Toxicology Reports 4 (2017) 408–414

Contents lists available at ScienceDirect

Toxicology Reports

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

Acute aflatoxin B1 – Induced hepatotoxicity alters gene expression and disrupts lipid and lipoprotein metabolism in rats

Oluwakemi Anuoluwapo Rotimi[a,*], Solomon Oladapo Rotimi[a], Chibueze Uchechukwu Duru[a], Ogheneworo Joel Ebebeinwe[a], Anthonia Obhio Abiodun[a], Boluwaji Oluwamayowa Oyeniyi[a], Francis Adedayo Faduyile[b]

[a] Biochemistry Unit and Molecular Biology Research Laboratory, Department of Biological Sciences, Covenant University, Ota, Ogun State, Nigeria
[b] Department of Pathology and Forensic Medicine, Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria

ARTICLE INFO

Keywords:
Aflatoxin B1
Liver
Lipid
Lipoprotein
Gene expression

ABSTRACT

In this study, alterations in lipid metabolism associated with acute aflatoxin B1 (AFB1) induced hepatotoxicity and gene expression changes underlying these effects were investigated. Rats were orally administered three doses (0.25 mg/kg, 0.5 mg/kg and 1.0 mg/kg) of AFB1 for seven days; after which blood was collected and liver excised. Lipid profiles of plasma and liver were determined spectrophotometrically while the expression of genes associated with lipid and lipoprotein metabolism was assayed by reverse transcriptase polymerase chain reaction. Acute exposure to AFB1 increased the levels of plasma and liver cholesterol, triglycerides and phospholipids. AFB1 at 0.5 mg/kg and 1.0 mg/kg resulted in a dose-dependent (1.2 and 1.5 fold, respectively) down-regulation of hepatic *Cpt1a* with a concomitant 1.2 and 1.5 fold increase in the level of plasma FFA, respectively. A similar observation of 1.2 and 1.3 fold increase was also observed in plasma triglyceride concentration, at both respective doses. AFB1 also decreased the relative expression of *Ahr, Lipc* and *Lcat* whereas, it upregulated *Scarb1* in a dose dependent manner. AFB1-induced dysregulation of the expression of lipid and lipoprotein metabolizing genes may be one mechanism linking AFB1 to altered lipid metabolism and ultimately risk for coronary heart disease.

1. Introduction

Aflatoxins are secondary metabolites synthesized by Aspergillus fungi particularly *Aspergillus flavus* and *Aspergillus parasiticus* [1,2]. They contaminate food and feedstuff most especially grains and nuts during pre - or post-harvest conditions in tropical regions specifically sub-Saharan Africa and Southeast Asia [1,3]. Among the naturally occurring aflatoxins that contaminate food significantly (aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2), aflatoxin B1 (AFB1) is the most common and most toxic, and the liver is its key target organ [4–6]. In the liver, AFB1 is biotransformed by microsomal cytochrome P_{450} to a highly reactive intermediate, AFB1-8, 9-epoxide which binds to nucleic acids to form adducts [4,7,8]. These adducts could block transcription and translation, thereby affecting the regulation of functional gene expression and ultimately causing hepatotoxicity [5]. AFB1-induced hepatotoxicity also results from accumulation of reactive oxygen species, which are precursors of hydroxyl radicals that interact with DNA and lead to mutations [9,10]. AFB1 also induces apoptosis, cytotoxicity and genotoxicity in human hepatocytes (HepG2 cells) [11,12].

Acute aflatoxicosis resulting from exposure to high doses of AFB1 through the diet over a short period causes hepatotoxicity while chronic aflatoxicosis resulting from exposure to low doses of AFB1 through the diet over a long period of time has been implicated in hepatocellular carcinoma [1,13]. Although acute aflatoxicosis is less common compared with chronic aflatoxicosis it occurs occasionally and such outbreaks have been reported in Kenya [14–16]. In an attempt to search for potential biomarkers- using transcriptomics and metabolomics- for earlier detection of AFB1 induced acute hepatotoxicity, Lu et al. [17] reported that gluconeogenesis and lipid metabolism disorders are major metabolic effects following acute AFB1 exposure.

