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Fumonisin B_1 exposure and its selective effect on porcine jejunal segment: Sphingolipids, glycolipids and trans-epithelial passage disturbance

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ABSTRACT

Fumonisin B_1 (FB_1) is a mycotoxin produced by *Fusarium verticillioides*, the cause of *Fusarium* kernel rot in maize. FB_1 is toxic in domestic and laboratory animals, including pigs. This study investigated the effects of a seven-days-exposure of 1.5 mg/kg b.w. FB_1 on the porcine intestinal epithelium. Statistically significant increases in the ratio of sphinganine to sphingosine, as well as alterations of the glycolipid distribution were observed in the jejunum. Using a porcine intestinal epithelial cell line (IPEC-1) derived from jejunum and ileum, we tested the effect of FB_1 *in vitro* in a time- and dose-dependent fashion. A significant increase in sphinganine concentration was observed after 2 days of FB_1 exposure at concentrations >100 μM, or from 6 days of FB_1 exposure at concentration >20 μM. We were also able to show that FB_1 exposure at 200 μM during 16 days increased the intestinal trans-epithelial flux of FB_1. These data indicate that, in pigs, this mycotoxin acts selectively on jejunum cells as follows: (i) FB_1 affects sphingolipid metabolism, as demonstrated by an increase of the amount of free sphingoid bases in a time- and dose-dependent manner, (ii) a depletion of the glycolipids in plasma membranes is observed, and (iii) an increase occurs in the trans-epithelial flux.

1. Introduction

Fumonisins are mycotoxins produced by *Fusarium verticilloides* (formerly *F. moniliforme*). Fumonisin B_1 (FB_1) is the most common and most of three related metabolites that occur in maize damaged by Fusarium kernel rot (FB_1, FB_2, FB_3). FB_1 occurs in maize grown in warm areas worldwide [1,2].

Fumonisin causes several mycotoxicoses in animals [2,3]. The manifestation of FB_1 toxicity shows very different clinical symptoms depending on the species. FB_1 induces leukoencephalomalacia in horses, nephrotoxicity in rats, rabbits and lambs, and hepatotoxicity in all examined species [4]. In pigs, FB_1 is responsible for pulmonary edema, liver failure and cardiovascular toxicity [3]. This toxin is carcinogenic in rodents [2] and may be a contributing factor in human esophageal cancers [1]. Fumonisin has also been shown to produce neural tube defects in rodents related to the effects of fumonisin on folate transport [5–7], which may also occur in

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humans [8]. Despite the range in response to acute and chronic exposure to fumonisin, elevation of free sphingoid bases leading in turn to a depletion of glycosphingolipids, among other sequelae, represent the common initial change.

Sphingolipids are components of plasma membrane representing 10–20% of membrane lipids. Sphingoid bases and complex sphingolipids play a crucial role in signaling pathways and regulating of several cellular functions including growing, differentiation, cellular death and immune response. Further, expression on the cell surface varies with the differentiation state and is affected by disease. Free sphingoid bases and complex sphingolipids are inter-related via the biosynthesis of ceramides by ceramide synthase and by glycoceramide synthase [9].

FB_1 is an inhibitor of the ceramide synthase. The inhibition of this enzyme causes an important increase in free sphinganine and a depletion in complex sphingolipids and ceramide [10]. Under this condition, an increase of the ratio of free sphingoid bases (sphinganine/sphingosine (Sa/So)) has been observed in several tissues and in plasma [11]. There is a relationship between the FB_1 exposure and the increase of the free sphingolipids ratio, and this change is used as a biomarker of experimental exposures [1].

The gastrointestinal tract represents the first barrier against ingested chemicals, food contaminants, and natural toxins [12]. After the ingestion of mycotoxin-contaminated food or feed, intestinal epithelial cells are exposed to a high concentration of toxin [13,14]. It is thus of interest to analyze the effects of fumonisins on the intestine [15–17]. The purpose of this study was to determine changes in sphingoid base ratio and glycolipid composition in intestinal epithelium of pigs after 7 days of exposure to 1.5 mg/kg b.w. FB_1. Additionally, we used a porcine epithelial intestinal cell line to determine the effects of FB_1 on intestinal cells: (i) by measuring the free sphingolipids after short-term exposure, (ii) the *in vitro* trans-epithelial flux after an exposure of 16 days.

2. Materials and methods

2.1. Toxins

Fumonisin B_1 (>98% pure by NMR and HPLC) used for *in vitro* studies was purchased from PROMEC/MRC (Tygerberg, South Africa) and diluted in sterile water. FB_1 (>98% pure by NMR, MS and HPLC) used in the short-term toxin exposure trial in swine was prepared as previously described [18].

