ISSN 0025-7680

ORIGINAL ARTICLE

MEDICINA (Buenos Aires) 2002; 62: 313-316

AFLATOXIN B1 CONTENT IN PATIENTS WITH HEPATIC DISEASES

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Abstract Aflatoxins are toxic metabolites of some *Aspergillus flavus, A. parasiticus* and *A. nomius* strains that occur in many foods and feeds. There are four major natural occurring aflatoxins: B1, B2, G1 and G2. These toxins can cause illness in human beings and animals. Aflatoxin B1 is the most abundant and toxic member of the family, and it is also the most potent hepatocarcinogen known. In order to estimate the potential human health risk of AFB1, it is useful to measure blood concentration. The presence of aflatoxin B1 in patients was evaluated by high-performance liquid chromatography, in serum samples, obtained from 20 patient volunteers with hepatic disease. Out of the 20 patients, the presence of AFB1 was detected in only one of them, in a concentration of 0.47 ng/cm^3. Nevertheless, this result should draw the attention of control organizations in Argentina to the need for a thorough food and feed inspection.

Key words: aflatoxin B1, hepatic diseases,serum samples, HPLC

Resumen ***Aflatoxina B1 en pacientes con enfermedades hep·ticas.*** Las aflatoxinas son metabolitos tóxicos producidos por cepas de *Aspergillus flavus, A. parasiticus* y *A. nomius,* presentes en alimentos y piensos. Las cuatro aflatoxinas principales son: aflatoxina B1, B2, G1 y G2. Dichas toxinas pueden causar enfermedades tanto en seres humanos como en animales. La aflatoxina B1 es la más abundante y la más tóxica del grupo y es también el más potente hepatocarcinógeno conocido. El objetivo de este trabajo fue detectar la presencia de aflatoxina B1 en sangre humana para estimar el riesgo potencial de la salud. La determinación de aflatoxina B1 fue realizada por cromatografía líquida de alto rendimiento, en suero de 20 pacientes voluntarios con enfermedades hepáticas. En sólo uno de estos pacientes se detectó la presencia de aflatoxina B1, en una concentración de 0.47ng/cm^3. Estos resultados deberían ser tenidos en cuenta por los responsables de la vigilancia y control de los alimentos en la Argentina.

Palabras clave: aflatoxina B1, enfermedades hepáticas, suero, HPLC

Aflatoxins are secondary metabolites produced by *Aspergillus flavus, A. parasiticus* and *A. nomius.* There are four naturally occurring aflatoxins: aflatoxin B1 (AFB1), B2, G1 and G2, all of them toxic, mutagenic and carcinogenic compounds, especially AFB1. The World Health Organization has recently classified AFB1 as a class 1 carcinogen[1]. Aflatoxins have been detected frequently in samples of corn, peanuts, cottonseeds, rice and other foodstuffs[1-3]. In Argentina, these mycotoxins have been detected in nuts, peanuts[4,5] and corn flour[6]. These data indicate that mycotoxins can enter the human diet via contaminated foodstuffs.

AFB1 is a well-documented hepatocarcinogen in animals, although species susceptibility varies greatly. In human beings, aflatoxins have been implicated in the etiology of hepatocellular carcinoma (HCC). Ecological studies performed in Africa and Southeast Asia have revealed a significant correlation between the aflatoxin exposure and incidence of human HCC[7,8]. The hypothesis that AFB1 has a causative role in the etiology of human HCC is also supported by some other findings in areas with high aflatoxin ingestion. A large fraction of the tumor tissue and adjacent non-malignant liver tissues from HCC patients contained an AGG to AGT mutation at codon 249 of the p53 tumor suppressor gene[9]. This mutation is also induced in cultured Hep G2 human hepatocytes exposed to AFB1[10] .

Since aflatoxin contaminated foodstuffs have been found in Argentina, we thought that it was important to determine whether or not detectable levels of AFB1 are present in human serum samples in our country. In order to estimate the potential human health risk of AFB1, we considered that it was important to measure its blood concentration. Several methods have been used in order to analyze feeds, foods and body fluids (human and animal plasma, serum, milk, etc). Analytical procedures, such us high performance liquid chromatography (HPLC),

Received: 22-III-2002 Accepted: 15-VII-2002

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thin layer chromatography (TLC) and enzymoimmunoassay (ELISA), are commonly used.

The aim of this study was to evaluate the presence of AFB1 in serum samples from twenty patient volunteers with hepatic disease.

Material and Methods

Patients and control sample

Ten milliliters of blood were taken from each of the twenty patients (Hospital Provincial del Centenario), all of whom suffered from hepatic diseases (hepatitis, cirrhosis or others and so on), and were from Rosario (Argentina).

