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Influence on functional parameters of intestinal tract induced by short-term exposure to fumonisins contaminated corn chyme samples

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ABSTRACT

The gut is a possible target toward mycotoxin fumonisins (FBs) exposure. The study aims to investigate the effects induced by FBs contaminated-corn chyme samples on functional parameters of human and rat intestine by using Ussing chamber. Fumonisins-contaminated corn and processed corn samples were undergone to *in vitro* digestion process and then added to luminal side. A reduction (about 90%) of short circuit current (Isc $\mu A/cm^2$) during exposure of human colon tissues to fumonisins-free corn chyme samples was observed, probably related to increased chyme osmolality. This hyperosmotic stress could drain water towards the luminal compartment, modifying Na^+ and Cl^- transports. The presence of FBs in corn chyme samples, independently to their concentration, did not affect significantly the Isc, probably related to their interference towards epithelial Na^+ transport, as assessed by using a specific inhibitor (Amiloride). The rat colon tract represents a more accessible model to study FBs toxicity showing a similar functional response to human. In the rat small intestine a significant reduction (about 15%) of Isc parameter during exposure to uncontaminated or FBs contaminated corn chyme samples was observed; therefore such model was not suitable to assess the FBs toxicity, probably because the prevalent glucose and amino acids electrogenic absorption overwhelmed the FBs influence on ionic transport.

1. Introduction

Fumonisins (FBs) are a group of structurally related mycotoxins produced by *Fusarium verticillioides*, *F. proliferatum* and *F. nyagamai* (Thiel et al., 1991) that are commonly found on corn. Fumonisin B_1 (FB_1) is the most abundant FBs occurring naturally in contaminated foods and feed and is believes to be the most toxic. Fumonisin B_1 causes various species-specific mycotoxicosis in farm animals, including equine leukoencephalomalacia and porcine pulmonary oedema and is hepatotoxic and nephrotoxic in various animals species. In humans, exposure to FBs has been linked to oesophageal cancer and neural tube defects (Soriano et al., 2005). The International Agency for Research on Cancer characterized the FBs as Group 2B carcinogens, i.e. a possible carcinogen to humans (Soriano et al., 2005). Controversies regarding the toxic and carcinogenic properties of the FBs were reported by Gelderblom and Marasas (2012) suggesting that further studies on modulating role of dietary constituents should be carried out. Fumonisins are structurally similar to sphingoid bases, such as sphingosine (So) and sphinganine (Sa) (which are components of the sphingolipid pathway) and are able to inhibit ceramide synthase with consequent disruption of sphingolipid metabolism (Soriano et al., 2005). Fumonisins are poorly absorbed *in vivo* and rapidly excreted. Fumonisin is excreted mainly in the faeces, either unchanged or depleted of one-ester-linked tricarballylic acid. Only trace amounts of aminopentol, the fully hydrolyzed FB_1, were found in faeces. Van der Westhuizen et al. (2011) reported an FB_1 excretion in human urines less than 0.075%, albeit interindividual variability of FB_1 urinary excretion was found by Riley et al. (2012). The poor absorption of this mycotoxin has been qualified by Shier (2000) as the "fumonisin paradox". Whether this poor absorption and consequent low

Abbreviations: FBs, fumonisins; Isc, short circuit current; DF, digestive fluids; AFBsC, artificially contaminated corn; FBsC, naturally contaminated corn; UC, uncontaminated corn; CC, cooked corn; NC, nixtamalized corn; SCb, spiked corn before digestion; SCa, spiked corn after digestion.