2.2. Animals

Six crossbred weanling piglets housed in floored indoor pens were used in this study. They were acclimatized for 1 week prior to being used in experimental protocols and were given *ad libitum* access to water and feed. They were cared for in accordance with the National Institutes of Health Guide and the French Ministry of Agriculture standards for the care and use of laboratory animals. During 7 days, to treated pigs were given 1.5 mg/kg body weight/day FB_1 diluted in water by gavage as already described [19]. Control animals received 4 ml of sterile water. At the end of the experiment, the animals were sacrificed and intestine samples were collected and divided in three parts corresponding to duodenum, jejunum and ileum tissues.

2.3. Brush border vesicles preparation

For each part, brush border vesicles were prepared following a modification of the procedure previously described [20]. Segments of small intestine were excised and thoroughly washed with ice-cold phosphate-buffered saline (PBS), pH 7.2. With a glass slide, the mucosa was scraped off in cold 5 mM EDTA, pH 8.0, and homogenized for 1 min in a blender (Waring Products, New Hartford, Conn.) at maximum speed. The homogenate was then filtered through a nylon mesh and centrifuged for 10 min at 200 × *g* to remove any whole cells or cell aggregates. Subsequently, the supernatant containing the intestinal brush borders was centrifuged for 10 min at 10,000 × *g* and 4 °C and the supernatant discarded. The pellet was washed twice in 5 mM EDTA as above. The final pellet was suspended in PBS and stored at −20 °C until use.

2.4. Sphingolipids and glycolipids samples' preparation

Sphingolipids and glycosphingolipids were extracted from brush border vesicles with three successive extractions: with methanol, methanol/chloroform (2:1) and with methanol/chloroform (1:2). The extracts were pooled and evaporated under nitrogen and hydrolyzed with base to recover free sphingoid bases and to remove phospholipids [21].

2.5. Total glycolipids analysis

The total glycolipids amount was estimated using orcinol to visualize the total glycolipids after chromatography on Silicagel TLC plate (without fluorescent indicator) with 5 μl spots of crude extract to obtain the relative amount in each extract by densitometry.

2.6. Sphingolipid liquid chromatography and mass spectrometry

Each sample was dried under vacuum and re-dissolved in 0.2 ml of mobile phases A and B at 80/20 (v/v), with A = ammonium acetate 5 mM–methanol–tetrahydrofuran (5:2:3, v/v/v) containing 0.01% acetic acid, and B = ammonium acetate 5 mM–methanol–tetrahydrofuran (1:2:7, v/v/v) containing 0.01% acetic acid. As only free sphingolipids rate Sa/So were compared between samples, no internal standard was used in these analyses.

Liquid chromatography (LC) was used to introduce 10 μl of sample into the ESI source at a 0.2 ml/min flow rate. This was carried out using a Thermo Surveyor MS pump (ThermoElectron) fitted with a 10 μl loop Rheodyne injector. The LC column used was an Ultracarb 5 μm ODS (30) column (150 mm × 2 mm) from Phenomenex (Sartrouville, France). Elution involved an isocratic elution at 100% A during 2 min, followed by a 20-min linear gradient to 100% B, held for 10 min, then decreased with a linear gradient to 100% A over 6 min and held at 100% A for 2 min in order to equilibrate the column for the next sample [21].

Sphinganine and sphingosine mass spectrometric analyses were performed on a Finnigan LCQ Deca XP MAX ion trap mass spectrometer (Thermo Electron, Les Ulis, France) equipped with an ESI source operated in the positive mode using the following conditions: needle voltage (5.0 kV); heated capillary temperature (375 °C); capillary voltage (15 V); tube lens offset (20 V). Nitrogen was used as the sheath gas at a flow rate of 69 (arbitrary LCQ units) and as the sweep gas at a flow rate of 14 (arbitrary LCQ units). All spectra were acquired under Automatic Gain Control conditions in the so-called ''zoom scan'' mode centered on 301.8.

2.7. Glycolipid ''mass fingerprint''

Glycolipids mass spectrometry analyses were performed on the above spectrometer operated in the negative mode under the following conditions: needle voltage (−4.0 kV); heated capillary temperature (375.0 °C); capillary voltage (−47 V); tube lens offset (−15 V); other conditions were as described above.

2.8. Cell culture and toxin

IPEC-1 is a newborn swine epithelial cell line that was derived from the small intestine of a newborn un-suckled piglet, cultured as described [22]. Briefly, the cells were maintained in serial passages in 75 cm^2 flasks at 37 °C, in a humidified incubator with a 5% CO_2 atmosphere. IPEC-1 were grown in complete DMEM/F-12 medium (Eurobio) supplemented with antibiotics, 5% FBS, 2 mM L-glutamine, 15 mM HEPES (Eurobio), epidermal growth factor (5 μg/l; Becton Dickinson Labware, Le Pont de Claix, France), and ITS (Premix, Sigma, St Quentin Fallavier, France). The ITS composition included insulin (5 μg/ml), transferrin (5 μg/ml), and selenium (5 ng/ml).

