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Calcium montmorillonite clay reduces urinary biomarkers of fumonisin B₁ exposure in rats and humans

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Fumonisin B₁ (FB₁) is often a co-contaminant with aflatoxin (AF) in grains and may enhance AF’s carcinogenicity by acting as a cancer promoter. Calcium montmorillonite (i.e. NovaSil, NS) is a possible dietary intervention to help decrease chronic aflatoxin exposure where populations are at risk. Previous studies show that an oral dose of NS clay was able to reduce AF exposure in a Ghanaian population. In vitro analyses from our laboratory indicated that FB₁ (like aflatoxin) could also be sorbed onto the surfaces of NS. Hence, our objectives were to evaluate the efficacy of NS clay to reduce urinary FB₁ in a rodent model and then in a human population highly exposed to AF. In the rodent model, male Fisher rats were randomly assigned to either FB₁ control, FB₁ + 2% NS or absolute control group. FB₁ alone or with clay was given as a single dose by gavage. For the human trial, participants received NS (1.5 or 3 g day⁻¹) or placebo (1.5 g day⁻¹) for 3 months. Urines from weeks 8 and 10 were collected from the study participants for analysis. In rats, NS significantly reduced urinary FB₁ biomarker by 20% in 24 h and 50% after 48 h compared to controls. In the humans, 56% of the urine samples analysed (n = 186) had detectable levels of FB₁. Median urinary FB₁ levels were significantly (p < 0.05) decreased by ≥90% in the high dose NS group (3 g day⁻¹) compared to the placebo. This work indicates that our study participants in Ghana were exposed to FB₁ (in addition to AFs) from the diet. Moreover, earlier studies have shown conclusively that NS reduces the bioavailability of AF and the findings from this study suggest that NS clay also reduces the bioavailability FB₁. This is important since AF is a proven dietary risk factor for hepatocellular carcinoma (HCC) in humans and FB₁ is suspected to be a dietary risk factor for HCC and oesophageal cancer in humans.

Keywords: HPLC; toxicology, animal study; clinical study; aflatoxins; fumonisins; mycotoxins

Introduction

Fumonisin B₁ (FB₁) is the most abundant of the naturally occurring fumonisins. FB₁ is produced by Fusarium fungi and it has been shown to be hepatotoxic, nephrotoxic and carcinogenic in a number of species (Voss et al. 2002). Epidemiological studies have correlated Fusarium spp. and fumonisin contamination of food sources with increased incidences of oesophageal cancer in regions of China and South Africa, neural tube defects along the Texas–Mexico border, and primary liver cancer in China (Chu and Li 1994; Ueno et al. 1997; Marasas et al. 2004; Shephard et al. 2007). However, there are no reports that definitively demonstrate a causative relationship (Stockmann-Juvala and Savolainen 2008). While methods assessing the hazard of fumonisins in foodstuffs exist, few are capable of determining the actual exposure of populations considered to be “at risk” (Shephard et al. 1996, 2007). Changes in sphingolipid ratio due to the inhibitory effect of FB₁ on de novo ceramide synthase activity are commonly utilised as biomarkers for FB₁ exposure (Voss et al. 2002; He et al. 2006; Sabourdy et al. 2008). Alterations in the sphinganine:sphingosine ratio have accurately reflected fumonisin exposure in non-human primates and swine have shown that excretion of FB₁ is mainly through the faeces with ≤1% urinary excretion (Shephard et al. 1994;
Fodor et al. 2008). Due to high dietary levels of FB1 in developing countries, urinary biomarkers have been successfully used to characterise exposure in human populations (Shetty et al. 1998; Turner et al. 1999; Gong et al. 2008; Van der Westhuizen et al. 2011). This biomarker has been applied to evaluate intervention strategies that could reduce exposure to fumonisins (Van der Westhuizen et al. 2011). Our laboratory has previously reported that a Ghanaian population is highly exposed to aflatoxins (AFs) due to the frequent consumption of AF-contaminated foods (Jolly et al. 2006; Phillips et al. 2008; Wang et al. 2008). It has been well-documented that AFs are contributors of immunosuppression, malnutrition and hepatocellular carcinoma (Wogan 1992; Williams et al. 2004; Jiang et al. 2005). Furthermore, in vitro and in vivo studies have demonstrated that FB1 can potentiate the effects of AFs (Carlson et al. 2001; IARC 2002; McKean et al. 2006). Kpodo et al. (2000) verified the co-occurrence of Fusarium spp. and fumonisins with AFs in maize samples from Ghanaian markets. Hence, it was postulated that participants from our previous study in Ghana, shown to be at high risk for aflatoxicosis, may be co-exposed to fumonisins. To reduce AF exposure, the use of NovaSil (NS) (a dioctahedral smectite clay) as an intervention plan for the enterosorption of the toxin has been shown to be safe and effective in humans (Phillips et al. 2008). Importantly, NS has also been evaluated in vitro for FB1 sorption and found to interact with this mycotoxin at interlayer surfaces at acidic pH (Lemke 2000). A possible mechanism for FB1 sorption to NS is protonation of the amino group at C2 on the molecule in acidic conditions (i.e. the stomach). The charged FB1 may be bound through a cationic exchange reaction at negatively charged surfaces of the clay.

