

Occupational exposure to aflatoxin B1 and ochratoxin A: Co-exposure in swine production

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ABSTRACT

The most common scenario in occupational settings is the co-exposure to several risk factors. This aspect has to be considered in the risk assessment process because can alter the toxicity and the health effects when dealing with a co-exposure to two or more chemical agents. A study was developed aiming to elucidate if there is occupational co-exposure to aflatoxin B1 (AFB1) and ochratoxin A (OTA) in Portuguese swine production. To assess occupational exposure to both mycotoxins, a biomarker of internal dose was used. The same blood samples from workers of seven swine farms and controls were considered to measure AFB1 and OTA. Twenty one workers (75%) showed detectable levels of AFB1 with values ranging from <1 ng/ml to 8.94 ng/ml and with significantly higher concentration when compared with controls. In the case of OTA, there wasn't found a statistical difference between workers and controls and the values for workers group ranged from 0.34 ng/ml to 3.12 ng/ml and 1.76 ng/ml to 3.42 ng/ml for control group. The results suggest that occupational exposure to AFB1 occurs. However, in the case of OTA results, seems that food consumption plays an important role in both groups exposure. The results claim attention for the possible implications on health of this co-exposure.

Keywords: mycotoxins, occupational exposure, co-exposure, risk assessment

1. INTRODUCTION

It is well established that people are normally exposed to a mixture of environmental stressors. However, toxicological studies normally focus on health effects resulting from exposure to a single chemical agent. Recent published work has showed that, in multiple simultaneous exposures, the most common case in occupational settings, toxicity is normally changed (Sexton and Hattis, 2007), and a specific chemical that don't have toxicity when exposure occurs alone can present toxicity when part of a mixture. Taking all this in consideration is of extreme relevance to refine the risk assessment methods to permit to contemplate the co-exposure scenario in occupational settings.

Mycotoxins are secondary metabolites produced by fungi and these low molecular weight natural contaminants can produce several health effects in humans and animals (Duarte et al., 2010). Moreover, most of the variables contributing to the presence or production of mycotoxins include environmental and ecological conditions that are normally difficult to control (Hussein and Brasel, 2001).

Aflatoxins are secondary metabolites produced predominantly by *Aspergillus flavus* and *Aspergillus parasiticus* fungi species (IARC, 1993). Among 18 different types of aflatoxins identified, major members are aflatoxins B1, B2, G1 and G2. Aflatoxin B1 (AFB1) is normally predominant in cultures as well as in food products and has been shown to be genotoxic and a potent hepatocarcinogen (IARC 1993).

Ochratoxins are also a relevant group of mycotoxins, which involve risk to the health of humans and animals. Due to a several toxic effects, ochratoxin A (OTA) is considered to be of especial interest to study due to the reported nephrotoxic, hepatotoxic, embryotoxic, teratogenic, neurotoxic, immunotoxic, genotoxic and carcinogenic effects (Pfohl-Leszkowicz and Manderville, 2007). In 2003, IARC classified OTA as human carcinogen of 2B group and is considered to be a possible human carcinogen on the basis of a wide evidence of carcinogenicity in several animal studies.

Although the existence of extensive literature on the ingestion of food contaminated with AFB1 and OTA, only a small number of studies explore the exposure in occupational settings (Halstensen, 2008) and a smaller number have paid attention to the cases of co-exposure to both mycotoxins. Considering this possibility of co-exposure, it must be ponder the additive effect reported in experimental studies regarding the interaction between these two mycotoxins and the significant risk that represents to human health (Speijers & Speijers, 2004).

Swine confinement buildings are prone to fungal and their metabolites contamination (Harting et al., 2012). Activities normally performed in swine farms involve high dust aerosolization and, consequently, result in wide spread of fungi and their metabolites, such as volatile organic compounds and mycotoxins (Tsapko et al., 2011).

Considering all the above mentioned and after taking in account data published recently about fungal contamination (Viegas et al., 2012) and AFB1 occupational exposure in swine production setting (Viegas et al., 2013), it was decide as relevant to assess also the occupational exposure to OTA.

Therefore this study was developed aiming to elucidate if there is occupational co-exposure to AFB1 and OTA in Portuguese swine production facilities.

2. MATERIALS AND METHOD

To assess occupational exposure to AFB1, a biomarker of internal dose was used as described in a previous research (Viegas et al. 2013). It was used the same blood samples from workers of seven swine farms and controls to measure OTA, although in different numbers: 24 workers instead of 28 and only 21 controls instead of 30 controls.

As justified previously (Viegas et al., 2013, 2014) the control group was considered in order to know the OTA background levels of the Portuguese population. This group was composed of subjects who conducted administrative tasks in an educational institution without any type of activity known to involve exposure to OTA. The same approach was followed by other authors (Mayer et al., 2003; Degen et al., 2011). The measurement of OTA in serum was performed by ELISA.