2.9. Cellular sphingolipids analysis

In order to evaluate the influence of FB_1 on cellular sphingolipid content, 10^5 cells IPEC-1 cells were seeded in 6-well plates (area: 9 cm^2, Polylabo-Nunc), in 3 ml of complete medium supplemented with 2% FBS. After 24 h of culture, the cells were treated with 20 or 100 μM FB_1. Every 48 h, cell culture medium was replaced by fresh complete medium supplemented with FBS and FB_1. After 2, 4 or 6 days of FB_1 exposure, the cells were scraped and suspended in 1.5 ml HBSS. An aliquot of 100 μl was used to determine protein concentration and 1.4 ml were used to analyze cellular sphingolipid contents. Sphingolipids were extracted in methanol/chloroform (1:1) by sonication and hydrolyzed as previously described [23]. After *O*-phtaldialdehyde derivatization in presence of thiomethanol and sodium borate, sphingosine and sphinganine were separated by HPLC on Daisogel C18-B column (2.1 mm × 150 mm; 5 μ) isocratically with 10% of water in methanol, and quantified by UV detection at 331 nm using a calibration curve.

2.10. Trans-epithelial electrical resistance (TEER)

IPEC-1 cells were seeded at 10^5 cells in 0.3 cm^2 transwell filters with 0.4 μm pores (Becton-Dickinson Labware). When the cells were confluent, apical and basal compartments were filled with serum-free complete DMEM/F-12 containing dexamethasone (50 μg/ml, Sigma) to allow the cells to differentiate. Treatments with FB_1 (0, 50, 100, and 200 μM) were started at the end of the differentiation process (when cells were fully differentiated, 10 days after the addition of dexamethasone). The integrity of tight junctions was assessed during 26 days by measuring the TEER using a Millicell volt-ohm meter (Millipore, Saint-Quentin en Yvelines, France). Experimental TEER values were expressed as $k\Omega \times cm^2$.

2.11. Measurement of trans-epithelial FB1 passage

Differentiated IPEC-1 cells were prepared as above. In this case, every 2 days, apical and basal culture media were changed. The trans-epithelial passage of FB_1 was assessed after 3, 10 and 16 days of exposure by measuring the FB_1 amount in apical and basal compartments by ion trap mass spectrometry. Briefly, culture medium was desalted and cleaned up using a 100 mg/ml Solid Phase Extraction Anionic column (SAX Supelco). The SAX column was conditioned with 1 ml of methanol and equilibrated with 1 ml of a 25 mM Tris–acetate buffer at pH 8.0. One microliter of sample was loaded on the column which was then washed with 1 ml of methanol/water (1:1). The toxin was eluted from the column with 1 ml of 1% acetic acid solution in methanol/water (1:1). This was carried out using a Thermo Separation P4000 pump (Thermo Quest) fitted with a 5 μl loop Rheodyne injector. The LC column used was an Ultrabase 5 μm C18 column (25 mm × 2 mm) from SFCC (Eragny, France). An isocratic elution at 0.2 ml/min flow rate with H_2O/CH_3OH (1:1) was used.

FB_1 mass spectrometry analyses were performed in the negative mode under the following conditions: needle voltage (−5.7 kV); heated capillary temperature (230 °C); capillary voltage (−4 V); tube lens offset (20 V). Nitrogen was used as the sheath gas at a flow rate of 80 (arb. units). MS/MS experiments were carried out using helium as collision gas. Collision energies were adjusted in order to get maximum structural information for each experiment, i.e., typically 25% (corresponding to 1.25 V_{p-p} excitation voltage) for MS/MS on the quasi-molecular species. In all MS^n experiments, ions were isolated at qz 0.8 with 1.5 u as ion isolation width and activated at qz 0.25.

2.12. Statistical analysis

ANOVA comparison with Fisher's PLSD, $p < 0.05$ was used to determine differences induced by FB_1 on free sphinganine cellular amount and on free sphingosine cellular amount.