A number of twenty-five normal healthy adult volunteers made up the control group. The patients were between 42 and 64 years old, and 65% were male.

All procedures using human samples were performed in accordance with the ethical standards established by the University of Rosario.

Each subject completed a questionnaire, which included questions on age, sex, occupation (i.e. peasant, factory worker, office employee, private business), residence (close to or far away from silos), hepatitis B antigen (HBsAg) status (carrier or non-carrier), smoking habits (smoking or non-smoking) and alcohol consumption.

The serum samples were stored at 4°C for up to one week.

Aflatoxin analysis

Serum samples in duplicate were extracted using chloroform and hexane. Extracts were evaporated to dryness under a stream of nitrogen, stored at 4°C for up to one week, and analyzed by HPLC, method according to Lamplugh[11].

HPLC was performed using a *Hewlett Packard* HPLC Series 1050 with autosampler and fluorescence detector. The detector was programmed to have excitation/emission wavelengths at 366 and 418 nanometers (nm) respectively. The column was a reverse-phase *Hypersil* C18 column (150 mm x 4.6 mm). A volume of 20 mm^3 of sample was injected into the instrument. The mobile phase consisted of a mixture of methanol-water-acetonitrile (25:25:50 v/v) eluted at a flow rate of 0.8 cm^3/min.

Calibration curve and blank serum samples were analyzed to detect fluorescent compounds, which might interfere with AFB1 analyses.

The identity confirmation test was performed with a serum sample spiked with a final concentration of standard *Sigma* A6636 of 1.6 ng/cm^3 and processed following the same protocol used with patients' samples.

Results

Data concerning the presence of AFB1 in serum and control samples, are summarized in Table 1.

Out of the 20 patients' samples, the presence of aflatoxin B1 was detected in only one of them (Fig. 1-a, Fig. 1-b). The concentration in this sample was 0.47 ng/cm^3. It was determined by comparing the peak height obtained with the calibration curve obtained from spiked serum samples (Fig. 1-c).

TABLE 1.– *Aflatoxin B1 in sera of patients and controls*

Level of aflatoxin B1 in serum	Patients	Controls
Detectable	1	0
Undetectable	19	25
Total	20	25

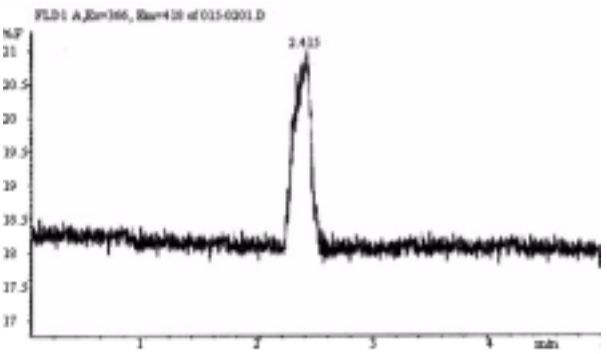

Fig. 1-a.– HPLC chromatogram of human serum extract from a patient with hepatic disease (AFB1: 0.47 ng/cm^3).

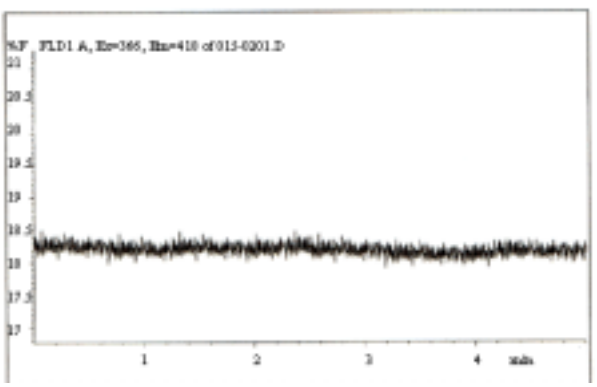

Fig. 1-b.– HPLC chromatogram of a healthy person who has not aflatoxin B1 in his serum.

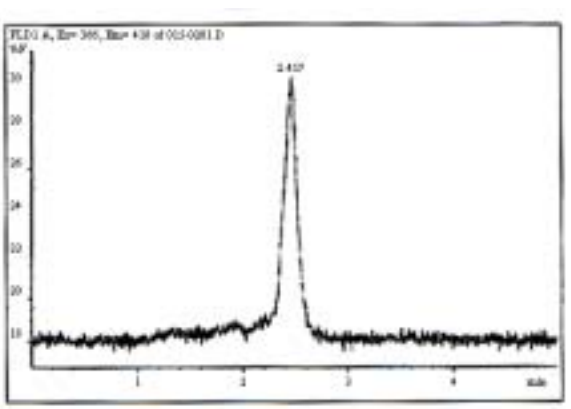

Fig. 1.c.– HPLC chromatogram of Aflatoxin B1 standard (1.6 ng/cm^3) extracted from spiked serum.

