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ORIGINAL ARTICLE

Occurrence of ochratoxin A in *Astragalus propinquus* root and its transfer to decoction

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Abstract

The aim of this study was to conduct a survey assessing (a) the ochratoxin A (OTA) content in different samples of *Astragalus propinquus* root (AR), one of the fundamental herbs in traditional Chinese medicine, and (b) the rate of OTA transfer to AR decoctions that are traditionally used to reduce general weakness and increase overall vitality. A validated method of high-performance liquid chromatography with fluorescence detection (HPLC-FLD) was used to determine OTA concentrations in AR samples and AR decoctions. The limit of quantification was 0.35 ng/g; the recovery of the HPLC method for AR samples was 82%; and the relative standard deviation (SD) of repeatability was 2.6%. All 40 tested AR samples were positive, with a mean value of 451.0 ng/g (range, 28.8–1700.0 ng/g). The transfer rate of OTA to decoctions, from a naturally contaminated and homogenized AR sample (internal reference material) with a concentration of OTA of 288.9 ng/g ± 12.3 (SD), was 83.4% ± 8.5 (SD). We believe it is necessary to continue OTA monitoring in AR and other herbal products, estimate the actual human usual intake, and perform health risk assessment.

Keywords *Astragalus propinquus* Schischkin · Herbal food supplement · Herbal products · HPLC-FLD · Ochratoxin A · Traditional Chinese medicine

Introduction

Astragalus propinquus Schischkin (syn. *Astragalus membranaceus* (Fisch.) Bunge) (*Fabaceae*) (reviewed by Roxas and Jurenka 2007) is one of the fundamental herbs in Traditional Chinese Medicine (Chang et al. 2012). It is used to treat different kinds of diseases and symptoms (Cho and Leung 2007; reviewed by Jiang et al. 2010), and its consumption has increased over the last decade (Huang et al. 2018). The plant root contains more than 100 bioactive components (Xu et al. 2006) such as triterpene saponins (primarily astragalosides) (reviewed by Ionkova et al. 2014), polysaccharides, isoflavonoids, and many others (Aldarmaa et al. 2010; reviewed by Fu et al. 2014). It has a wide range of beneficial effects: cardiotonic and analgesic (reviewed by Rios and Waterman 1997; Verotta and El-Sebakhy 2001), immunomodulating (Bedir et al. 2000), anti-inflammatory (Choi et al. 2007), antihyperglycemic (Chan et al. 2009), antioxidant and antiviral (Zhu et al. 2009), and hepatoprotective (Chinese Pharmacopoeia Commission 2010). Despite these beneficial effects, we found it necessary to ensure the quality and safety of these herbs (reviewed by Chen et al. 2017), frequently contaminated during growth, collection, transportation, and storage, by toxigenic microfungi that can produce mycotoxins (Halt 1998).

A preliminary study on toxigenic microscopic fungi in the *Astragalus propinquus* root reported the presence of *Aspergillus* section *Nigri*, a known producer of ochratoxin A (OTA) (reviewed by Samson and Pitt 2000). In terms of public health, OTA is an important mycotoxin of concern (reviewed

Chemical compound studied in this article: ochratoxin A (PubChem CID: 442530)

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by Bhat et al. 2010). Consequently, in line with our previous study of long-term monitoring of OTA in foodstuffs performed in the Czech Republic (Ostry et al. 2015), we undertook to study in the same country the OTA content of this new potential exposure source that is the *Astragallus propinquus* root.

OTA presents many toxic effects, such as carcinogenicity, genotoxicity, nephrotoxicity, hepatotoxicity, immunotoxicity, neurotoxicity, embryotoxicity, and teratogenicity (reviewed by Pfohl-Leszkowicz and Manderville 2007; reviewed by Heussner and Bingle 2015). OTA has been classified by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to humans (group 2B) on the basis of sufficient evidence in experimental animals for its carcinogenicity (IARC 1993). OTA reclassification from group 2B to group 2A (probably carcinogenic to humans) has been recently discussed (reviewed by Ostry et al. 2017).