3. Results

3.1. Effect of FB1 on porcine intestinal epithelium through the free sphingoid base exploration

Using a sphingosine (So) and a sphinganine (Sa) standard solutions, a good separation was obtained using the conditions described (Fig. 1). So and Sa had retention times of 18.3 min (*m/z* 300.5) and 19.2 min (*m/z* 302.5), respectively. These results were obtained using the same amount of So and

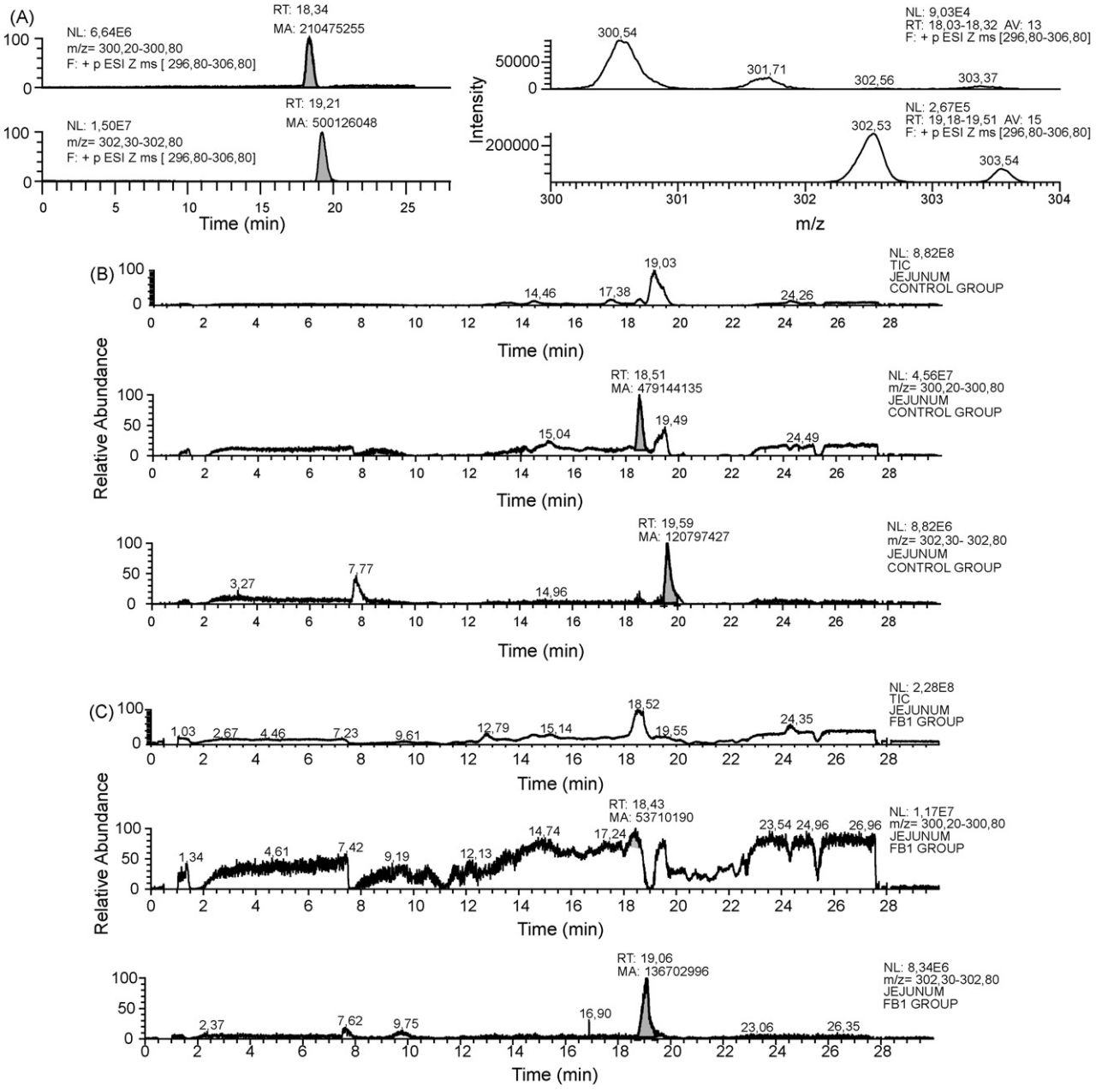

Fig. 1 – Free sphingoïd bases investigation by positive electrospray ionization in zoom scan mode. (A) LC-MS analysis of 30 pg of sphingosine (up) and sphinganine (bottom) standards. (B) Sphingosine and sphinganine LC-MS analysis of jejunum segment of the control group and (C) sphingosine and sphinganine LC-MS analysis of the same segment in the FB_1-treated group. For both panels from up to bottom: TIC chromatogram, extracted ion mass chromatogram of *m/z* 300.5 ± 0.3 for sphingosine identification, extracted ion mass chromatogram of *m/z* at 302.5 ± 0.3 for sphinganine identification.

Sa injected on column. Under these conditions, the peak areas obtained were not identical, probably due to a difference in ionization. The peak areas of Sa were higher than those of So, in a ratio of 2.37.

