epidemiology and molecular pathology of gallbladder cancer. *CA Cancer J Clin*, **51**, 349-64.

Ministry of Health, Labour and Welfare (2008). Handling of foods containing mycotoxins (aflatoxins), shoku-an No. 0728004, Tokyo.

Olsen JH, Dragsted L, Autrup H (1988). Cancer risk and occupational exposure to aflatoxins in Denmark. *Br J Cancer,* **58**, 392-6.

Serra I, Yamamoto M, Calvo A, et al (2002). Association of chili pepper consumption, low socioeconomic status and longstanding gallstones with gallbladder cancer in a Chilean population. *Int J Cancer*, **102**, 407-11.

Sieber SM, Correa P, Dalgard DW, et al (1979). Induction of osteogenic sarcomas and tumors of the hepatobiliary system in nonhuman primates with aflatoxin B1. *Cancer Res,* **39**, 4545-54.

Strom BL, Soloway RD, Rios-Dalenz JL, et al (1995). Risk factors for gallbladder cancer. An international collaborative case-control study. *Cancer*, **76**, 1747-56.

Tsuchiya Y, Terao M, Okano K, et al (2011). Mutagenicity and mutagens of the red chili pepper as gallbladder cancer risk factor in Chilean women. *Asian Pac J Cancer Prev*, **12**, 471-6.

Wistuba II, Gazdar AF (2004). Gallbladder cancer: lessons from a rare tumour. *Nat Rev Cancer*, **4**, 695-706.

World Health Organization (2002). IARC monographs on the evaluation of carcinogenic risks to humans. *Aflatoxins*, **82**, 171-300.