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bioavailability is due to poor transport across the epithelium of the intestine or due to the strong association of fumonisin with the intestinal content remains uncertain. Fumonisins are excreted in bile, and even though absorption is low, some enterohepatic recirculation occurs. Previous data have indicated an interaction between FB_1 and cholesterol and/or bile salts, which may lead to the incorporation of dietary FB_1 into mixed micelles, as proposed by Mahfoud et al. (2002). The gastrointestinal tract may also be a target of FBs. As reported by Lèssard et al. (2009), direct toxic effects of FB_1 on the gut documented changes in intestinal structure and cellularity. The consumption of an extract rich in FBs (1.5 mg/kg body weight) for 9 days has the potential to alter intestinal porcine physiology, villus architecture and enzyme activities (Lèssard et al., 2009). *In vivo* exposure for 7 days to FB_1 induced an increase in the ratio of Sa/So as well as alterations of the glycolipid distribution observed in porcine jejunum epithelium (Loiseau et al., 2007). After *in vivo* exposure of small porcine intestine an additional immunomodulation effect, observed as down-regulation of IL-8 expression (Bouhet et al., 2006), FB_1 reduction of *in vivo* antigen presenting cells maturation (Devriendt et al., 2009) and increased colonization of porcine intestine by an extraintestinal pathogenic *Escherichia coli* strain (Oswald et al., 2003), was found. The toxic FB_1 activity was also observed on large intestine; in fact, Prelusky et al. (1996) reported an high accumulation of radio-labelled FB_1 in the colon in comparison with the stomach or the small intestine; these authors estimated that after 24 days of feeding pigs with a feed contaminated (3 mg/kg feed from 1 to 12 days, followed by 2 mg/kg feed during days 13–24), the concentrations of FB_1 and its metabolites reached 160, 65, 40, 33 and 583 ng/g tissue for liver, kidney, stomach, small intestine and colon respectively. Lallès et al. (2010) reported high sensitivity of colon to the deleterious effects of this mycotoxin in FB_1-treated pigs. In fact in colon tract FB_1 induced a mild increase in stress proteins and inflammatory stimuli, measured as enhanced αB crystalline and COX-1.

Although the intestine is recognized as a possible target for FB_1 toxicity and is in contact with mycotoxins ingested with the food (Bouhet and Oswald, 2007), no information to date for supporting any effects of FB_1 on human gastro-intestinal tract is available.

In order to reproduce a conventional way of mycotoxin exposure, the aim of this study was to investigate the influences of chymes obtained from *in vitro* digestion process of different FBs-contaminated corn samples on intestinal functionality by using Ussing chamber. Fumonisin-free corn samples were used for control experiments. The study was carried out on human healthy sigmoid colon tissues, obtained from patients undergoing subtotal colectomy for colon cancer, and on small and large intestine samples from White Wistar female rats in order to check if this laboratory animal could have similar intestinal sensitivity to FBs compared to human intestine and could to be used as *in vitro* model.

2. Materials and methods

2.1. Chemicals and reagents

Acetonitrile (ACN), methanol (MeOH) (both for HPLC purpose), glacial acetic acid were purchased either from Mallinckrodt Baker (Milan, Italy). Ultrapure water was produced by a Millipore Milli-Q system (Millipore, Bedford, MA, USA). Standard powder of FB_1 and FB_2, Amiloride and Mannitol, were from Sigma–Aldrich (Milan, Italy). C18 columns containing 500 mg of sorbent were from Waters Corporation (Waters, Milford, MA). Paper filters (No. 4) were from Whatman (Maidstone, UK); Micro Spin Filter Tubes (0.20 mm, regenerated cellulose, GRACE) were from Alltech (Deerfield, IL, USA).

2.2. Food samples

A total of 5 unprocessed corn samples, including 2 blank (FBs-free) samples (UC1 and UC2) and 3 corn samples naturally contaminated with FBs (FBsC1, FBsC2 and FBsC3) were purchased from Italian retail markets. One corn sample (AFBsC) had been artificially inoculated with a toxigenic strain of *F. verticillioides*. Two processed corn samples i.e. an alkaline-cooked (nixtamalized corn, NC) and corn boiled (cooked, CC), naturally contaminated with FBs, were produced within the MycoRed Project (EC KBBE-2007-222690-2 MYCORED).

