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Short communication

Physiologically based toxicokinetics of serum aflatoxin B_1-lysine adduct in F344 rats

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ABSTRACT

Aflatoxin B_1-lysine adduct (AFB-Lys) is a reliable biomarker for aflatoxin exposure; however, a systematic toxicokinetic evaluation has not been reported. In this study, male F344 rats were orally exposed to single, or repeated, doses of AFB_1 and the toxicokinetics of serum AFB-Lys that followed treatments were investigated. A single-dose of AFB_1 increased serum AFB-Lys levels rapidly peaking at 4 h, followed by first-order elimination, through which the half-life was estimated to be 2.31 days. A physiologically based pharmacokinetic model showed that approximately 3.00–3.90% and 1.12–1.98% of the administered AFB_1 doses were converted to serum AFB-Lys adducts at 2 h and 24 h post treatment, respectively. Repeated AFB_1 exposure at 5–25 μg/kg body weight linearly increased serum AFB-Lys levels for 5 weeks in animals, resulting in a 1–1.5 times higher AFB-Lys level overall. This indicates the potential of this adduct as a reliable biomarker for repeated low dose exposure. Higher dose exposure at 75 μg/kg increased the level of AFB-Lys to a maximum at 2 weeks, followed by a gradual decrease to near plateau level up to 5 weeks. In conclusion, this study systematically evaluated the toxicokinetics of serum AFB-Lys adduct in F344 rats using a physiologically based pharmacokinetic model and robust statistical modeling analysis and provided a firm and clear understanding of the toxicokinetics of this biomarker.

1. Introduction

Aflatoxins (AF) are ubiquitous food contaminants produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus*. Humans exposed to AF contaminated food are subject to a wide range of health effects, including acute poisoning (aflatoxicosis), hepatocellular carcinoma (HCC) and stunting in children (IARC, 1993; Liu and Wu, 2010; Smith et al., 2012; Williams et al., 2004). The uncovering of the etiological role of AF exposure is closely associated with the development and application of molecular markers relevant to AF exposure or risk (Kensler et al., 2011; Qian et al., 1994).

The toxicology of aflatoxin B_1 (AFB_1) has been detailed elsewhere (Eaton and Groopman, 1994). AFB_1 is metabolically activated by liver cytochrome P450 (CYP450) 1A2 and 3A4 to AFB_1-8,9-epoxide which can readily bind DNA to form AFB_1-N^7-guanine adduct (Essigmann et al., 1977) or react with albumin to produce AFB_1-albumin adduct if not conjugated with glutathione (Sabbioni et al., 1987). It has been shown that albumin is the only protein in serum that binds AFB_1 to a significant level (Skipper et al., 1985). Lysine is the primary amino acid group for the covalent binding of AFB_1 to albumin. Under enzyme digestion, AFB_1-lysine (AFB-Lys) is released from the adducted albumin (Sabbioni et al., 1987). This biomarker has been widely used to indicate human exposure status (Azziz-Baumgartner et al., 2005; Wang et al., 1996) and to assess human chemoprevention strategies (Tang et al., 2008; Wang et al., 2008). Several previous studies have reported the partial toxicokinetics of the AFB-Lys adduct in animal models (Sabbioni et al., 1987; Scholl et al., 2006; Wild et al., 1986, 1996). However, a complete study design relevant to human exposure (route, dose and duration) is lacking, and further studies are warranted to demonstrate the physiologically based toxicokinetics of the serum AFB-Lys biomarker.

Abbreviations: AF, flatoxins; AFB_1, aflatoxin B_1; AFB-Lys, aflatoxin B_1-lysine; CYP450, cytochrome P-450; DMSO, dimethyl sulfoxide; GST, glutathione S transferase; HCC, hepatocellular carcinoma; HPLC, high performance liquid chromatography; SPE, solid phase extraction.