Thus, the main objectives of this study were to: (1) determine the efficacy of NS to reduce urinary FB1 in Fischer 344 rats; (2) assess exposure to fumonisins in a Ghanaian population highly exposed to AFs using the FB1 urinary biomarker; and (3) assess the efficacy of NS to reduce fumonisin exposure in this population. Importantly, strategies that reduce co-exposures to AFs and fumonisins are highly desirable for populations at risk for both agents.

**Materials and methods**

**Chemicals**

FB1, o-phthalaldehyde (OPA), methanol, 2-mercaptoethanol, sodium phosphate dibasic and phosphoric acid were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Naphthalene 2,3-dicarboxaldehyde (NDA), potassium cyanide (KCN), sodium tetraborate (STB), phosphate-buffered saline (PBS) and potassium hydroxide (KOH) were also obtained from Sigma. Fumonitest WB columns were purchased from VICAM (Watertown, MA, USA). All of the experiments were done using filtered and deionised water (18.2 MΩ cm) (Millipore, Milford, MA, USA), and all other chemicals and reagents were purchased commercially at the highest degree of purity available.

**Preparation of FB1 standard and derivatisation solutions**

FB1 standard solution (1 mg ml⁻¹) was prepared in 50:50 (v/v) methanol/water. NDA and OPA solutions were made daily and stored at 4 °C in the dark. NDA was prepared as described by He et al. (2005) and OPA was prepared according to Cai et al. (2007). These solutions were prepared according to the above mentioned methods, except during the fluorescence comparison analysis in which equal molar concentrations of NDA and OPA were used. For the experiments comparing sensitivity of the derivatising agents, excitation and emission parameters were set at 335 and 440 nm for OPA and 252 and 483 nm for NDA.

