Preliminary study of Ochratoxin A in human plasma in agricultural zones of Chile and its relation to food consumption

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Abstract

Ochratoxin A (OTA) is a mycotoxin produced by different species of Aspergillus and Penicillium fungi. The presence of OTA in human blood has been reported in many studies, especially in Europe, however none have been done in South America. In this study, 88 blood samples from healthy donors from two different Chilean agricultural zones were analyzed. In parallel with sample collection, the donors filled a questionnaire regarding food intake during the last three months. The blood samples were collected in Colbún in March and July of 2004 and in San Vicente de Tagua – Tagua in October of 2004. The extraction procedure was done in the solid phase with a Sep-Pak® RP-18 cartridge and a final purification with immunoaffinity Ochrprep® columns. The presence of OTA was confirmed by the formation of Ochratoxin A methyl ester. Fifty four percent of the samples collected in Colbún and 91% of samples from San Vicente de Tagua – Tagua were positive to OTA at ranges of 0.07–2.75 ppb and 0.22–2.12 ppb, respectively. The OTA levels in serum did not show a good correlation with normal dietary consumption.

Keywords: Ochratoxin A; Plasma; Mycotoxin; Chile; HPLC

1. Introduction

Mycotoxins are toxic metabolites produced by fungi under specific conditions. These metabolites have been found as contaminants in several types of food (Pittet, 1998). Ochratoxin A (OTA) is a metabolite produced by what are known as “store” fungi, such as Aspergillus ochraceus (Harris and Mantle, 2001), Aspergillus niger (Blumenthal, 2004), Aspergillus carbonarius, Penicillium verrucosum, etc. OTA has been found in different foods like pork meat (Jørgensen and Petersen, 2002), coffee (Studer-Rohr et al., 1995), barley and associated products like beer (Visconti et al., 2000), wheat, rice, oats (Solfrizzo et al., 1998) and wine (Blesa et al., 2004; Serra et al., 2004).

OTA produces an inhibition of protein synthesis and lipid peroxidation by oxidative processes (Gautier et al., 2001; Omar et al., 1996). These mechanisms may generate nephrotoxic, neurotoxic and immunotoxic effects (Luhe et al., 2003; Schaaf et al., 2002; Álvarez et al., 2004). OTA is considered by the International Agency for Research on Cancer as possibly carcinogenic for humans (group 2B) (IARC, 1993), and also teratogenic and carcinogenic effects have been described in some animal species (Veselá et al., 1983; Lioi et al., 2004). The toxic-kinetic parameters of OTA have not been well established in humans, but in some animal studies OTA showed high availability after oral administration (Fuchs and Hult, 1992; Omar et al., 1996). OTA binds rapidly and with high affinity to plasma proteins with increased T1/2 values (Mally et al., 2005). The metabolism in humans is basically produced through cytochrome P-450 (Dortant et al., 2001) generating less toxic metabolites (Fazecas et al., 2005) which are eliminated in urine.
and feces (Li et al., 2000, 1997). OTA can cross the placenta and is also excreted in animal milk (Valenta and Goll, 1996; Breitholtz-Emanuelsson et al., 1993).

In many countries, OTA analysis has been done to detect its presence in the human population and has frequently been found in blood and milk samples. These results indicate a continuous and widespread distribution of this mycotoxin (Assaf et al., 2004; Rizzo et al., 2002; Ueno et al., 1998; Thuvander et al., 2001). For that reason, maximum tolerance levels were established for some foods such as cereal and its derivatives which have a range of 0.5–10 ppb (EC, 2005). The relationship between OTA in human blood and dietary intake of the toxin has not been totally investigated. This research is a preliminary study of OTA presence in human plasma in central-south agricultural zones of Chile and its possible relation with food intake.

2. Materials and methods

2.1. Collection of blood samples

Eighty eight blood samples were collected from healthy blood donors during one year in three stages. The samples were taken in different seasons in the central-south agricultural area of Chile. The chosen zones were Colbún and San Vicente de Tagua – Tagua. Forty four samples from the Colbún zone were taken at two time periods: March 2004 and July 2004, and the 44 samples from San Vicente de Tagua – Tagua were taken in October 2004.

Blood samples were taken by venous withdrawal using a syringe and Vacutainer® tubes previously conditioned with the anticoagulant K3EDTA. Plasma was separated by centrifugation at 3000 rpm for 15 min at room temperature. Samples were decontaminated by heating at 60 °C for 2 h. The plasma samples were stored in plastic Eppendorf® tubes at −20 °C.

2.2. Analysis of Ochratoxin A

Ochratoxin A standard (CAS number 303-47-9, Sigma–Aldrich Co, USA) was dissolved in methanol and calibrated spectrophotometrically at 333 nm using the extinction coefficient 6400 M−1 cm−1 (Pohland et al., 1992). OTA analyses were done by high performance liquid chromatography (HPLC) using the method of Scott et al. (1998). The methodology consists of a solid extraction phase with Sep-Pak® RP-18 cartridges and further purification with an immunoaffinity Ochraprep® column.