Due to the toxic effects of OTA, a tolerable weekly intake (TWI) of 120 ng/kg body weight (bw) has been set by the EFSA (2006), and the limits for OTA in foodstuffs in the European Union were set by Commission Regulation (EC) No. 1881/2006 and its supplements the following years (EU 2006). The Expert Committee meeting on Agricultural Contaminants in food (European Commission, DG Health and Food Safety) is considering setting limits for herbal teas, infusions, and decoctions.

The present work consisted in determining the OTA concentration in *Astragallus propinquus* roots and assessing the OTA transfer rate to decoctions, using a high-performance liquid chromatography method with fluorescence detection (HPLC-FLD). Decoctions were studied because they are traditionally used to reduce general weakness and increase overall vitality (Ma et al. 2011) and because ochratoxins are generally relatively heat stable: boiling has no significant effect, while baking and roasting can reduce the toxin content by 20% (Puntaric et al. 2001; reviewed by Bullerman and Bianchini 2007).

Material and methods

Chemicals and immunoaffinity columns

A stock standard solution of OTA (Sigma-Aldrich, s.r.o., Prague, Czech Republic) was prepared by dissolving 1000 μg of OTA standard in 100 mL of pure methanol, obtaining a 10-μg OTA/mL solution. This stock solution was diluted with methanol in order to obtain three appropriate work solutions (0.40, 4.0, and 40 ng/mL). The OTA solutions were stored in amber vials at 4 °C, until analysis by liquid chromatography coupled with a fluorescence detector (LC-FLD). Acetonitrile, methanol, water (all of LC grade), and acetic acid were purchased from Merck (Prague, Czech Republic). Ultrapure water was prepared by Milli-Q Plus (Millipore, Billerica, MA, USA). Other information about chemicals and reagents or materials are described in detail in Malir et al. (2014).

Immunoaffinity columns Ochraprep® were manufactured by R-Biopharm (Darmstadt, Germany) and delivered by Jemo Trading s r.o. (Bratislava, Slovak Republic).

Sample collection

A total of 40 randomly chosen *Astragalus propinquus* root (AR) samples were purchased from drugstores from 25 different locations in the Czech Republic from 2015 to the first third of 2016, to obtain root samples of different batches over 15 months. AR samples were purchased in different forms—chopped, crushed, or powdered. The origin of the samples is most probably China, but we were unable to confirm the traceability by requests made to suppliers. The samples were preserved in dry atmosphere and in the dark, according to the manufacturer's recommendations, and the time limit for storage was not exceeded. All samples were homogenized for HPLC analysis.

In order to assess OTA transfer to AR decoction, it was prepared 400 g of internal reference material (naturally contaminated and homogenized AR sample) in which the concentration of OTA was 288.9 ng/g ± 12.3 (SD). OTA concentration was measured by HPLC and a Cochran test of homogeneity was done: the matrix was confirmed as homogenized (statistical test indicated $C = 0.32$, which is lower than the 5% critical value $C\alpha = 0.56$).

Preparation of AR decoction

Each decoction was prepared by addition of 3 g of internal reference material of AR to 300 mL of water, bringing to a boil, and boiling was continued for 15 min on the basis of recommendations of suppliers and following pharmaceutical procedures. Subsequently, the decoction was mixed and filtrated.

OTA extraction and cleanup of AR sample and AR decoction

Extraction was performed according to the method of Zimmerli and Dick (1995) modified by Malir et al. (2014). The same method was used for the extraction of OTA from decoctions, except that—for the first step—5 mL of stirred decoction was extracted directly by chloroform.

High-performance liquid chromatography determination OTA in AR samples and AR decoctions

The validated method of reversed-phase high-performance liquid chromatography with fluorescence detection (HPLC-FLD) was used for OTA determination in AR samples and AR decoctions. Chromatographic conditions are described in Malir et al. (2014). The retention time of OTA was around 8.2 min. The OTA concentrations were quantified using the calibration curve method as described in Dohnal et al. (2013). The recovery of the HPLC method for AR samples was 82%, and the average relative standard deviation of repeatability was 2.6%. The limit of detection (LOD) for OTA in AR was 0.1 ng/g, and the limit of quantification (LOQ) was 0.35 ng/g. LOD and LOQ of OTA were calculated by taking the average noise signal and adding 3 and 10 standard deviations of the noise, respectively.

