Microbial Pathogenesis 107 (2017) 309–312

Contents lists available at ScienceDirect

Microbial Pathogenesis

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

Aflatoxins produced by *Aspergillus parasiticus* present in the diet of quails increase the activities of cholinesterase and adenosine deaminase

Aleksandro Schafer da Silva [a, b, *], Janio M. Santurio [c], Lenilson F. Roza [a], Nathieli B. Bottari [b], Gabriela M. Galli [a], Vera M. Morsch [b], Maria Rosa C. Schetinger [b], Matheus D. Baldissera [c], Lenita M. Stefani [a], Willian M. Radavelli [a], Thainã Tomasi [a], Marcel M. Boiago [a, *]

[a] Graduate Program in Animal Science and Department of Animal Science, Universidade do Estado de Santa Catarina (UDESC), Chapecó, SC, Brazil
[b] Graduate Program in Toxicological Biochemical and Department of Biochemistry and Molecular Biology, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil
[c] Department of Microbiology and Parasitology, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil

ARTICLE INFO

Article history:
Received 2 March 2017
Received in revised form
30 March 2017
Accepted 30 March 2017
Available online 31 March 2017

Keywords:
Aspergillus parasiticus
Adenosine deaminase
Aflatoxins
Cholinesterases
Quails

ABSTRACT

The aim of this study was to evaluate the effects of aflatoxins on cholinesterases (acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), and adenosine deaminase (ADA) activities in quails. For this, twenty male quails were randomly distributed into two groups (n = 10 each): the group A was composed by quails that received feed without aflatoxin (the control group); while the group B was composed by quails that received feed contaminated with 200 ppm/kg of feed of aflatoxin. On day 20, the animals were euthanized to measure the activities of AChE (total blood and brain), BChE (serum) and ADA (serum, liver, and brain), as well as for histopathological analyses (liver and intestine). AChE, BChE, and ADA levels increased in animals intoxicated by aflatoxin compared to the control group. The presence of aflatoxin lead to severe hydropic degeneration of hepatocytes and small focus of hepatocyte necrosis. In conclusion, aflatoxins poisoning increased AChE, BChE, and ADA activities, suggesting the involvement of these enzymes during this type of intoxication, in addition to the fact that they are well known molecules that participate in physiological and pathological events as inflammatory mediators. In summary, increased AChE, BChE and ADA activities contribute directly to the inflammatory process and tissue damage, and they might be involved in disease development.

1. Introduction

Mycotoxins are considered toxic secondary metabolites naturally produced by several filamentous fungi [1]. Fungi are commonly found in grains used to feed several species of animals, however the production of mycotoxins occurs when there is a stressful environment to these agents, such as alterations on temperature or humidity [2]. Among the different types of mycotoxins, the aflatoxin B_1 (AFB_1) is the most pathogenic, and it is produced mainly by species of *Aspergillus flavus* and *Aspergillus parasiticus* [3]. Recently, a study demonstrated that mycotoxins in chickens negatively affect growth rate, feed conversion, and reproductive efficiency of these animals, causing damage in different tissues, such as liver, spleen, heart, and bursa of Fabricius [4], and thus, increase the cost of production for poultry industries. These alterations in tissue weight and size contribute to economic losses in the poultry industry [5–7].

Some alterations in the integrity of cell membranes caused by toxic agents can be determined by measuring certain enzymes released by damaged cells [8]. The cholinesterases, for example, catalyze the hydrolysis of acetylcholine (ACh) in choline and acetic acid. Theses enzymes are divided into two types: the

* Corresponding authors. Department of Animal Science, Universidade do Estado de Santa Catarina, Rua Beloni Trombeta Zanin, 680E, Chapecó, Zip: 89815-630 SC, Brazil.
E-mail addresses: aleksandro.silva@udesc.br (A.S. da Silva), mmboiago@gmail.com (M.M. Boiago).

http://dx.doi.org/10.1016/j.micpath.2017.03.041
0882-4010/

acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8). These enzymes play important roles during the immune and inflammatory responses, due to ACh anti-inflammatory effect [9]. Recently, some studies have reported that BChE is a novel marker of tissue inflammation and acute inflammatory response [10]. The adenosine deaminase (ADA) is another important enzyme used as marker of tissue damage, being considered a key enzyme in the purine metabolism, catalyzing the deamination of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively, by regulating the concentration of extracellular adenosine [11]. ADA activity plays an important role on immune and inflammatory response by regulating adenosine levels, a molecule of the anti-inflammatory process, and thus, protecting the host tissue from damage [12]. Considering tissue damage caused by aflatoxins in intoxicated animals and taking into account that their mechanisms of action are not yet fully understood, the aim of this study was to assess whether aflatoxins in the diets used to feed quails (*Coturnix coturnix*) could influence cholinesterases and ADA activities.