The plasma samples were analyzed by HPLC using a Merck-Hitachi system composed of: quaternary pump Lachrom L-7000, Rheodyne injector with 20 µl loop, fluorescence detector F-1000 and data acquisition system with Varian Star 4.0 Software. Mobil phase: acetonitrile + methanol + o-phosphoric acid 0.15 M; (1 + 1 + 1 v/v/v) at a flow rate 0.8 ml/min. Column Waters Symmetry™ C18, 3.9 × 150 mm, 5 µm. Fluorescence detection was done with an excitation and emission wavelength of 333 nm and 470 nm, respectively. Sample confirmation of OTA was done following the method of Zimmerli and Dick (1995) for formation of the methyl ester derivative. The retention time was 5.6 min for OTA and between 8.5 and 9 min for OTA methyl ester using the same chromatographic conditions.

2.3. Calculation of Ochratoxin A intake based on plasma levels

The mean of OTA level in blood plasma was expressed as continuous dietary intake according to the Klassen equation (Directorate General-Health and consumer protection, 2002; Thuvander et al., 2004):

\[
K_d = \frac{C_l \times C_p}{A} = 1.97 \times C_p
\]

\[
K_d \quad \text{continuous dietary intake (ng/kb bw/day)}
\]

\[
C_l \quad \text{plasma clearance (ml/kg bw/day)}
\]

\[
C_p \quad \text{plasma concentration of OTA (ng/ml)}
\]

\[
A \quad \text{bioavailability, estimated as 50%}
\]

2.4. Collection of food consumption data

All volunteer donors answered a food questionnaire with 26 questions about their alimentary habits during the last three months previous to blood sample withdrawal. The food questionnaire was prepared based on the local alimentary habits. The questions included foods susceptible to contamination with OTA. The answers were based on frequency of food intake (daily, once per week; once per two weeks and once per month) and dose (portions) and expressed in g/day of food. Finally, the foods chosen were pork meat, chicken meat and all kinds of cereals, including cereals as raw material and their derivatives because these kinds of foods showed the highest consumption in the present study and furthermore, they agree with the European Union Ochratoxin A-report that indicates the contribution of these foods to OTA intake is near to 50%. Consumption of other foods, including fruits, considered in this questionnaire did not show a significant intake, therefore, they were not relevant to the purpose of this study. Since no information exists concerning food commodity contamination in Chile, this point will be a topic for further studies.

2.5. Statistical analysis

STATS® software was used for the statistical analysis. Previous to the correlation analyses of OTA levels in blood and food intake, the differences between women and men in relation to OTA levels in plasma were also studied. The Pearson correlation was used to determine the correlation between plasma levels and food consumption.

3. Results

3.1. Validation parameters

The calibration curve showed a linear range between 0.1 ppb and 37.5 ppb with a determination coefficient (r^2) of 0.999. Sensitivity was estimated by the Standard Error method with the limits of detection (LOD) and quantification (LOQ) being 0.1 ppb and 0.4 ppb, respectively. The recovery levels were calculated at three points: 1 ppb, 2 ppb and 5 ppb; each level with three replicates resulting in recoveries of over 95% for all levels. Repeatability and reproducibility studies were performed with five replicates at a spiking level of 5 ppb and presented a relative standard deviation of 2.7% and 5.7%, respectively (Chan et al., 2004; Miller and Miller, 2002). The chromatographic conditions used were adequate for analysis, OTA and OTA methyl ester (Fig. 1).

3.2. OTA plasma levels

OTA was detected in 62 of the 88 samples (Table 1) with a mean level of 0.44 ppb and 0.77 ppb for Colbún and San Vicente de Tagua – Tagua, respectively. The levels in San Vicente de Tagua – Tagua were significantly higher in relation to the Colbún zone with a probability of significant difference (PSD) of 99.99% (t = 4.06481). Both zones showed a different distribution; the main group in Colbún...
was in the range of 0–0.4 ppb while those in San Vicente de Tagua – Tagua were between 0.41 ppb and 1.20 ppb (Graphic 1). It should be noted that the samples from both zones were not collected at the same time.

In relation to the differences between women and men, no significant differences were found in the zone of Colbún (PSD = 10.02%, \(t = 0.1266\)), however, in the zone of San Vicente de Tagua – Tagua the mean from women was significantly higher than that from men (PSD = 98.78%, \(t = 2.6193\)).

### 3.3. OTA continuous dietary intake calculated from plasma levels and food consumption

The OTA continuous dietary intake was expressed as ng/kg bw/day. The results obtained from plasma levels were calculated using the Klassen equation. The OTA continuous dietary intake in all cases was lower than the Tolerable Daily Intake (TDI) defined by the International Scientific Committee on Ochratoxin A in 2002 (Table 2) (EC, 2002). The means for each zone were 0.84 ng/kg bw/day and 1.40 ng/kg bw/day, with San Vicente de Tagua – Tagua having a higher average (PSD = 99.91, \(t = 3.4489\)).

