Food and Chemical Toxicology xxx (2015) 1–7

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Biomonitoring of concurrent mycotoxin exposure among adults in Sweden through urinary multi-biomarker analysis

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ARTICLE INFO

Article history:
Received 29 January 2015
Received in revised form
20 May 2015
Accepted 21 May 2015
Available online xxx

Keywords:
Concurrent exposure
Diet
Mycotoxins
Urinary biomarkers
Human biomonitoring

ABSTRACT

Mycotoxin producing moulds may contaminate numerous agricultural commodities either before harvest or during storage. A varied diet consisting of different foods may therefore be contaminated with a range of mycotoxins. The aim of the present study was to study concurrent exposure to mycotoxins through urinary multi-biomarker analysis, as well as its possible associations with the diet.

Urinary samples from 252 adults, participating in the Swedish national dietary survey Riksmaten 2010–11, were collected together with a 4-day diet record. Concurrent mycotoxin exposure was studied using a multi-biomarker LC-MS/MS method. The results revealed that exposure to mycotoxins is common and concurrent exposure to more than one toxin was found in 69% of the study population. However, when comparing the number of toxins detected with the reported consumption data it was difficult to distinguish food patterns which would indicate an increased risk of exposure to many mycotoxins simultaneously.

This is the first study to investigate concurrent mycotoxin exposure and urinary levels of fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), nivalenol (NIV), ochratoxin A (OTA), zearalenone (ZEA), α-zearalenol (α-ZOL), β-zearalenol (β-ZOL) and de-epoxydeoxynivalenol (DOM-1) among adults in Sweden.

1. Introduction

Mycotoxins are naturally occurring secondary metabolites of fungi commonly found to contaminate large volumes of staple food (Magan and Olsen, 2004). Although there are geographical and climatic differences as well as substantial year to year fluctuations in the occurrence of mycotoxins in foodstuffs, exposure to these natural contaminants may be unavoidable (Bennett and Klich, 2003; Magan and Olsen, 2004). Mycotoxins are important from a health perspective as they display a wide range of deleterious effects, including hepatotoxicity, nephrotoxicity, teratotoxicity, haematotoxicity, immunotoxicity and hormonal or reproductive effects but with different potencies depending on species and sex (Cortinovis et al., 2013; el Khoury and Atoui, 2010; Marin et al., 2013; Voss et al., 2007; Zinedine et al., 2007). Some mycotoxins, such as the main aflatoxins, aflatoxin B_1, B_2, G_1 and G_2 (AFB_1, AFB_2, AFG_1 and AFG_2) have been classified by the International Agency for Research on cancer (IARC) as carcinogenic to humans whereas OTA and FB_1 have been classified as possibly carcinogenic to humans (International Agency for Research on Cancer, 1993, 2002). Some mycotoxins, such as deoxynivalenol (DON), also cause acute effects and have been suggested as an etiological factor in several cases of human gastroenteritis (Bhat et al., 1989; Li et al., 1999; Yoshizawa et al., 1983).

However, humans are often exposed to more than one mycotoxin at the same time. This simultaneous exposure is due to several factors including the ability of some fungi to produce a number of mycotoxins simultaneously, as well as the fact that food commodities may be contaminated by several fungi. Moreover a varied diet consists of several food items and may therefore be contaminated with a range of toxins. It is therefore relevant from a public health perspective to study simultaneous mycotoxin exposure. In recent years there has been a swift development in

Abbreviations: AFB_1, aflatoxin B_1; AFB_2, aflatoxin B_2; AFG_1, aflatoxin G_1; AFG_2, aflatoxin G_2; AFM_1, aflatoxin M1; DON, deoxynivalenol; DOM-1, de-epoxydeoxynivalenol; FB_1, fumonisin B_1; FB_2, fumonisin B_2; NIV, nivalenol; OTA, ochratoxin A; ZEA, zearalenone; α-ZOL, α-zearalenol; β-ZOL, β-zearalenol.