The results from the FB_1-treated and control animals are shown in Fig. 1. A change in the Sa/So ratio took place in the jejunum segment in the treated animals. The tissues from the control group had a Sa/So ratio of 0.1 (Fig. 1, panel B), whereas tissues in the FB_1-treated-animals showed a Sa/So ratio of 1.1 (Fig. 1, panel C). No other significant Sa/So perturbations were observed in the duodenum and in the ileum (data not shown).

3.2. *Effect of FB1 on porcine intestinal epithelium through the study of the mass fingerprints of glycolipids*

All three tissues from the FB_1-treated pigs had lower glycolipids than the control group ($p < 0.05$). No differences

in response were observed between the duodenum, the jejunum and ileum (data not shown).

In order to examine this more carefully, LC-MS analyses were performed using the procedure of Mano et al. [21]. To obtain a global view, data have been treated as ion maps. In such an approach, each *m/z* ratio was considered as an independent variable regardless of the glycolipid. That means that several fragments were considered according to the sugar and fatty acid components of the various structures. As noted above, all intestinal segments of the treated group contained 20–50% less glycolipid than in the corresponding samples from the control group. Apart the decrease in total glycolipid content, changes in glycolipid composition (''mass fingerprint'') were seen only in jejunum, whereas no changes were observed in the duodenum and ileum (not shown). The comparison of glycolipid mass fingerprint in the jejunum,