2.1 Blood Sample Preparation

All blood samples were subjected to centrifugation to obtain serum then stored at -20°C until analysis. Five hundred microliters of serum was incubated for 18 h with pronase (Calbiochem, 50 U per 5 mg protein) before application to pre-wet C18 column (RIDA C18 column, R-Biopharm). The column was washed with 5 ml 5% methanol to remove small peptides and amino acids. The fraction containing ochratoxin was eluted with 80% methanol, which was posteriorly evaporated under a nitrogen stream and diluted to reach a 10% methanol solution. The eluate was then applied to an immunoaffinity column (Ochraperp Ochtratoxin; R-Biopharm) and the Ochtratoxin-containing fraction was eluted with 1 ml methanol in phosphate buffer 0.1 M, pH 7.4 (1:1), after rinsing the column with phosphate-buffered saline (PBS).

2.2 ELISA Assay

For ochratoxin quantification, the RIDASCREEN Ochtratoxin A30/15 enzyme-linked immunosorbent assay (ELISA; R Biopharm) was used, and was calibrated with Ochtratoxin standards from 0 to 1800 ng/L. For testing, samples or standards were pipetted into the wells already coated with specific antibodies against OTA. Free and enzyme conjugated OTA compete for the OTA antibody binding sites. Any unbound enzyme conjugate is then removed in a washing step. Indicator color was obtained by adding a substrate/chromogenic solution to each well and the reaction was stopped after 15 min with a termination solution. Absorbance was measured at 450 nm and results were assessed with Ridasolf Win software version 1.73 (R Biopharm).

2.3. Statistical Analysis

The statistical analysis of the data was performed using SPSS version 22.0. In the statistical analysis we used the Spearman correlation analysis, since the assumption of normality (the Shapiro-Wilk test) data was lacking. For comparison of blood contamination with AFB1 and the OTA used the Wilcoxon Signed Ranks Test. The results are considered significant at a significance level of 5%.

3. RESULTS AND DISCUSSION

As already published, twenty one workers (75%) showed detectable levels of AFB1 with values ranging from <1 ng/ml to 8.94 ng/ml and with significantly higher concentration when compared to controls (all below $<$ LOD) (Viegas et al., 2013). In the case of OTA results, there wasn't found a statistical difference between workers and controls and the values for workers group ranged from 0.34 ng/ml to 3.12 ng/ml and 1.76 ng/ml to 3.42 ng/ml for controls group. Considering only the workers group, there was no significant correlation of blood contamination between AFB1 and OTA ($r_s = 0.291$, $p = 0.168$).

Additionally, statistically significant differences by AFB1 and by OTA were detected, verifying that AFB1 has significantly higher levels ($z = -2.072$, $p = 0.019$) (Table 1).

Table 1- Results of descriptive measures and Wilcoxon signed Ranks Test

	Count	Median	Interquartil range	Ranks			Test Statistics ^d		
				N	Mean Rank	Sum of Ranks	z	p (one sided)	
AFB1	24	1.67	1.28	Negative Ranks	17 ^a	13.09	222.50	-2.072 ^e	.019
				OTA - AFB1 Positive Ranks	7 ^b	11.07	77.50		
OTA	24	1.40	0.43	Ties	0 ^c				
				Total	24				

a. OTA < AFB1
b. OTA > AFB1
c. OTA = AFB1

d. Wilcoxon Signed Ranks Test
e. Based on positive ranks.

A biomarker of internal dose was used providing information regarding exposure to both mycotoxins and also exposure intensity and absorption rates (Iavicoli et al., 2002; Viegas et al., 2013).

The obtained results suggest that occupational exposure to AFB1 occurs and can be related with different causes and contamination sources present in this occupational setting (Viegas et al., 2013). Moreover, our findings lead to the conclusion that exposure is happening essentially by inhalation. Probably, the high contamination by dust found previously on these farms units (Viegas et al., 2013) is promoting AFB1 exposure since dust acts as carrier of AFB1 to the workers breathing zone and mouth. Regarding OTA results, probably food consumption plays an important role in both groups exposure. In previous studies developed in Portugal (Pena et al., 2006; Duarte et al., 2010), in order to evaluate population exposure to this mycotoxin, found OTA in a high prevalence in biological fluids. Other research work presented data showing the presence of OTA in Portuguese food products, such as wine grapes and wine (Serra et

al., 2006) and wheat and maize bread (Juan et al., 2008; Duarte et al., 2010). For instance, the high consumption of bread in Portuguese population probably contributes significantly to the OTA exposure found in the individuals enrolled on the study (Duarte et al., 2010).

AFB1 and OTA are both genotoxic, but with important differences in the mechanism of action of each one (Corcuera et al., 2011). Previously, in vitro studies showed that the mixture showed cytotoxic additive effects and also a slight increase in DNA fragmentation as compared to mycotoxins taken in separately experiments (El Golli-Bennour et al., 2010). However, more recently, one in vitro study showed different results: OTA significantly decreased DNA damage promoted by AFB1 in Hep G2 cells. The author's claimed the possibility of AFB1 and OTA compete for the same metabolic pathway leading to less AFB1 adducts (Corcuera et al., 2011). Further studies need to be developed due to probable common co-exposure to these mycotoxins in different occupational settings and the fact of exposure occurring by different exposure routes (inhalation for AFB1 and ingestion for OTA) must be explore.

4. CONCLUSIONS

This is the first research work developed in Portugal that tries to assess occupational co-exposure to two genotoxic mycotoxins. The results claim attention for the complexity of the exposure scenarios and the possible implications on health of this co-exposure.

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6. REFERENCES

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