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2. Materials and methods

2.1. Chemicals and materials

Unlabelled aflatoxin B_1 (>98% purity), dimethyl sulfoxide (DMSO), albumin determination reagent (bromocreosol purple), and normal rat serum were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO). Pronase (25 kU, Nuclease-free) was purchased from Calbiochem (La Jolla, CA). Protein assay dye reagent concentrate and protein standards were purchased from Bio-Rad Laboratories Inc. (Hercules, CA). Mixed mode solid phase extraction (SPE) cartridges were purchased from the Waters Corp. (Milford, MA). Authentic AFB-Lys was synthesized as previously described (Sabbioni et al., 1987). All other chemicals and solvents were of highest grade and purity available.

2.2. Animals

Male F344 rats (120–130 g) were purchased from Harlan Laboratories Inc. (Indianapolis, IN) and maintained under controlled conditions of temperature (22–25 °C), humidity (50–70%), and light/dark cycles (12 h/12 h). Animals were fed with AIN-76A diet (Teklad, Madison, WI) and acclimated for one week before treatment. Feed and water were provided *ad libitum*. Animal use and care and the experimental protocol were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Georgia.

2.3. Experimental design

For the single-dose study, rats (5 per group) were gavaged with 0, 50, 250, or 1000 μg AFB_1/kg body weight (BW). AFB_1 was dissolved in DMSO with a gavage volume of 50 μL/100 g BW. Animals were anesthetized and blood was collected by cardiac puncture at 2, 4, 8 h, and 1, 3, 5, 7, 14, and 21 days after treatment. For the repeated-dose study, rats (25 per group) were gavaged daily with 0, 5, 10, 25, or 75 μg/kg BW for up to 5 weeks (5 days per week). Animals (5 per group) were anesthetized and blood samples were collected at weekly intervals and 24 h after the last dose. Serum samples were prepared and stored at -20 °C until analysis.

2.4. Serum sample processing and quantification of AFB-Lys adducts

The method for serum processing and quantification of AFB-Lys adducts has been reported previously (Qian et al., 2010). Albumin and total protein levels in serum were determined as previously described (Wang et al., 1996). Aliquots of each serum sample (150 μL) were digested by pronase (pronase:total protein, 1:4, w:w) at 37 °C for 3 h to release AFB-Lys adducts. The digests were purified using Waters MAX SPE cartridges. After priming with methanol and equilibration with water, the loaded cartridge was sequentially washed by water, 70% methanol, and 1% ammonium hydroxide in methanol at a flow rate of 1 mL/min. The eluate in 2% formic acid in methanol was vacuum-dried with a Labconco Centrivap concentrator (Kansas City, MO) and reconstituted for HPLC-fluorescence detection. The average recovery rate was 90%.

The Agilent 1200 HPLC-fluorescence system (Santa Clara, CA) was used for quantification of the AFB-Lys adducts. The mobile phases consisted of buffer A (20 mM $NH_4H_2PO_4$, pH 7.2) and buffer B (100% Methanol). The Zorbax Eclipse XDB-C18 reverse phase column (5 μm, 4.6 × 250 mm) was used and 100 μL was injected at a flow rate of 1 mL/min. A gradient was generated to separate the AFB-Lys adduct within 25 min and the retention time of AFB-Lys was equal to approximately 12.3 min. AFB-Lys adduct was detected by fluorescence at the excitation and emission wavelengths of 405 nm and 470 nm, respectively. Quality assurance and quality control procedures were taken during analyses, which included simultaneous analysis of one authentic standard and a quality control sample daily. The limit of detection was 0.4 pg/mg albumin. The serum AFB-Lys level of each sample was adjusted by its albumin content accordingly.

2.5. Statistical analysis

The curves of serum AFB-Lys formation and elimination after a single dose were fitted with zero-, first-, and second-order kinetic functions, which were used to determine the appropriate model for AFB-Lys kinetics. The lowest-order model with acceptable residuals ($r^2 > 0.90$) was considered to optimally describe the elimination curve. All modeling and residual determinations were performed with Sigma Plot 10.0 software (San Jose, CA) using the least sum of squares method. The half-life was computed from the fitting of the model selected.