2. Materials and methods

2.1. Aflatoxin

Aflatoxin was produced by fermentation of converted rice under constant stirring and controlled temperature. For this, NRLL strain of *A. parasiticus* was used according to the methodology described by Shotwell et al. [13], and improved by West et al. [14]. After autoclaving, the rice was dried with hot air and grounded in a laboratory mill. The aflatoxin concentration was determined by high performance liquid chromatography (HPLC) [15]. A total of 100 mg/kg of aflatoxins was obtained, which contained approximately 83% of aflatoxin B_1, 9% of aflatoxin B_2, 5% of aflatoxin G_2, and 3% of aflatoxin G_1. Powder rice was added to the diet in the adequate proportion, never exceeding 1% of the total diet.

2.2. Experimental design and animals

Eggs of Japanese quails (*C. coturnix*) were incubated under adequate conditions for hatching in the Experimental Poultry Farm at the Universidade do Estado de Santa Catarina (UDESC), Brazil. After hatching, the quails were housed in cages in a room under controlled temperatures using thermal radiation lamps. At 75 days of age, 20 males with 160 ± 20 g were selected and allocated in metallic cages with ten animals each. The diet provided was formulated according to Rostagno [16].

2.3. Study design

The experimental design was completely randomized, with two treatments and ten repetitions, being one male quail considered one experimental unity. Groups were divided as follows: the group A composed by quails that received control feed (the control group); and the group B with quails that received feed with aflatoxins (200 ppm/kg of feed). Feed was supplied *ad libitum*. The dose of aflatoxin used was defined in a previous study, aiming the intoxication of the animals at day 20 of experiment (unpublished data).

2.4. Sampling

On day 20 after the beginning of the experiment, all quails were euthanized by cervical dislocation. Blood samples were collected by cardiac puncture in tubes with anticoagulant (EDTA) and without (to obtain serum). Blood samples collected without anticoagulant were centrifuged at 3500 rpm during 10 min, and sera were separated for analyses. Liver and brain were also collected. These tissues were homogenized in a flask with Tris-HCl buffer (10 mM, pH 7.4), and centrifuged (10.000×*g* at 4 °C) for 10 min. Aliquots of the supernatants were stored at −20 °C until use. A fragment of liver, brain and small intestine (duodenum and jejunum) was collected for histopathological analyses. Protein content was measured by the Coomassie method [17] using albumin as the standard.

2.5. Enzymatic activities

2.5.1. AChE activity in total blood and brain

AChE activity in total blood was measured according to Ellman et al. [18], and as previously described by Worek et al. [19]. The samples were hemolyzed with phosphate buffer (pH 7.4) containing Triton X-100 stored at 30 °C. The hydrolysis of acetylcholine (ACh) was measured at 436 nm. The specific activity of whole blood AChE was calculated from the quotient between AChE activity and hemoglobin content, and the results were expressed as mU/umol HB.

AChE enzymatic activity in the brain homogenates was determined by a modified spectrophotometric method of Ellman et al. [18]. The reaction mixture was composed of 1.0 M of potassium phosphate buffer (pH 8.0) and 10 mM of DTNB. This method is based on the increased yellow color of the nitrobenzoate (TNB), an ion produced from thiocoline after reaction with 5,5-dithio-bis-acid-nitrobenzoic (DTNB) ion, measured by absorbance at 412 nm after 2 min of incubation at 30 °C. The reaction was initiated by adding 0.78 mM acetylcholine iodide as substrate. All samples were analyzed in duplicate and the enzymatic activity was expressed in μmol AcSCh/h/mg of protein.

2.5.2. Seric BChE activity

Seric BChE activity was determined as described by Ellman et al. [18], using butyrylcholine (BCh) as substrate. The assay was performed in a medium containing sodium phosphate buffer 0.1 M (pH 7.4), 0.30 mM of DTNB, and 15 μL of sample. Pre-incubation was done at 37 °C for 3 min, and reading on a spectrophotometer (412 nm) at intervals of 20-20 s for 2 min. The analysis was carried out in duplicate, and the enzymatic activity was expressed as μmol BcSCh/h/mg of protein.

2.5.3. ADA activity in serum, liver and brain

The activity of ADA in serum was determined spectrophotometrically, according to the method described by Giusti and Gakis [20]. The reaction started by the addition of adenosine (substrate) to a final concentration of 21 mMol/L; incubations were carried out for 1 h at 37 °C. The reaction stopped by adding 106 mMol/L 0.16 mMol/L of phenolnitroprusside/mL. The reaction was mixed immediately with 125 mMol/L and 11mMol/L of alkaline hypochlorite (sodium hypochlorite). Ammonium sulfate (75 μmol/L) served as ammonium standard. The ammonium concentration is directly proportional to the absorption of indophenol at 650 nm. The specificity activity was reported as U/L.

ADA activity in the liver and brain was measured according to Giusti [21], which is based on the direct measurement of ammonia produced when the enzyme acts on the adenosine. A volume of 100 μL of liver or brain homogenate was used. The enzymatic reaction was started by the addition of 500 μL of 21 mM adenosine as substrate and stopped by adding 1.5 mL of 106/0.16 mM phenol-sodium nitroprusside to the reaction mixture, which was immediately mixed with 1.5 mL of 125/11 mM alkaline-hypochlorite (sodium hypochlorite). Released ammonia reacted with alkaline-hypochlorite and phenol in the presence of the catalyst-sodium