**Rodent model experimental design**

A total of 18 male Fisher 344 rats (5-week-old male, 110–130 g body weight, bw) were purchased from Harlan (Houston, TX, USA) and maintained on nutritionally complete powdered feed (Teklad rodent diet 8604; Harlan, Madison, WI, USA) and water ad libitum. The rats were randomised into three equal groups: (A) Absolute control; (B) FB1 control; and (C) FB1 plus 2% (w/w) NS inclusion in the feed. The dietary NS clay concentration was based on the highest level previously determined to be safe in a chronic rodent study (Afriyie-Gyawu et al. 2005). After a brief acclimation period (1 week), the rats were gavaged and placed in metabolism cages (1 rat per cage) for 8 days and daily urine samples were collected. Groups B and C were administered 3.75 mg FB1 per rat (25 mg kg⁻¹ bw based on an average of 150 g bw) via aqueous gavage. The gavage solution for group C also contained 2% NS (the daily amount inclusion based on an average of 20 g feed consumed per day). Group C also received dietary NS (2% wet weight, ww) during the acclimation week and the week after gavage. All rats were housed in a climate-controlled environment (temperature 22–25°C) that was artificially illuminated (12-h dark/light cycle) and free from chemical contamination. Rats were inspected daily for general appearance, behavioural changes and signs of morbidity and mortality. Body weights were measured prior to acclimation, prior to gavage, and every 2 days during sample collection.
Stored urine samples were available from our previous Phase IIa clinical trial assessing the safety and efficacy of NovaSil for reducing AF exposure in a Ghanaian population (Afriyie-Gyawu et al. 2008; Phillips et al. 2008). Volunteers were recruited from six communities within the Ejura-Sekyedumase district in the Ashanti Region of Ghana. Biomarker data regarding exposure to fumonins was unknown for this area; however, maize samples from neighbouring districts in Ghana had been shown to contain fumonins (Kpodo et al. 2000). To our knowledge, this is the first study to measure FB₁ in a human population in Ghana. A total of 177 volunteers (male and female) were selected to participate in the NS intervention study based on predetermined inclusion criteria. Participants met the following conditions: signed consent form, healthy status based on physical examination, age 18–58 years, intake of corn and/or groundnut-based foods at least four times per week, blood AFB₁-albumin adduct levels >0.5 pmol AFB₁ mg⁻¹ albumin, no history of chronic disease(s), no use of prescribed medications, non-pregnant and non-breastfeeding females, normal ranges of haematological parameters and normal liver and renal function indicators (blood and urine parameters). The FB₁ levels in food consumed by our study participants were not measured and hence could not be correlated with urinary excretion patterns.

**Human study design and protocol**

The study protocol was approved by the Institutional Review Boards at Texas A&M University and Noguchi Memorial Institute for Medical Research in Ghana for Ethical Clearance. All participants were provided written informed consent, as well as an oral explanation prior to beginning the study. The overall study design adhered to guidelines set for a randomised, double-blind, placebo controlled Phase IIa clinical trial (Sangare-Tigori et al. 2006). Participants were randomly divided into one of three groups: High dose (NS 3.0 g day⁻¹), low dose (NS 1.5 g day⁻¹) or placebo control (1.5 g day⁻¹; microcrystalline cellulose) for a period of 3 months. Trained study monitors collected blood and urine samples (morning urine) from each participant at multiple time points. Aliquots of the urines were stored separately in polypropylene tubes and shipped frozen to Texas A&M University where they were stored at −80°C prior to biomarker analysis. Samples collected at weeks 8 and 10 from all three treatment groups (186 samples) were used in this preliminary study to assess fumonisin exposure and intervention efficacy. Baseline urine samples were not available for analysis.

**FB₁ urine analysis**

Frozen urine samples were thawed at room temperature and centrifuged at 500 g for 5 min. Portions (10 ml) of the urine samples were passed through Fumonistest WB columns (VICAM, Watertown, MA, USA) at a flow rate of approximately 1 ml min⁻¹). Columns were washed with 6 ml phosphate-buffered saline and 6 ml of water before elution with 2 ml of 100% methanol. Samples were dried at 50°C under nitrogen gas and reconstituted in 150 μl of 50:50 (v/v) methanol/water. Samples were derivatised with NDA as previously described by Cho et al. (2002). Briefly, aliquots of NDA solution (300 μl) were added to samples which were vortexed and heated (50°C) for 10 min. Analyte extracts were injected (200 μl) onto a 250 × 4.6 mm C₁₈ LUNA column, 5 μm particle size (Phenomenex, Torrance, CA, USA) with mobile phase consisting of 77:23 (v/v) methanol/sodium phosphate dibasic (0.1 M, pH 3.35) at a flow-rate of 1 ml min⁻¹. FB₁ analysis was performed using a Waters HPLC System equipped with an auto-injector (model 717A) and fluorescence detector (model 2475). Excitation and emission parameters were set at 252 and 483 nm for detection of NDA-derivatised samples. Creatinine concentrations in animal urine samples were measured at the Texas Veterinary Medical Diagnostic Laboratory in College Station (TX, USA). Creatinine concentrations in human urine samples were measured at St. Joseph’s Regional Health Center Laboratory in Bryan (TX, USA). The identity of the urine metabolite was confirmed via MALDI-TOF on a Kratos Kompact Probe MALDI-MS instrument (Kratos Analytical). A saturated solution of alpha-cyano-4-hydroxycinnamic acid (in methanol) was used to facilitate ionisation of the sample as previously described by Neupert et al. (2009).

