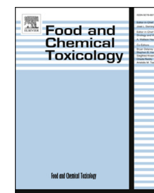
Food and Chemical Toxicology 98 (2016) 210–219

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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



Aflatoxin B_1-lysine adduct in dried blood spot samples of animals and humans

Kathy S. Xue [a, b], Wenjie Cai [a], Lili Tang [a], Jia-Sheng Wang [a, b, *]

[a] Department of Environmental Health Science, College of Public Health, University of Georgia, Athens, GA, USA
[b] Interdisciplinary Toxicology Program, College of Public Health, University of Georgia, Athens, GA, USA

ARTICLE INFO

Article history:
Received 6 September 2016
Received in revised form
30 October 2016
Accepted 1 November 2016
Available online 2 November 2016

Keywords:
Aflatoxin
Dried blood spots

ABSTRACT

Dried blood spots (DBS) were proposed as potentially viable method for exposure assessment of environmental toxicants in infant and young children. For this study, we validated an experimental protocol to quantify AFB_1-lysine adduct in DBS samples of AFB_1-treated F344 rats, as well as samples from human field study. Significant dose-response relationships in AFB_1-lysine adduct formation were found in DBS samples of rats treated with single- and repeated-dose AFB_1. AFB_1-lysine levels in DBS samples were highly correlated with corresponding serum sample levels. The Person coefficients were 0.997 for the single-dose exposure, and 0.996 for the repeated-dose exposure. Levels of AFB_1-lysine adduct had also good agreement between DBS and serum samples as shown by Bland-Altman plot analysis. For human field study samples ($n = 36$), a Pearson correlation coefficient of 0.784 was found between AFB_1-lysine adduct levels of DBS and corresponding serum samples. Bland-Altman plots showed the distribution of the log differences between DBS and serum AFB_1-lysine levels are within 95% confidence intervals. These results showed AFB_1-lysine adduct levels in DBS cards and serum samples from animals and human samples are comparable, and the DBS technique and analytical protocol is a good means to assess AFB_1 exposure in infant and children populations.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Aflatoxins, produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus*, have been one of the most well-known risk factors to liver cancer worldwide, and aflatoxin B_1 (AFB_1) has been classified as group 1 human carcinogen by International Agency for Research on Cancer (IARC, 1993, 2012). Toxic effects of AFB_1 have been widely explored in both animal studies and human epidemiological studies. Acute exposure to high levels of AFB_1 can lead to lethal aflatoxicosis, while chronic low level exposures have been linked with high incidence of hepatocellular carcinoma as well as growth stunting (Asim et al., 2011; Chao et al., 1994; Etzel, 2014; Groopman et al., 1996; Henry et al., 2002; Kensler et al., 2011; Knipstein et al., 2015; Tillett, 2010; Van Rensburg et al., 1985; Wang et al., 1996, 2001). As a ubiquitous contaminant among groundnuts- and corn-based foods, the primary route of exposure has been through oral consumption of food contaminated with AFB_1, which has been a major food safety issue and public health concern for decades, particularly in developing countries. Studies in recent years have suggested a strong link between childhood stunting, a developmental disorder with various short-term and long-term adverse health outcome, and chronic dietary exposure to mycotoxins, particularly AFB_1 (de Onis and Branca, 2016; Groopman et al., 2014; IARC, 2015; Watson et al., 2015). These increased concerns over AFB_1 exposure and its adverse health effect have placed new demands on the efficiency, viability, and convenience of the exposure assessment methods among the young populations.

Serum AFB_1-lysine adduct is considered to be the most reliable biomarker for assessing chronic exposure to AFB_1. As shown by Fig. 1, this adduct is formed as a part of metabolic pathway of AFB_1. Following absorption, AFB_1 is activated by CYP1A2 and 3A4 in human liver into reactive AFB_1-8,9-epoxides, which subsequently react with DNA to form AFB-N^7-guanine adduct, a highly unstable DNA adduct which underwent depurination readily and are excreted as the guanine base adduct in urine (Guengerich et al., 1998; Kensler et al., 2011). By-products of epoxides can also bind to albumin, the only protein shown to bind AFB_1 on a significant level, with lysine the primary amino acid group for the covalent

Abbreviations: AF, aflatoxins; AFB_1, aflatoxin B_1; AFB_1-lysine, aflatoxin B_1-lysine; CYP450, cytochrome P-450; DMSO, dimethyl sulfoxide; SPE, solid phase extraction.

* Corresponding author. 150 Green Street, Athens, GA 30602, USA.
E-mail address: jswang@uga.edu (J.-S. Wang).

http://dx.doi.org/10.1016/j.fct.2016.11.002
0278-6915/© 2016 Elsevier Ltd. All rights reserved.