The confirmation of the presence of OTA was realized by derivatization (esterification) of OTA with BF_3-methanol leading to the formation of the OTA methyl ester under the same HPLC chromatographic conditions (Creppy et al. 1993), and the retention time of the OTA methyl ester was shifted to 17.5 min instead of 8.2 min for OTA.

Results and discussion

Occurrence of OTA in AR samples

One hundred percent of AR samples ($n = 40$) were OTA positive; concentrations ranged from 28.8 to 1700.0 ng/g, with a mean value of 451.0 ng/g, median value of 210.0 ng/g, and 90th percentile value of 1618.0 ng/g. These results document the high OTA concentrations and contamination frequency observed in samples of *Astragallus propinquus* root sold in the Czech Republic. Concentrations were higher compared with the range of 87.7–158.7 ng/g reported in the study of Yang et al. (2010) performed on three samples (described as strongly moldy) from three provinces in China. A more recent study by Zhou et al. (2016) reported only one positive sample (out of 9) from China at a level of 3.98 ng/g.

Occurrence of OTA in contaminated AR decoctions

Results of OTA occurrence in 12 decoctions prepared from the internal reference material (OTA concentration, 288.9 ng/g ± 12.3 [SD]) were as follows: mean concentration, 2.41 ng/mL ± 0.25 SD; median, 2.41 ng/mL; 90th percentile, 2.68 ng/g.

The percentage transfer rate was calculated based on the formula:

$$T_p = \frac{C_f \cdot V}{C_m \cdot a} \cdot 100$$

where T_p is percentage transfer rate, C_f final concentration of OTA in decoction (ng/mL), V final amount of decoction (300 mL), C_m concentration of OTA in internal reference material (288.9 ng/g), and a amount of used matrix (3 g).

The OTA average transfer rate from the AR to the decoctions was calculated to be 83.4% ± 8.5 (SD). The results of this study confirm that a significant amount of OTA can be transferred to the decoctions similarly to tea or coffee beverages (Malir et al. 2014).

At present, WHO estimates that over 100 million Europeans use traditional Chinese medicine, and one fifth of them use it regularly for health care (reviewed by WHO 2013). AR can be consumed by most of the population age groups, but traditional herbalists recommend that AR should not be used for more than 3 weeks without close follow-up and careful monitoring. Indeed, the dose range per day is broad, because it may vary according to the type and severity of the conditions treated and to the individual patient condition (reviewed by Kemper and Small 1999). Different suppliers and sources recommend 4–30 g of dry AR as a decoction per day (reviewed by Bone and Mills 2013).

Considering the results of our study, with 451 ng OTA/g AR (mean OTA concentration from our samples), the transfer rate, and assuming a daily consumption of 9 g dry AR through decoctions, we can estimate a OTA usual intake of 0.024 mg/person/week. Assuming a 60-kg bw consumer (EFSA 2006), this intake already exceeds the TWI more than three times. For the highest consumption and contamination levels, the estimated OTA intake of 0.30 mg OTA/person/week would exceed more than forty times the TWI which can be a problem due to exposure to OTA from other dietary exposure sources (e.g., foodstuffs) which can also be major contributors to OTA exposure.

This is an early warning for pharmacists, physicians, and manufacturers, distributors, and consumers of *Astragalus propinquus*-based herbal products and a call to continue not only OTA, but also other mycotoxins' monitoring and estimate of their actual human usual intake. Due to the precautionary principle, we believe it is necessary to continue OTA monitoring in AR and other herbal medicines and food supplements. In addition, it is also necessary to ensure the quality and safety of those products prepared "as consumed" (e.g., decoction) and not only "as purchased" (e.g., AR).

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Compliance with ethical standards

Conflict of interest The authors confirm that there are no known conflicts of interest associated with this publication.

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