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Acute toxicity of a single gavage dose of fumonisin B_1 in rabbits

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ABSTRACT

The aim of this study was to determine the clinical, pathological and mycotoxicological effects of oral administration of fumonisin B_1 (FB_1) in rabbits. Eighteen rabbits were randomly assigned to two experimental groups: control group, 0 mg FB_1; fumonisin group, 31.5 mg FB_1/kg body weight, corresponding to about 630 mg FB_1/kg diet. Fumonisin administered as a single oral dose to rabbits resulted in acute toxicity, significantly interfering with body and liver weight. Serum biochemical analysis revealed a significant increase of total protein, alkaline phosphatase (AP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), urea and creatinine in the group receiving FB_1 compared to control animals, a finding characterizing hepatic and renal injury in this group. Urinary protein concentrations were markedly elevated at 12, 24, 48 and 72 h after dosing, although visible pathological abnormalities were not observed, probably because of rapid repair of the damage. FB_1 was detected in feces, with a maximum concentration at 24 h after administration, indicating that the enterohepatic circulation is important in rabbits. FB_1 concentrations found in urine were low, with peak elimination at 12 h after intoxication. The highest FB_1 concentrations were observed in feces compared to urine and liver, demonstrating that feces are the main routes of excretion.

1. Introduction

Fumonisins are secondary metabolites produced by *Fusarium verticillioides* (Sacc.) Nirenberg (=*F. moniliforme* Sheldon) and *F. proliferatum* (Matsushima) Nirenberg. These mycotoxins are found worldwide and can be isolated from corn and corn-based food naturally contaminated with *Fusarium* [1,2]. Among the known fumonisins, fumonisin B_1 (FB_1) is the most abundant and corresponds to about 70% of the total fumonisin concentration detected in maize [3]. Fumonisins are inhibitors of ceramide synthase, a key enzyme in the de novo biosynthesis of ceramide and more complex sphingolipids. Sphingolipids and sphingolipid metabolites are known to be important signaling molecules [4].

Ingestion of FB_1 causes a variety of toxicoses in animals, including leukoencephalomalacia in horses [5], pulmonary edema in swine [6], and hepatocarcinoma and liver disease in rats [7,15]. Both weight gain and loss have been demonstrated in chicks fed increasing doses of FB_1 [8]. Hepatic and renal toxicity has been observed in several species, including rats, mice, broilers, turkeys, ducks, and rabbits [9–14]. Additionally, FB_1 in foods has been associated with a high incidence of human esophageal cancer [16,17]. On the basis of existing toxicological evidence, the International Agency for Research on Cancer (IARC) has indicated FB_1 as a possible carcinogen to humans (Group 2B) [15].

Toxicokinetic studies of FB_1 have been conducted on rats [18], vervet monkeys [19], swine [20,21], and cows [22]. According to these authors, FB_1 is poorly absorbed and rapidly excreted, with small amounts being detected in liver and kidneys. FB_1 is also excreted in bile and, despite low absorption, some enterohepatic recirculation may occur [23]. Based on these considerations, the purpose of the present study was to determine the clinical, pathological and mycotoxicological effects of oral administration of FB_1 in rabbits.

2. Materials and methods

2.1. Mycotoxins

Purified FB_1 (>95%) administered to rabbits was obtained from South Africa through the Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC, Tygerberg, South Africa).

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2.2. Animals and diet

Eighteen 50-day-old male white New Zealand rabbits (*Oryctolagus cuniculus*) weighing an average of 1.7 kg were acclimatized in individual metabolic cages for 10 days before the beginning of the experiment. Feed and water were provided *ad libitum*. The diets, especially prepared for laboratory rabbits by Nuvital Nutrientes S/A, contained no detectable levels of aflatoxins, zearalenone [24] or fumonisins [25]. The study was approved by the institutional Committee on the Care and Use of Laboratory Animals of the University of São Paulo.

2.3. Experimental protocol

Eighteen animals were randomly assigned to two groups. Six rabbits only received sterile saline (control group) and 12 animals were treated by gavage with 31.5 mg FB_1/kg body weight, corresponding to about 630 mg FB_1/kg diet. Blood samples for analysis of the biochemical profile and feces samples were obtained 12, 24, 48 and 72 h after gavage. The animals were euthanized on day 7 and organs were submitted to mycotoxicological (liver) and histopathological (liver and kidney) analysis.

2.4. Clinical alterations and body weight

The rabbits were clinically evaluated at 8-h intervals and were weighed before and 7 days after the experiment. Absolute and relative liver and kidney weights were also determined after sacrifice of the animals on day 7.

2.5. Clinical–pathological analysis

After 7 days, blood samples were collected and aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), gamma-glutamyltransferase (GGT), total protein, albumin, creatinine, urea, cholesterol, and total bilirubin (direct and indirect bilirubin) were determined according to Birgel [26]. Serum analysis was performed with a Technicon RA-100 analyzer (Bayer, Tarrytown, NY, USA) using commercial kits from Boehringer-Mannheim France SA (Meylan, France), Roche Diagnostics (Mannheim, Germany), and Bayer. For the determination of urinary protein, urine samples collected at 12, 24, 48 and 72 h after dosing were pooled and the total volume was recorded. Urinary protein was measured as described by Doetsch [27].