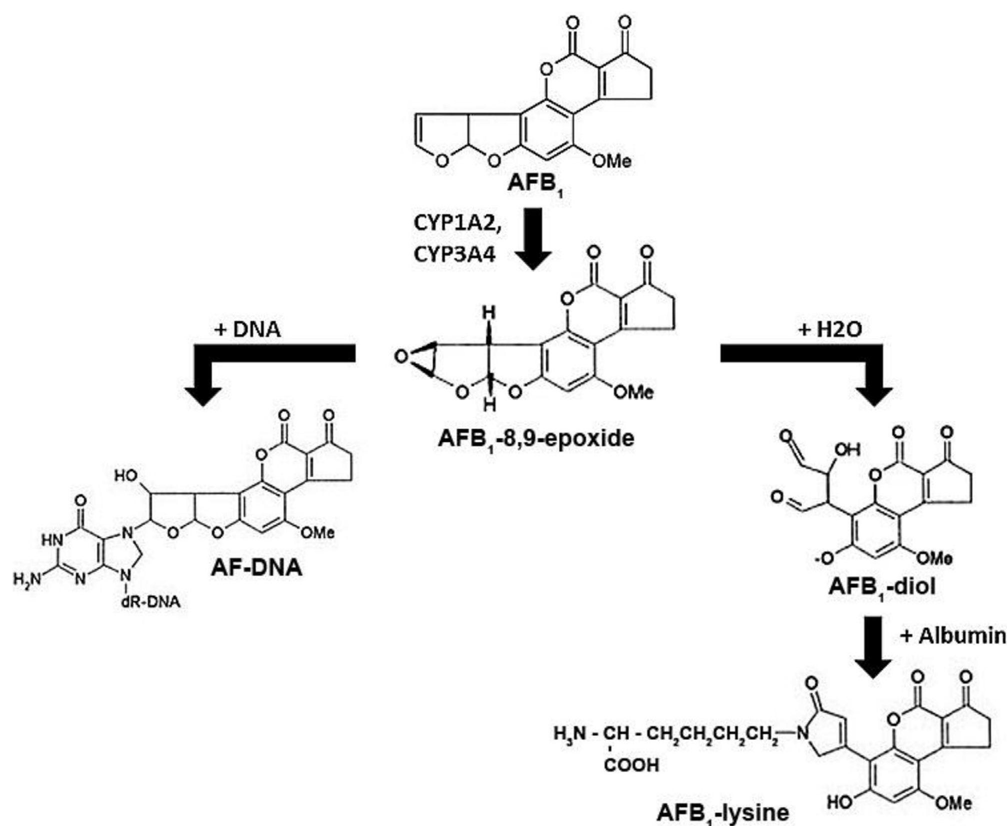


Fig. 1. Metabolic pathway and major DNA and protein adducts of AFB_1. Depicted here including phase I metabolic activation and formation of DNA adduct, AFB-N^7-guanine, and the albumin adduct, AFB_1-lysine.

binding of AFB_1-diol (Sabbioni et al., 1987). Due to the longer half-life as compared to AFB-N^7-guanine, AFB_1-lysine adduct has been considered as a reliable biomarker for assessment of chronic AFB_1 exposure (Kensler et al., 2011). Upon enzymatic digestion, AFB_1-lysine adducts were released from albumin in blood and can be quantified using various methods, including immunoassays, high-performance liquid chromatography (HPLC) with fluorescence detection, and isotope dilution mass spectrometry (IDMS) (McCoy et al., 2008; Sabbioni et al., 1987, 1990). Studies have found significant association between AFB_1 exposure and serum AFB_1-lysine levels in both experimental animals and human studies (Qian et al., 2013; Sabbioni et al., 1990; Wild et al., 1992, 1996). This biomarker has also been used to evaluate various intervention strategies (Groopman et al., 2014; Kang et al., 2015; Kensler et al., 2011; Leroy et al., 2015; Mitchell et al., 2016; Tang et al., 2008; Xu et al., 2010).

Dried blood spots (DBS) have emerged as an attractive alternative to serum sample collection. This technique, popularly used in new-born congenital disease screening first developed by Robert Guthier (Sharma et al., 2014), was proposed as a potential means for screening biomarkers for exposure assessment and epidemiology, due to its less invasive nature, reduced health hazards, and ease of storage and shipment (Lehmann et al., 2013; McDade, 2014). However, to our knowledge, there are currently few studies on assessing aflatoxin exposure using DBS samples in young populations. The aims for the current study is thus to validate a previously developed method for quantification of AFB_1-lysine levels in DBS samples, using both samples from animals treated with single- and repeated-doses of AFB_1, as well as human DBS samples of known AFB_1 exposure.

2. Materials and methods

2.1. Reagents and chemicals

Unlabelled aflatoxin B_1 (>98% purity), dimethyl sulfoxide (DMSO), albumin determination reagent (bromocreosol purple), and normal rat serum were purchased from Sigma Aldrich Chemical Company (St. Louis, MO). ELISA kit for human albumin analysis (ab108788) was obtained from Abcam (Cambridge, MA). Pronase (25 kU, Nuclease-free) was purchased from Calbiochem (La Jolla, CA). Protein assay dye reagent concentrate and protein standards were from Bio-Rad Laboratories Incorporation (Hercules, CA). Mixed mode solid phase extraction (SPE) cartridges were purchased from the Waters Corporation (Milford, MA). All other chemicals and solvents were of highest grade and purity available. Normal human whole blood purchased from Biological Specialty Corporation (Colmar, PA, 18915). AFB_1-lysine standard used in this study was synthesized as previously described by Sabbioni et al. (1987). AFB_1-lysine has been confirmed via both UV spectrophotometry and LC/MS. Confirmation and quantification of AFB_1-lysine standard is established using UV spectrum, where absorbance at 400 nm was used and adjusted with published molar extinction coefficient of 30,866 ($Lmol^{-1}cm^{-1}$) as described by Sabbioni (1990). The contents are further confirmed via LC/MS, with selective reaction monitoring transition of 457 (m/z)–394 (m/z) for AFB_1-lysine, as described in figure caption of supplemental Fig. 1.

2.2. Sample preparation and extraction

Seven commercial DBS cards from different commercial sources

Download English Version:

https://daneshyari.com/en/article/5560411

Download Persian Version:

https://daneshyari.com/article/5560411

Daneshyari.com