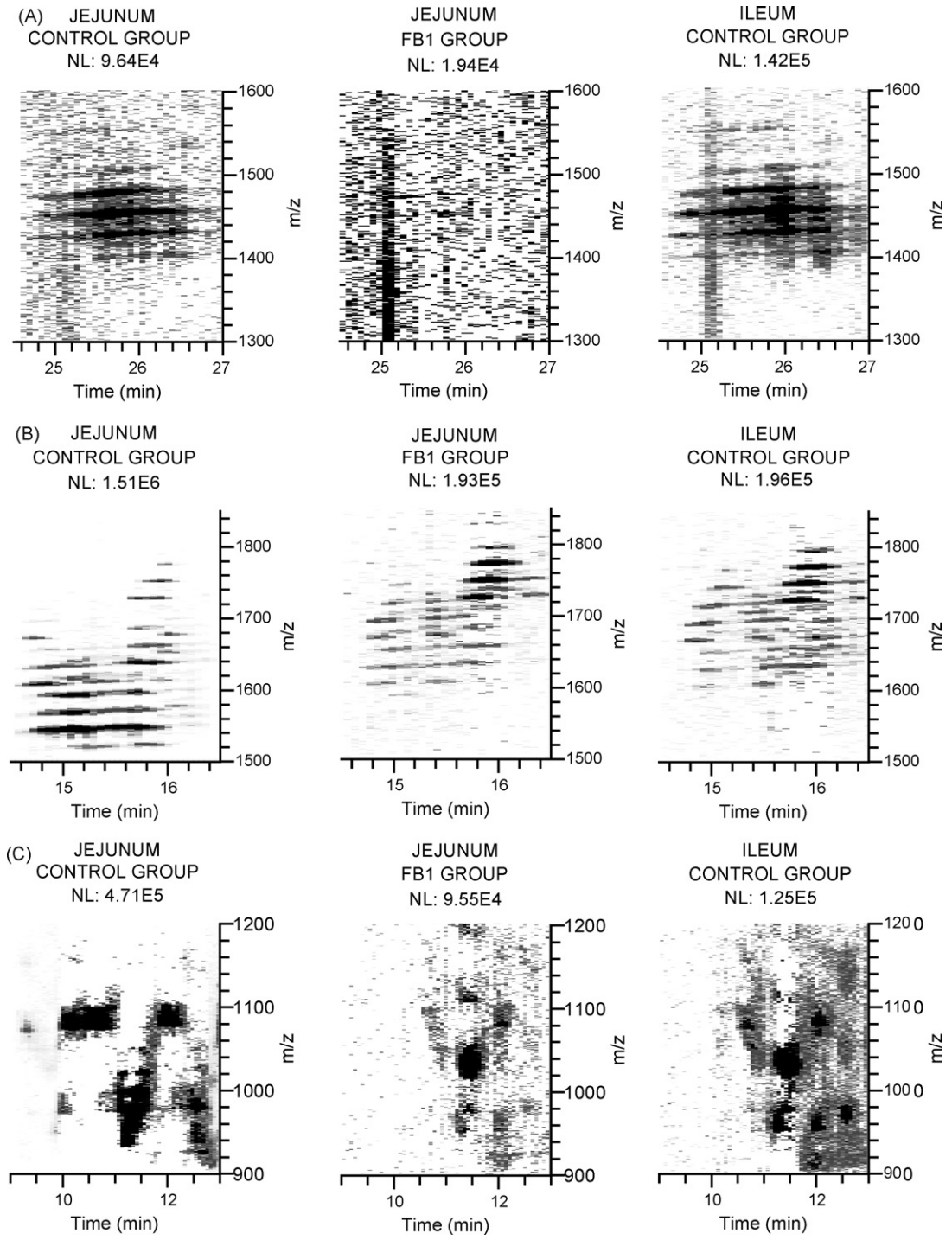

Fig. 2 – Glycolipids mass fingerprints investigation. From left to right, in jejunum control group, jejunum FB1-treated group, and ileum control group under negative electrospray ionisation. From top to bottom: ion maps for apolar (Panel A), medium polarity (Panel B) and polar glycolipids (Panel C), respectively.

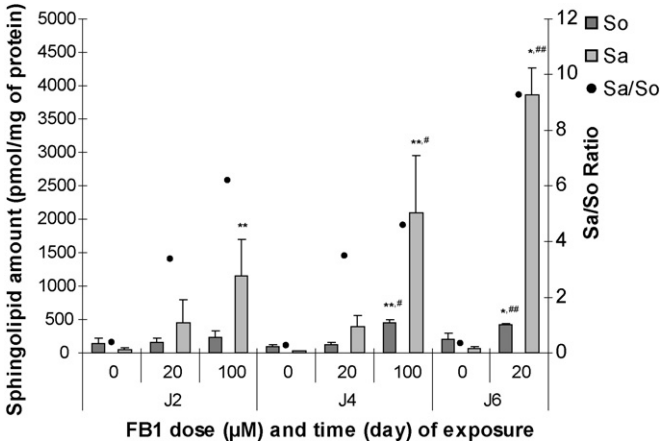

Fig. 3 – Modulation of sphinganine (Sa), sphingosine (So) and sphingolipids ratio in IPEC-1 cells depending on the dose and the time of FB_1 exposure. Values are expressed as mean ± S.D. of three independent experiments. *Values significantly different from the FB_1 dose of 0 μM for the same treatment day, $p < 0.05$. **Values significantly different from the FB_1 dose of 0 and 20 μM for the same treatment day, $p < 0.05$. #Values significantly different from the same FB_1 dose for the day 2, $p < 0.05$. ##Values significantly different from the same FB_1 dose for the day 2 and the day 4, $p < 0.05$.

shows important differences in three areas compared to the controls. The most hydrophobic area (Panel A, Fig. 2) corresponds to the area for retention times ranging from 24.5 to 27.0 min and for *m/z* between 1300 and 1600. In this region, whereas no glycolipids were detected in the FB_1-treated group, three mass spots were present in the control group at *m/z* 1429, 1455 and 1480, respectively. The medium polarity region (Panel B, Fig. 2) corresponded to retention times from 14.5 to 16.5 min and *m/z* ratios between 1500 and 1850. The glycolipids in the jejunum control tissue were mainly below *m/z* 1700. However, the major glycolipids observed in the jejunum FB1-treated group were in the *m/z* 1700–1800 range as observed on the ileum control group map. This modified glycolipids composition of the jejunum segment could be explained by a specific effect of the FB_1 on the jejunal epithelial cells. Indeed, during FB_1 exposure, jejunal epithelial cells might be dedifferentiated in ileal epithelial cells to increase the resistance against this mycotoxin. Finally, for the most polar compounds (Panel C, Fig. 2), from 9 to 13 min and for *m/z* between 900 and1200, a compound appeared at *m/z* 1030 in the FB_1-treated group. This spot was also observed on the ileum control group map. There were few glycolipids observed in this region in the control group.

3.3. *In vitro effect of FB_1*

We have observed a dose- and time-dependent effect of FB_1 on the amount of free sphingolipids in intestinal porcine epithelial cells (IPEC-1). A significant increase in the sphinganine levels was observed in cells treated with 100 μM of FB_1 at day 2, whereas an exposure of 20 μM of FB_1 resulted in a significant increase of sphinganine after 6 days of treatment. A significant increase in sphingosine was shown after 4 days in the 100 μM of FB_1 treatment and by an exposure of 20 μM of FB_1 after 6 days. A significant difference in Sa/So ratio was found only for 20 μM of FB_1 treatment versus the control group after 6 days (Fig. 3).