2.6. Autopsy, gross pathology and histopathology

Seven days after the administration of the toxin, the rabbits were anesthetized with pentobarbital, bled by cardiac puncture and autopsied for the investigation of gross lesions. Any macroscopic alterations found in the organs and tissues were recorded. The liver and kidneys were removed and weighed, and samples were collected in 10% neutral buffered formalin, pH 7.8. Tissue sections (4 μm-thick) were stained with hematoxylin and eosin and used for microscopic histological evaluation.

2.7. Analysis of fumonisin content in feces, urine and liver

Feces and urine samples were collected 12, 24, 48 and 72 h after the beginning of the experiment, and liver samples were obtained 7 days after dosing. For quantification of FB_1, samples were stored at −20 °C until the time of analysis.

(a) *FB_1 in urine* [28]: A 250-μL aliquot of urine was diluted in 700 μL distilled water and 3 mL methanol. The samples were applied to a Bond-Elut SAX solid-phase extraction cartridge conditioned with 5 mL methanol and 5 mL methanol:water (3:1, v/v). The samples were rinsed with 1 mL methanol:water (3:1, v/v) and 5 mL methanol. The toxin was then eluted with 10 mL 5% acetic acid in methanol and dried at 60 °C under a stream of nitrogen. The extracts were derivatized with reactive OPA. The OPA reagent was prepared by dissolving 40 mg OPA in 1 mL methanol, followed by the addition of 5 mL 0.1 M sodium borate and 50 μL 2-mercaptoethanol. Derivatives were prepared by mixing a 50 μL aliquot of sample extract with 200 μL OPA reagent and analyzed by HPLC on a Supelcosil LC reverse-phase column using a mobile phase of methanol:0.05 M sodium dihydrogen phosphate (53:47, v/v) adjusted to pH 3.4 with orthophosphoric acid. The injection volume was 20 μL derivatized sample. FB_1 was detected by fluorescence at excitation and emission wavelengths of 335 and 440 nm, respectively. The identity of the peaks assigned to FB_1 was confirmed by comparing test chromatograms with the standard, with special attention to retention time. Samples presenting a peak during fumonisin retention were confirmed by the addition of standard and reprocessing. The average recovery was 91%.

(b) *FB_1 in feces* [19]: A subsample of 1.5 g was extracted by vortexing with 15 mL 0.1 M ethylenediaminetetraacetic acid (EDTA) at pH 5.2. The mixture was centrifuged at 2000 × *g* for 10 min and the supernatant was removed. Extraction was repeated five times. The supernatants were combined and acidified to pH 2.9 with 5 M hydrochloric acid and centrifuged at 4000 × *g* for 10 min. A 6 mL aliquot was applied to a Bond Elut C_{18} solid-phase extraction cartridge conditioned with 5 mL methanol and 5 mL water. The samples were rinsed with 5 mL water, 5 mL methanol:water (1:3, v/v) and 3 mL methanol:water (1:1, v/v). FB_1 was eluted with 15 mL methanol and the extracts were dried at 60 °C under a stream of nitrogen. The residue was redissolved in 500 μL methanol immediately before derivatization with reactive OPA and analyzed by HPLC as described for the urine samples. The average recovery was 80%.

(c) *FB_1 in liver* [29]: Five grams of the sample was extracted with 7 mL acetonitrile:water (1:1, v/v) for 30 min. The mixture was centrifuged at 4000 × *g* for 10 min, and 1 mL of the supernatant was combined with 8 mL methanol:water (3:1, v/v) and applied to a Bond-Elut SAX solid-phase extraction cartridge (Romer Labs 2400) conditioned with 10 mL methanol and 10 mL methanol:water (3:1, v/v). The samples were rinsed with 4 mL methanol. The toxin was then eluted with 15 mL methanol:acetic acid (95:5, v/v). The eluted samples were dried at 60 °C under a stream of nitrogen. The extracts were derivatized with reactive OPA and analyzed by HPLC as described for the urine samples. The average recovery was 70%.

2.8. Statistical analysis

The individual data on body weight gain; serum biochemical parameters and relative organ weight (g/100 g body weight) were subjected to descriptive statistics (mean and relative standard deviation), followed by analysis of variance (ANOVA). Treatment means were compared by the Tukey test using the Statgraphics software, Version 5.1 (Statgraphics Manugistics, Rockville, MD, USA). Statistical significance was set at $P < 0.05$.

3. Results

3.1. Fumonisin B_1-treated animals

FB_1-treated animals presented clinical signs of intoxication such as apathy, anorexia, lethargy and ruffled fur. A significant ($P < 0.05$)