**Statistics**

Data generated from HPLC analyses were transferred into an Excel database for management. Mean, median, confidence interval (CI) and range were calculated for concentrations of FB₁. To show the effect of NS ingestion on FB₁ levels, statistical evaluation focused on the comparisons among different treatment groups in the rodent model and human population. Two time points were utilised to further evaluate the efficacy of the NS sorbent in the human clinical intervention trial. For statistical analysis, non-detectable samples = LOD/2. The Kruskal–Wallis and Wilcoxon rank-sum non-parametric tests were used to compare the differences among and between treatment groups. A p-value ≤0.05 (two-tailed) was considered significant. Statistical analyses were done using SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA).
Results

Comparison of OPA versus NDA derivatisation sensitivity

Detection of both OPA and NDA derivatised FB1 was linear (0–480 ng), with NDA having a stable lower LOD (0.242 ng) (Figure 1A–D). The detected relative intensity of NDA derivatised FB1 was two to five times the intensity of OPA derivatised FB1 (Figure 1A, B). Thus, NDA derivatisation was used and favoured for analysis of urine samples in both the rodent model and human study.

NovaSil efficacy in the rodent model

Urines collected at 24, 48 and 72 h post-gavage were analysed for FB1. Most of the FB1 dosed was eliminated in urine within 72 h (Figure 2). At 24 h, the mean total urinary FB1 excretion for rats in group B was 864.7 ng FB1 ml⁻¹ urine (94.1 ng FB1 mg⁻¹ creatinine). After 48 h, excretion of the biomarker was reduced to 183.1 ng FB1 ml⁻¹ urine (9.4 ng FB1 mg⁻¹ creatinine). Rats in group C showed a mean total urinary excretion of 625.7 ng FB1 ml⁻¹ urine (9.4 ng FB1 mg⁻¹ creatinine). Rats in group A were used to verify the lack of urinary FB1 (Table 1). There was a statistically significant difference (p < 0.05) in the median (Mdn) levels of urinary FB1 when all three treatment groups were compared. At 24 h post-gavage, median value of urinary FB1 was significantly reduced by 34% in the NS treatment group (Mdn = 590.4 ng ml⁻¹ urine) compared to the FB1 control group (Mdn = 892.4 ng ml⁻¹ urine) (p < 0.05). When standardised with creatinine, urinary FB1 was still significantly reduced by 20%. At 48 h post-gavage, there was a significant reduction of 50% in urinary FB1 in the NS treatment group when compared to the FB1 control group.

NovaSil efficacy in the human study

A total of 186 urine samples from study participants at week 8 and week 10 were analysed for the presence of FB1. Mean and median levels of FB1 are presented in Table 2. When placebo groups were compared, 60.9% of week 8 (14/23) and 72.1% of week 10 (31/43), samples were found to be positive for the presence of FB1 with a median urinary FB1 concentration of 2.00 and 2.91 ng mg⁻¹ creatinine, respectively. Comparison of the week 8 high and low dose NS treatment groups to placebo revealed a decreased percentage of
participants with detectable urinary FB$_1$ (39.1 and 45.5%, respectively). A similar numerical reduction was noted when week 10 high and low dose treatment groups were compared to placebo with 54.3 and 52.5% being positive for FB$_1$, respectively. Median FB$_1$ levels for the high dose treatment group were found to be significantly lower than the placebo group at week 8 ($p < 0.05$). Median levels for the low dose group were reduced but not found to be significant. Similarly, week 10 median urinary FB$_1$ levels from both, high and low dose groups were significantly reduced ($p < 0.05$) when compared to placebo (Figures 3 and 4; Table 2).