3.4. *Trans-epithelial passage of FB_1*

In order to evaluate the trans-epithelial passage through an *in vitro* cellular model of intestinal pig epithelium, transfer of FB_1 from the apical cell culture medium to the basolateral compartment was determined by tandem mass spectrometry. The negative ESI produced $[M - H]^-$ ion from FB_1 (*m/z* 720) was used as the precursor ion in the LC-MS/MS method based on the monitoring of the *m/z* 562 product ion trace for quantitative measurements. Under these conditions, a linear response was found between 2 and 100 μM ($r^2 = 0.98$). The detection limit was 100 nM, corresponding to 720 pg FB_1 injected on the LC column. We verified that FB_1 did not adsorb on the tissue culture plates and on the Transwell filters (data not shown).

In vitro trans-epithelial measurement passage measurements were performed at three time points following addition

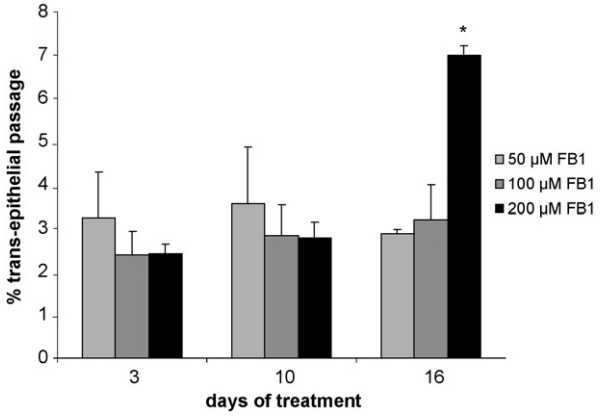

Fig. 4 – Intestinal FB_1 trans-epithelial passage measurements during a chronical exposure of this own mycotoxin. Values are expressed as mean ± S.D. of three independent dosages. *Values significantly different from the other groups, $p < 0.05$.

of FB_1 post differentiation (Fig. 4). At 3 and 6 days post differentiation, FB_1 flux was maintained at the level of 2.5–3% of the mass amount of FB_1 added. After 16 days, intestinal FB_1 flux increased only for the maximum dose tested (200 μM). At this concentration, *in vitro* intestinal permeability was ~3 times greater compared to the intestinal epithelium treated by the lowest dose of FB_1 (50 μM). A comparison of the trans-epithelial passage data and of the TEER data showed that the FB_1 passage was significantly increased when TEER was low (Fig. 5), suggesting damage of the epithelium that could be associated with tight junction dysfunction.

4. Discussion

Systemic uptake of fumonisin from oral exposures is poor (2–6% of dose [1]). However, the consumption of low quality of maize-based foods and feeds may result in very high exposure of the digestive system tissues. This would be expected to be more important in monogastric animals (including humans). Considering domestic animals, recommended swine diets can contain *ca.* 75% maize. For swine, oral FB_1 bioavailability is ~4% [24]. After 72 h, 91.7% of the radiolabeled FB_1 is excreted in faeces under native or metabolised form, with approximately 1.2% of the dose eliminated via the bile, and only 0.8% is recovered in urine. Enterohepatic circulation could increase the exposure of intestinal cells to this mycotoxin.

In the present study, we observed significant *in vivo* and *in vitro* effects of FB_1 on intestinal epithelium. *In vivo* effects were determined after an exposure of 1.5 mg/kg b.w. during 1 week, which corresponds to a feed contaminated with 24 μg/g. *In vitro* effects on epithelial intestinal cells were determined at concentrations of 20–200 μM. Although this is difficult to estimate because real exposures would be long in duration, this would notionally correspond to a feed contaminated with 16–160 μg/g (or 1–10 mg/kg b.w. in whole pig). Such concentrations are within the range commonly observed in contaminated feed throughout the world [4]. For example, FB_1 was found in 50% of maize samples collected between 1988 and 1991 from the Midwestern United States [25]. In that survey, 10% of the samples had toxin levels between 10 and 50 μg/g.

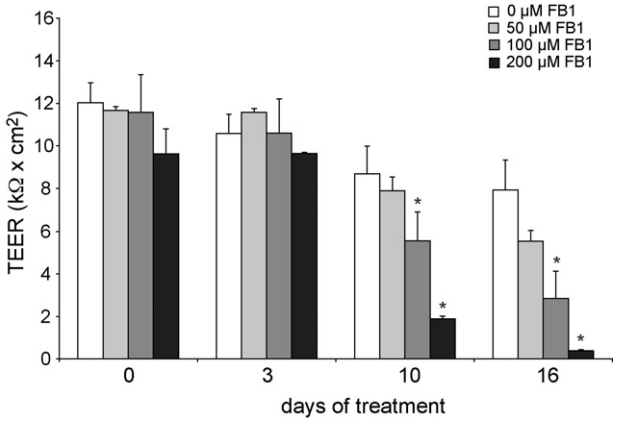

Fig. 5 – Time-course of FB_1-induced TEER decrease in IPEC-1 cells. IPEC-1 cells cultured on Transwell filters were incubated for various times in the absence or presence of FB_1 added into the apical compartment. Treatment with FB_1 began on fully differentiated cells. The values are expressed as the mean ± S.D. of three independent wells. *Values significantly different from the control group (0 μM) for the same treatment day, $p < 0.05$.