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http://dx.doi.org/10.1016/j.fct.2015.05.023
0278-6915/

analytical methods resulting in the possibility of studying concurrent exposure through method assays capable of determination of concomitant mycotoxins in foods, as well as mycotoxin biomarkers in blood or urine (Capriotti et al., 2012; Solfrizzo et al., 2011, 2014; Warth et al., 2012, 2013). Biomarkers is a valuable tool in measuring exposure at the individual level while avoiding the problems associated with dietary registration and the heterogeneous contamination of mycotoxins in food which otherwise may hamper exposure assessments (Bryden, 2007; Solfrizzo et al., 2011; Timbrell, 1998; Turner et al., 2012, 2011).

Recently, a study concerning the levels of urinary DON showed that exposure to DON was very common (>90%) in the Swedish adult population (Wallin et al., 2013). To further explore the levels and possible patterns of mycotoxin exposure among the Swedish population, a multi-biomarker assay including six different mycotoxins or metabolites (aflatoxin M_1 (AFM_1), DON, DOM-1, FB_1, FB_2, NIV, OTA, ZEA, α-ZOL and β-ZOL) was used. Consumption data from the latest Swedish national survey investigating dietary habits among adults, Riksmaten 2010–11 (National Food Agency, 2012), was used in this study to analyse dietary patterns associated with the number of mycotoxins found in the urinary samples.

2. Material and methods

2.1. Study design and population

The study population took part in Riksmaten 2010–11, the latest Swedish national survey (National Food Agency, 2012) investigating dietary habits among adults. The Riksmaten study was conducted over one year, between May 2010 and July 2011, to capture seasonal variations. Participants completed a self-assisted web based diet record over four consecutive days and a questionnaire covering background information and information about foods less frequently eaten. The participants also donated spot samples of urine which were collected on average 19 ± 34 days after the first day of dietary recording. Urine samples were collected at the Occupational and Environmental Medicine Centers in seven regions in Sweden. An equal number of individuals were randomly selected in each region independent of the population size. In total, 300 individuals provided spot urine samples (participation rate 30%). All participants gave oral informed consent before entering the study. The study was approved by the regional ethical committee in Uppsala (National Food Agency, 2012). The study population, dietary registration and sample collection is described in detail by Bjermo and collaborators and Wallin and collaborators (Bjermo et al., 2013; Wallin et al., 2013).

2.2. Urine analysis

Samples were sent frozen from the regional centres and distributed via the National Food Agency (Uppsala, Sweden) for analysis. The samples were sent by courier to the Institute of Sciences of Food Production (ISPA) in Bari (Italy) in May 2013 for mycotoxin (DON, NIV, FB_1, FB_2, ZEA, and OTA) and metabolite (AFM_1, DOM-1, α-ZOL and β-ZOL) analysis. Due to insufficient amounts of urine or miscoding only 252 samples were included for further analyses of the results.

2.3. Determination of urinary mycotoxin biomarkers

Urinary biomarkers (DON, DOM-1, AFM_1, FB_1, ZEA, α-ZOL, β-ZOL and OTA) were determined as previously described (Solfrizzo et al., 2011, 2014) with some modifications. Briefly, a 5 mL urine sample, instead of 6 mL, was incubated at 37 °C for 18 h with 250 μL of β-glucuronidase/sulfatase type H-2 from *Helix* pomatia (Sigma Aldrich, Milan, Italy), diluted with 5 mL of water, instead of 6 mL, and purified on a Myco6in1^{+TM} IAC (Vicam, Watertown, MA, USA) and OASIS® HLB (Waters, Milford, MA, USA) SPE column connected in tandem. The OASIS® HLB column was previously conditioned by passing 2 mL MeOH and 2 mL ultrapure water. After sample application and elution, the two columns were separated, the Myco6in1^{+TM} was washed with water (4 mL, discarded portion) and eluted with methanol (3 mL) and water (2 mL) and collected in a vial. The OASIS® column was washed with methanol/water (20:80, 1 mL) and was discarded. DON that had passed through the Myco6in1^{+TM} and retained on the OASIS® was eluted with methanol/water (40:60, 1 mL) that was collected in the vial containing the eluates from the Myco6in1^{+TM} column. The combined eluates were dried under air stream at 55 °C and reconstituted in methanol/water (20:80, 200 μL) containing 0.5% acetic acid. Purified extract was filtered through a Minisart® RC 4 syringe filter (Sartorius, Muggiò, MB, Italy) and a volume of 10 μL (equivalent to 0.25 mL urine sample) was analysed by UPLC-MS/MS.