![Chromatograms for OTA sample (2.5 ng/ml) and OTA sample methyl ester.](image)

**Table 1**

Ochratoxin A levels in the agricultural zones of Colbún and San Vicente de Tagua – Tagua

<table>
<thead>
<tr>
<th></th>
<th>Colbún</th>
<th>San Vicente de Tagua – Tagua</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td>Mean (ng/ml)</td>
<td>0.42 ± 0.72</td>
<td>0.47 ± 0.81</td>
</tr>
<tr>
<td>Positive samples (%)</td>
<td>16/28 (57%)</td>
<td>6/16 (38%)</td>
</tr>
<tr>
<td>Range</td>
<td>0.07–2.75</td>
<td>0.10–2.75</td>
</tr>
<tr>
<td></td>
<td>0.88 ± 0.40</td>
<td>0.51 ± 0.38</td>
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<tr>
<td></td>
<td>24/25 (96%)</td>
<td>16/19 (84%)</td>
</tr>
<tr>
<td></td>
<td>0.29–2.12</td>
<td>0.22–1.31</td>
</tr>
</tbody>
</table>

### 3.4. Analysis of food consumption

The differences between women and men in relation to the consumption in g/day of food were previously calculated. According to the statistical test, cereal consumption in men from Colbún was significantly higher than in women with a PSD of 99.64% \(t = 3.0829\). The same situ-
ation was observed with pork consumption (PSD = 83.57%, t = 1.4154). With respect to chicken consumption, a difference was also observed (PSD = 87.00%, t = 1.5445) but in this case the consumption was higher in women.

In the San Vicente de Tagua – Tagua zone, no significant difference in cereal consumption between women and men was found (PSD = 37.70%, t = 0.4953). Pork consumption was higher in men (PSD = 91.47%, t = 1.7624) and chicken consumption did not present a significant difference (PSD = 10.36%, t = 0.1310) (Table 3).

The higher cereal consumption in the Colbún zone may be a consequence of the economic activity of this zone (cereal crop) and a rural population of >70% (CENSO Chile, 2002).

3.5. Correlation between OTA and food consumption

OTA levels in plasma from women in Colbún did not show a significant correlation with cereals (r = 0.2361), pork (r = 0.1662) or chicken (r = 0.3282) consumption. In the case of men, a slight correlation for cereal consumption (r = 0.4501) was seen, but the data only showed a tendency. No correlation with other foods was found (r < 0.1). Similar results were found in the region of San Vicente de Tagua – Tagua. There was no correlation between OTA plasma levels and consumption of cereals and pork (all results with r < 0.2) in both women and men. Concerning chicken consumption, a tendency was found (r = 0.5580) in the men’s group.

4. Discussion

Our results have allowed us to establish the presence of OTA in human plasma in the central-south agricultural region of Chile. In both populations, the incidences of positive values for OTA in blood were over 50%. With respect to the levels found, the averages were similar to those reported in other countries, except in San Vicente de Tagua – Tagua where the women’s group presented values higher than other reports (Ueno et al., 1998; Thuvander et al., 2001; Grosso et al., 2003).

Both zones have different economic activities; the Colbún zone is characterized for its cereal crop, for the presence of small mills and for a large rural area. Nevertheless, the mean OTA plasma levels were lower than San Vicente de Tagua – Tagua, a city with a high activity in fruit crops and a rural population of only 45% (CENSO Chile, 2002). There are no significant differences in OTA levels between woman and men from the Colbún zone, but in the San Vicente de Tagua – Tagua zone, the OTA levels were significantly higher in woman than in men.

Table 3
Daily average intake of pork and chicken meat and all cereals and derivatives in grams of food/day

<table>
<thead>
<tr>
<th></th>
<th>Colbún</th>
<th>San Vicente de Tagua – Tagua</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Women (g/day)</td>
<td>Men (g/day)</td>
</tr>
<tr>
<td>Cereals</td>
<td>229.40 ± 98.71</td>
<td>329.56 ± 110.13</td>
</tr>
<tr>
<td>Pork</td>
<td>14.79 ± 8.67</td>
<td>22.83 ± 28.67</td>
</tr>
<tr>
<td>Chicken</td>
<td>52.49 ± 29.08</td>
<td>39.37 ± 24.62</td>
</tr>
</tbody>
</table>
Correlations between OTA levels in plasma and food consumption were not significant, with the exception of cereal consumption by men in Colbún and chicken consumption in San Vicente de Tagua – Tagua. It was not possible to identify the specific food responsible for the OTA contribution. A slight correlation was observed between cereal consumption in Colbún men and chicken consumption in San Vicente de Tagua – Tagua men.

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References