Lipids are molecules that play key roles in metabolic pathways and the lipids of clinical and physiological significance are fatty acids, triglycerides, cholesterol and phospholipids [18]. These lipids are transported in the blood as lipoproteins which are made of a hydrophobic

Abbreviations: Ahr, aryl hydrocarbon receptor; *Cpt1a*, carnitine palmitoyl transferase 1A; *Lipc*, hepatic lipoprotein lipase; *Lcat*, lecithin – cholesterol acyltransferase; *Scarb1*, scavenger receptor class B member 1

* Corresponding author.
E-mail address: kemi.rotimi@covenantuniversity.edu.ng (O.A. Rotimi).

http://dx.doi.org/10.1016/j.toxrep.2017.07.006
Received 2 March 2017; Received in revised form 19 July 2017; Accepted 21 July 2017
Available online 24 July 2017
2214-7500/ © 2017 Published by Elsevier Ireland Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).

core surrounded by a hydrophilic layer [18,19]. Disturbances in the homeostasis of these lipids and lipoproteins resulting in dyslipidemia characterized by hypertriglyceridemia, low HDL-cholesterol and elevated LDL- cholesterol are associated with various diseases including cardiovascular disease [20–25]. Specifically abnormalities in lipoprotein levels and oxidation of LDL have been found to play a role in the development of cardiovascular diseases in humans [26,27].

AFB1 has been reported to cause alterations in plasma and liver lipid levels [2,4,17]. However, the doses at which these effects occur and the mechanisms underlying these alterations need further exploration. Lu et al. [17] reported that gene expression analysis and metabolite profiling are more sensitive than general toxicity studies for the detection of earlier hepatotoxicity induced by AFB1. The LD_{50} of AFB1 has been reported to be 2.71 mg/kg and the authors reported that no mortality was observed in rats treated with 1.0 mg/kg AFB1 [28]. Therefore, this study investigated the effects of acute oral exposure to three doses (0.25, 0.5 and 1.0 mg/kg) of AFB1, the doses were selected to ensure there was no death during the experimental period, on lipid and lipoprotein metabolism in rats and assessed expression of genes in pathways relevant to lipid metabolism.

2. Materials and methods

2.1. Chemicals

AFB1 was a product of Sigma-Aldrich (St. Louis, MO). Reagent diagnostic kits were products of BioSino Biotechnology & Science Inc. (Beijing, China). RNAlater® and RNA extraction spin column kit were products of Aidlab Biotechnologies Co. Ltd (Beijing, China) while TransGen EasyScript® one-step RT-PCR kit was a product of TransGen Biotech Co. Ltd (Beijing, China). All other chemicals used in this study, unless otherwise stated, were products of Sigma-Aldrich (St. Louis, MO).

2.2. Animals

Twenty 10-week old inbred male albino rats weighing between 100 and 150 g were used for this research. The rats were housed in clean cages, subjected to standard 12-h light and dark cycles and had access to feed and clean tap water *ad libitum*. The animals were allowed to acclimatize to their environment for one week before the experiment started. The experiment was conducted, and animals cared for in accordance with the declaration of Helsinki.

2.3. Treatment protocol and tissue collection

The rats were randomly distributed into four treatment groups of five rats each and treated with 0, 0.25, 0.5 or 1.0 mg/kg body weight AFB1. AFB1 in olive oil was administered by oral gavage for seven days while the control rats received equal volume of olive oil alone. The rats were sacrificed on the eighth day, after an overnight fast, under anesthesia and blood collected by cardiac puncture. Blood and liver were processed as previously described by Rotimi et al. [29] while a portion of the left lobe was preserved in RNAlater® and another portion kept in 10% formalin for histological studies. Plasma was obtained from whole blood by centrifugation at 3000 rpm for 15 min.