The analyses were performed on a triple quadrupole API 5000 mass spectrometer (Applied Biosystems, Foster City, CA, USA), equipped with a ESI interface and an Acquity UPLC system comprising a binary pump and a microautosampler from Waters (Milford, MA, USA). The analytical column was an Acquity UPLC BEH phenyl column (2.1 × 150 mm, 1.7 μm particles; Waters) set at 40 °C. The flow rate of the mobile phase was 250 μL/min. For multi-biomarker separation a linear gradient of MeOH (containing 0.5% acetic acid) in water (containing 0.5% acetic acid) was used as mobile phase as follows: 20–80% MeOH in 5 min, 80% MeOH for 5 min, 80%–20% MeOH in 0.5 min and left to equilibrate for 4.5 min before the next run. For UPLC-MS/MS analyses, the ESI interface was used in positive ion mode for AFM_1, FB_1 and OTA and in negative ion mode for DON, DOM-1, ZEA, α-ZOL and β-ZOL.

Due to the cross reactivity of Myco6in1^{+TM} for NIV and FB_2, these mycotoxins were included in the panel of analytes determined by UPLC-MS/MS and were monitored in negative and positive mode, respectively. The mass spectrometer operated in MRM (multiple reaction monitoring) mode. MS/MS conditions of all analytes are reported elsewhere (Lattanzio et al., 2014, 2007). The biomarkers were quantified using matrix-matched standards and results were corrected for method recovery. To prepare matrix-matched calibration solutions, urines from 21 individuals were mixed and 5 mL aliquots were purified according to the protocol reported above. After purification, the combined eluates were spiked with appropriate amounts of each standard solution. The spiked extracts were dried down and reconstituted in 200 μL of methanol/water (20:80) with 0.5% acetic acid and filtered through a Minisart® RC 4 syringe filter. The ranges of equivalent biomarker concentrations in urine for the five matrix-matched calibration solutions were: DON 0.2–120.4 ng/mL, DOM-1 0.89–29.82 ng/mL, AFM_1 0.01–7.00 ng/mL, FB_1 0.01–12.19 ng/mL, ZEA 0.01–12.00 ng/mL, α-ZOL and β-ZOL 0.04–24.72 ng/mL, OTA 0.006–5.010 ng/mL. NIV and FB_2 were quantified with a single point of matrix-matched calibration solution specifically prepared for these two mycotoxins. Urinary creatinine was determined with Test™ Creatinine Enzymatic (Instrumentation Laboratory, Milan, Italy).

The analytical method used in the present study is a multi-biomarker method designed to include six mycotoxins (DON, ZEA, FB_1, FB_2, OTA & NIV) and four metabolites (AFM_1, DOM-1, α-ZOL & β-ZOL). All samples with detectable levels and <LOQ were considered as positives. When calculating the mean in all samples, values reported as < LOQ were assumed to be at LOQ. In the assessment of concurrent mycotoxin exposure, metabolites were considered as an indicator of exposure to the mother mycotoxin, i.e. samples where α-ZOL or β-ZOL were present but not ZEA were regarded as samples positive for the mother mycotoxin.

Please cite this article in press as: Wallin, S., et al., Biomonitoring of concurrent mycotoxin exposure among adults in Sweden through urinary multi-biomarker analysis, Food and Chemical Toxicology (2015), http://dx.doi.org/10.1016/j.fct.2015.05.023