2.3. Tissue samples

Human healthy sigmoid colon mucosal sections obtained from 23 patients (10 females, 13 males ranging between 35 and 85 years; median age 63.6 years) undergoing subtotal colectomy for colon cancer, were used. The specimens were provided by the surgery Departments at the Policlinico of Bari. Prior to surgery, all patients had given their fully informed and written consent about the aims of the surgical intervention. All the intestinal samples utilized in this work, were made anonymous. Upon removal of the tissue and before inclusion in buffered formalin for routine pathology examination, a strip (about 5 × 1.5 cm) of full thickness colonic wall was isolated within the redundant healthy area surrounding the pathologic region, stored in ice-cold Krebs solution, and transported to the laboratory for electrophysiological studies.

Small or large intestine samples from fifty-three White Wistar rats were used to evaluate FBs effect on electrophysiological parameters by using Ussing chamber. White Wistar female rats (6–12 months; 250 g) bred in the approved facility at the University of Bari, were housed individually in appropriate cages on a 12 h light–dark cycle with food and water ad libitum. Rats were anesthetized with $CHCl_3$, sacrificed and intestine samples were removed by sharp dissection and used for experiments with Ussing chamber.

All experiments were performed to international guidelines on the ethical use of animals and were designed to minimize their suffering. Experiments in this study were approved by the Italian Health Department (Art. 9 del Decreto Legslativo 116/92).

2.4. In vitro digestion process

Corn samples were *in vitro* digested according to the protocol described by Versantvoort et al. (2005). This model, validated for mycotoxins contaminated food, simulates the digestion process in the gastrointestinal tract in humans in a simplified manner by applying physiologically based conditions, i.e. chemical composition of digestive fluids (DF), pH and residence time periods typical for each compartment. Briefly, digestion process consisted of three steps: short incubation (5 min) of 4.5 g corn matrix with 6 ml saliva, addition of 12 ml gastric juice followed by a two hours incubation and, finally, addition of 12 ml duodenal juice and 6 ml bile followed by a two hours incubation. All intestinal fluids were prepared one day before the experiments following the protocol described by Versantvoort et al. (2005). All incubation times were performed at 37 ± 2 °C and the samples were rotated head-over-heels by using a stirring rotating plate (1xg) in order to simulate peristaltic movements. After the last incubation step, the tubes were centrifuged (2900×*g* for 5 min at 25 °C) and the supernatants, representing the intestinal chyme samples, were used for chemical analysis and for Ussing chamber.

2.5. Evaluation of stability of fumonisins during in vitro digestion process

To evaluate the stability of FBs during *in vitro* digestion process, a blank (FBs-free) corn sample was spiked at EU maximum level of the $FB_1 + FB_2$ (i.e. 4 μg/g) in unprocessed cereals (Commission Regulation, 2007). In particular spiking levels were 3.1 μg/g for FB_1 and 0.77 μg/g for FB_2. Then the spiked corn was digested as described in Section 2.3 and the supernatant, representing the intestinal chyme sample, was used for LC–MS/MS analysis and for Ussing chamber. Triplicate independent spiking experiments were performed.

2.6. Fumonisins analysis in chyme samples and recovery experiments

Chyme corn samples, obtained by digestion process, were analyzed following the protocol described by Dall'Asta et al. (2010). The concentration of FBs was determined in chyme corn samples after a desalting step. Briefly, an aliquot of 2 mL of chyme corn samples was cleaned-up through C18 columns (Waters) preconditioned with 2 mL of methanol followed by 2 mL of bidistilled water. Then, column was washed with 2 mL of bidistilled water and FBs were eluted with 2 mL of water/acetonitrile 1:1 v/v. A 1-mL portion of eluted extract was evaporated to dryness under a stream of nitrogen, and the residue was redissolved in 1 mL of water/methanol, 30:70 v/v and analyzed by LC–MS/MS according to De Girolamo et al. (2013). Limit of detection (LOD) and limit of quantification (LOQ) of the method were 0.1 μg/g for FB_1 and 0.075 μg/g for FB_2 and 0.35 μg/g for FB_1 and 0.27 μg/g for FB_2, respectively.

To estimate the amount of FBs retained from the column, chyme blank (FBs-free) corn sample was spiked with 3.1 μg/g FB_1 and 0.77 μg/g FB_2 and cleaned-up through the C18 column. Triplicate independent spiking experiments were performed.