TABLE 2.– *Different characteristics of patients (p) and controls (c)*

	Age			Sex		HbsAg Carrier		Smoking		Alcohol drinking	
	30-44	45-54	55-64	F	M	Yes	No	Yes	No	Yes	No
P	15%	40%	45%	35%	65%	15%	85%	75%	25%	30%	70%
C	20%	40%	40%	40%	60%	0%	100%	40%	60%	20%	80%

Storage of extracts of spiked sera as well as extracts obtained from patients' samples at 4°C for periods up to one week did not affect the recovery of AFB1.

The limit of detection for AFB1 determined by analyzing serum samples spiked with decreasing concentrations of aflatoxin B1, was 250 pg/cm^3.

No fluorescent compounds were detected in blank serum samples. The waste solvents were also analyzed, showing no presence of AFB1. The patient whose serum contained AFB1 suffered from cirrhosis.

Table 2, shows the characteristics of the patients and control group, whose samples were analyzed according to occupation, residence, HBsAg carrier condition, smoking and alcohol consumption habits.

The patient whose serum contained aflatoxin B1 was 53 years old, masculine, lived far away from silos, suffered from cirrhosis; he was HBsAg-negative, a non-smoker and an alcohol drinker.

Discussion

A number of clinical reports on acute aflatoxicosis and its relation to chronic hepatic injury and hepatocellular carcinoma due to aflatoxin contaminated food have appeared[12-14] in the world. Aflatoxins are also known to affect and suppress the immune responses of experimental animals[15, 16]. However, no previous report has been made to our knowledge of the detection of aflatoxins in Argentinian patients.

Under natural conditions, exposure to the aflatoxins may occur orally (by food ingestion) and by tracheal and bronchial absorption (by the inhalation of contaminated dust). It is a well-documented fact that absorption following oral exposure is complete[17, 18]. After absorption, the AFB1 is transported via the blood and not via the lymphatic system[19, 20]. Hsieh and Wong found that over 50% of radio-labelled toxin disappeared from the duodenum within one hour, appearing in the venous blood as water-soluble metabolites, protein adducts and free toxin[21]. Protein adducts (in which the AFB1 is linked to the lysine component of serum albumin) represented over 50% of the total radio-activity; the water-soluble metabolites occurred in only minor quantities and free AFB1 represented less than 33% of the labelled material[22, 23]. This could explain the low concentration detected in the patient in question.

The finding of AFB1 in the human serum in an Argentinian patient, to the best of our knowledge, is the first such report. However, it could not reach significance probably due to the small number of samples involved.

The finding of only one patient with AFB1 in serum, did not allow to study the correlation between mycotoxicosis and some other factors, such as age, sex, hepatic disseases, habits, etc.

The patient with positive result neither was a peasant nor lived close to silos, so we can conclude that the patient was not exposed to aflatoxins by contaminated dust. The detection of AFB1 in his serum may suggest that the patient ate food contaminated with this micotoxin.

Detection of the contamination of food products by filamentous fungi excludes them from the food chain in developed countries. Unfortunately, this action is infrequent in many developing countries, and contaminated foodstuffs enter into the diet. If it were possible, it would be ideal to avoid its entry into commerce in every country.

Since some toxins may persist even when the fungi are no longer there, it is important to consider the fact that in foodstuffs it must beenanalyzed not only for the presence of toxigenic fungi but also for the presence of mycotoxins. Besides, the presence of toxigenic fungi in foods must alert the autorities about a potential hazard in foodstuffs that are not immediately consumed and could be stored in inadequate conditions.

Our data indicate that, as in many other countries, AFB1 is present in food or feed products available in Argentina, and in order to protect the health of consumers, their regular control is desirable.

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De todas las máquinas que ha construido el hombre, la más interesante, es, a mi juicio, el reloj, artefacto específicamente humano, que la mera animalidad no hubiera inventado nunca. El llamado *homo faber* no sería realmente *homo* si no hubiera fabricado relojes. Y en verdad, tampoco importa mucho que los fabrique; basta con que los use, menos todavía, basta con que los necesite. Porque el hombre es el animal que mide su tiempo.

Antonio Machado (1875-1939)

Juan de Mairena II (1943). 4ta. Edición. Buenos Aires: Losada, 1968. Capítulo XI, p 9