3. Results

3.1. Single dose study

Following a single-dose of AFB_1, the levels of AFB-Lys in serum rapidly increased at 2 h and reached a peak at 4 h, followed by a non-linear decrease (Fig. 1). Linear dose–response relationships were found between AFB_1 doses and serum AFB-Lys levels at both 2 and 24 h post treatment (see supplementary Fig. 1A and B, $r^2 = 0.98$ and 0.99, respectively). In addition, the elimination of serum AFB-Lys followed first-order kinetics for both 50 ($r^2 = 0.9829$, $p < 0.001$) and 250 μg/kg groups ($r^2 = 0.9745$, $p < 0.0001$) (see supplementary Table 1). Non-linear decreases of serum AFB-Lys after 4 h were demonstrated based on model analysis for the 50 and 250 μg/kg groups (Fig. 2A and B). Significant linear decreases were also observed with the log (AFB-Lys) over time for the 50 and 250 μg/kg groups. Half-lives of AFB-Lys were estimated to be 2.35 and 2.27 days from the curve fittings of the data from the 50 and 250 μg/kg groups, respectively. The half-life was estimated based on the integrated slope (λ, as the regression coefficient of the fitted model) of the log (AFB-Lys) over time curves, *i.e.*, $t_{1/2} = 0.693/\lambda$ (Fig. 2C and D). The conversion ratios of AFB_1 to AFB-Lys following a single dose treatment were determined to be 3.00–3.90% at 2 h or 1.12–1.98% at 24 h post treatment (see supplementary Tables 2 and 3), as estimated by a physiologically based pharmacokinetic model.

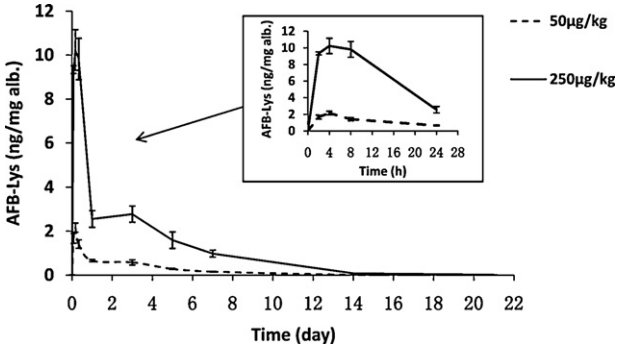

Fig. 1. Serum concentrations of AFB-Lys adduct after a single-dose of AFB_1 exposure. The inset is an enlarged view of 0 to 24 h. Values shown are mean ± SD ($n = 5$).

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3.2. Repeated dose study

Repeated dosing with AFB_1 increased serum AFB-Lys for up to 5 weeks at all doses (Fig. 3). A dose-dependent temporal increase in the AFB-Lys level was found in animals that received 5–25 μg/kg AFB_1 over a 5-week study period. The level of AFB-Lys reached a maximum after 2 weeks, followed by a gradual decrease over time in the 75 μg/kg group. To further examine the kinetics of the serum AFB-Lys after the repeated-dose treatment, we conducted different model analyses and found that the linear regression model best fit the data for the 5 ($r^2 = 0.96$, $p < 0.01$, Fig. 4A), 10 ($r^2 = 0.97$, $p < 0.01$, Fig. 4B) and 25 μg/kg groups ($r^2 = 0.98$, $p < 0.01$, Fig. 4C), respectively. A Gaussian curve was the best fit for the 75 μg/kg group ($r^2 = 0.86$, $p = 0.1634$, Fig. 4D). Linear dose-response relationships were also found between cumulative doses and serum AFB-Lys levels (see supplementary Fig. 2A, $r^2 = 0.99$, $p < 0.01$), as well as between the cumulative doses and the conversion ratio (AFB-Lys formation)/(AFB_1 dosed) after a 1-week treatment (see supplementary Fig. 2B, $r^2 = 0.99$, $p < 0.01$).

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4. Discussion

In this study we evaluated the toxicokinetics of serum AFB-Lys adduct in F344 rats with special focus on doses relevant to acute (single) or chronic (repeated) human exposure. The peak concentration of serum AFB-Lys was found at 4 h in the current study.