2.4. Biochemical analysis

2.4.1. Plasma lipid profiles

Total cholesterol and triglyceride concentrations in the plasma were determined using commercially available kits according to the manufacturer's instructions. Total HDL and HDL_3 were recovered from the plasma using dextran sulfate-$MgCl_2$ precipitation at the final concentration of 10 mg/mL dextran sulfate, 0.5 M $MgCl_2$, 0.05% NaN_3 and 19.1 mg/mL dextran sulfate, 1.95 M $MgCl_2$, 0.05% NaN_3, respectively [30,31]. The supernatant, containing the lipoprotein, was recovered after centrifugation at 1500 *g* for 30 min. Cholesterol and triglyceride concentrations were determined in this supernatant with the same commercial kits used for total cholesterol and triglyceride.

Plasma phospholipids were determined as described by Rifai et al. [30], using 1-amino-2-naptho-4-sulphonic acid reagent, while free fatty acids (FFA) were determined spectrophotometrically at 620 nm as described by Rotimi et al. [32].

2.4.2. Liver lipid profiles

Lipids were extracted from the liver according to the method of Folch et al. [33] and aliquots of the extract was used for determining cholesterol, triglycerides and phospholipids as earlier described by Rotimi et al. [34].

2.5. RNA extraction

RNA was extracted from RNAlater® – stabilized liver using the Aidlab spin column RNA extraction kit according to the instructions of the manufacturer. Concentration and purity of extracted RNA was determined at 260 nm and 280 nm using a NanoDrop® 2000 spectrophotometer (Thermo Scientific). RNA samples were kept at −80 °C until gene expression analysis.

2.6. Expression of lipid metabolizing genes

The levels of expression of some lipid metabolizing genes were assessed in the liver using semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR) as previously described by Rotimi et al. [35]. Briefly, the RT-PCR was carried out with 500 ng RNA template using TranGen EasyScript one-step RT-PCR kit according to manufacturer's instructions. The RNA samples were subjected to an initial 30 min incubation at 45 °C for cDNA synthesis after which PCR amplification was carried out, using gene specific primers (GSP) (Table 1), at 94 °C for 5 min followed by 40 cycles of 94 °C for 30 s, 5 min at the annealing temperature of GSP, and 1 min at 72 °C. All amplifications were carried out in C1000 Touch™ Thermal Cycler (Bio-Rad Laboratories, Hercules, CA). After PCR, amplicons were visualized on 1.2% agarose gel in 1X Tris Borate EDTA buffer using UVP BioDoc-It™ Imaging system (Upland, CA, USA). The intensity of the bands were analyzed using Image J software [36]. Results were presented as relative expression (ratio of intensity of each gene to that of β-actin, *Actb)* of each gene in comparison with a housekeeping (β-actin, *Actb*) gene. There were no changes in the expression of the housekeeping gene across the treatment groups.

2.7. Histopathology

A portion of liver samples were fixed in 10% formalin immediately after harvesting. The tissues were processed by cutting pieces in tissue

Table 1
Sequences of gene – specific primers.

Gene	Sequence (5′–3′)	Template
Ahr	Forward: GGGCCAAGAGCTTCTTTGATG Reverse: GCAAGTCCTGCCAGTCTCTGA	NM_001308255.1
Lipc	Forward: GAGCCCAGTCCCCCTTCA Reverse: ATGTCATTCTTTGCTGCGTCTC	NM_012597.2
Scarb1	Forward: GGCAAATTTGGCCTGTTCGT Reverse: CCACAGCAATGGCAGGACTA	NM_031541.1
Lcat	Forward: AACTGGCTGTGCTACCGAAA Reverse: TAGGTCTTGCCAAAGCCAGG	NM_017024.2
Cpt1a	Forward: AAGTCAACGGCAGAGCAGAG Reverse: ACGCCCAAGTATTCACAGGG	NM_031559.2
Actb	Forward: GTCAGGTCATCACTATCGGCAAT Reverse: AGAGGTCTTTACGGATGTCAACGT	NM_031144.3

Download English Version:

https://daneshyari.com/en/article/5558683

Download Persian Version:

https://daneshyari.com/article/5558683

Daneshyari.com