The primary biochemical effect of fumonisin B_1 is to inhibit ceramide synthase, leading to the accumulation of sphingoïd bases and to the depletion of more complex sphingolipids [10]. Increases in free sphinganine concentration and in the Sa/So ratio have been observed in several cell lines as well as in different tissues including mouse intestine [15]. In pigs, the Sa/So ratio had been studied in several organs such as lung, liver, kidneys and pancreas [26] but not in the intestine. In 2004, Bouhet et al. [22] demonstrated that a prolonged (10–15 days) exposure to modest concentrations (50 μM) FB_1 alters the behaviour of IPEC-1 cells. This prompted the present study to assess whether this effect occurred *in vivo* and to better understand the mechanism.

This is the first report that FB_1 affects the concentrations of free sphingoid bases and the glycolipid content of porcine intestinal epithelial cells *in vivo*. The Sa/So ratio increased by 10-fold in jejunum, with a variation from 0.1 in the control group to 1.1 in the FB_1-treated group. In contrast, no change in free sphingoid base content was detected in the duodenum and ileum. FB_1 treatment further resulted in a significant decrease in the total glycolipid content in each segment. In both duodenum and ileum, the qualitative distributions were conserved, whereas in jejunum, the distribution of glycolipids in the treated animals was more similar to that of the ileum than to the jejunum of untreated animals, particularly in the medium and high polarity ranges. This result was seen in all three treated animals, confirming that FB_1 acts more specifically on the jejunal segment than on the other compartments of the small intestine (Fig. 2). These major changes in membrane composition would likely have a number of consequences. These might include alterations in toxin or nutrients uptake.

As noted above, FB_1 bioavailability is poor, *ca.* 4% for swine [27]. However, the study by Prelusky et al. was performed with a single administration of FB_1. In this and our previous study [22], we had shown that a chronic exposure to FB_1 diminishes trans-epithelial movement at least of the toxin itself. During the first 10 days of exposure and regardless of the FB_1 exposure, only 3% of FB_1 was taken up in the basal compartment. After 16 days, there was a thresholded change in this response. The two lower FB_1 concentrations tested (50 and 100 μM) did not influence the passage rate of this mycotoxin; whereas high concentrations (200 μM) had a large increase (Fig. 4). These results offer some confirmation of the hypothesis that FB_1 can affect overall transport most likely to many substances, including the toxin itself. This suggests that the low uptake of FB1 from short-term exposures [24] requires further investigation in a chronic exposure study to determine if the AUC changes with chronic exposure.

The time lag between the initiation of cellular exposure to FB_1 and increases in free sphingolipids in the IPEC-1 cells as well as the lag in detecting changes in the *in vitro* trans-epithelial passage can be explained by the turnover of complex sphingolipids [15]. The present data confirm the progressive

influence of FB_1 on the increase of the intestinal transepithelial passage of this mycotoxin along the time. There are many possible explanations of these effects. These include perturbation of the lipid raft microdomain resulting in the inhibition of the extracellular flux of this mycotoxin, or by tight junction disruption, increasing paracellular passage. The latter hypothesis is supported by evidence that glycosphingolipids are structural components of the tight junctions and/or contribute to the ''raft-like'' environments around these regions [28].

In summary, we have demonstrated that in swine, fumonisin acts selectively on the jejunum segment causing large changes in membrane composition related to both sphingolipids and total glycolipid composition. This is in good agreement with our previous data [29] where we have shown that these changes may contribute to the increased colonization of the intestinal tract by pathogenic bacteria. Indeed, as shown by Svensson et al. in 2003 [30] glycolipids are closely implied in the bacterial attachment capability on the cell surface. Studies done using IPEC-1 cells demonstrate that this changed the uptake of FB_1. This tissue-specific response may be explained by a characteristic fatty acid transporter family located in the jejunum and ileum [31]. All these results confirm that chronic exposure to FB_1 causes functional damage to intestinal tissue, a consequence of fumonisin exposure that has not been widely investigated. Considering the chronic exposures to fumonisin that can occur when this toxin is a common contaminant of maize used for feeding swine (and humans), this would reasonably be expected to result in two important consequences. The first would be to increase systemic exposure compared to that predicted by the short-term experimental data currently available. Secondly, it is reasonable to speculate that this would affect nutrient uptake as well as change the response of the jejunum to the disease. All of these consequences may have important implications for human and animal health.

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