**MALDI-TOF analysis**

MALDI-TOF mass spectrometry (MTMS) analysis of parent (M + H$^+$), NDA derivatised, and a NDA derivatised + sodium ion (M + Na$^+$) FB$_1$ confirmed a mass/charge of 723.0, 906.5, and 928.3, respectively (Figure 5A–C). MTMS analysis of FB$_1$ peaks collected from HPLC analysis of randomly selected positive urine samples verified the presence and identity of FB$_1$, with peaks at 907.4 (M + H$^+$) and 928.5 (M + Na$^+$) (Figure 5C). The lack of parent peak (~722.8) in MTMS analysed samples may provide an indirect indicator of the efficiency of the NDA derivatisation reaction.

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**Table 1. Reduction in FB$_1$ levels with NovaSil treatment in Fischer 344 rats.**

<table>
<thead>
<tr>
<th>Urine collection 24h post-gavage</th>
<th>Absolute control</th>
<th>FB$_1$ control</th>
<th>FB$_1$ + 2% NS treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>% detectable FB$_1$ samples</td>
<td>0 ($n = 6$)</td>
<td>100 ($n = 6$)</td>
<td>100 ($n = 6$)</td>
</tr>
<tr>
<td>Mean FB$_1$ (95% CI)</td>
<td>N.D.</td>
<td>864.68 (719–1010.14)</td>
<td>625.77 (554.17–696.83)</td>
</tr>
<tr>
<td>Median FB$_1$ (ng ml$^{-1}$ urine)</td>
<td>N.D.</td>
<td>892.35</td>
<td>590.41*</td>
</tr>
<tr>
<td>Mean FB$_1$ (95% Cl)</td>
<td>N.D.</td>
<td>194.07 (161.55–226.59)</td>
<td>158.17 (138.21–178.14)</td>
</tr>
<tr>
<td>Median FB$_1$ (ng mg$^{-1}$ creatinine)</td>
<td>N.D.</td>
<td>191.95</td>
<td>154.32*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Urine collection 48h post-gavage</th>
<th>Absolute control</th>
<th>FB$_1$ control</th>
<th>FB$_1$ + 2% NS treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>% detectable FB$_1$ samples</td>
<td>0 ($n = 6$)</td>
<td>100 ($n = 6$)</td>
<td>100 ($n = 6$)</td>
</tr>
<tr>
<td>Mean FB$_1$ (95% Cl)</td>
<td>N.D.</td>
<td>183.07 (110.16–255.98)</td>
<td>83.29 (18.40–148.19)</td>
</tr>
<tr>
<td>Median FB$_1$ (ng ml$^{-1}$ urine)</td>
<td>N.D.</td>
<td>183.65</td>
<td>91.92*</td>
</tr>
<tr>
<td>Mean FB$_1$ (95% Cl)</td>
<td>N.D.</td>
<td>9.38 (6.03–12.72)</td>
<td>4.42 (1.00–7.85)</td>
</tr>
<tr>
<td>Median FB$_1$ (ng mg$^{-1}$ creatinine)</td>
<td>N.D.</td>
<td>9.91</td>
<td>4.95*</td>
</tr>
</tbody>
</table>

Notes: Data represent the mean, confidence interval (CI) and median from each treatment group.
* Significantly different from FB$_1$ control group, $p < 0.05$ (Wilcoxon rank-sum test).
N.D., not detected.

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**Figure 2.** NovaSil reduction of total excreted and creatinine standardised urinary FB$_1$ in Fischer 344 rats. Data represents the cycle of urinary FB$_1$ excretion following 25 mg kg$^{-1}$ dose by gavage. Urinary FB$_1$ was reduced by 27% in the NS treated group. This reduction was statistically significant ($p < 0.05$) when NS treated and non-treated groups were compared using the Kruskal–Wallis and Wilcoxon rank-sum test. Data represents the means and standard error. Non-detectable levels of FB$_1$ were documented for the control group at all time points.
Discussion

Human exposure to mycotoxins has received increased attention, and recent studies in West Africa have confirmed the co-occurrence of AFs and fumonisins in maize-based foodstuffs found in Cote d’Ivoire and Ghana (Kpodo et al. 2000; Sangare-Tigori et al. 2006). While surveys have demonstrated that maize-based products are often co-contaminated with diverse mycotoxins; there is a paucity of published data measuring human exposure to fumonisins and AFs. Use of urinary FB1 as a biomarker of exposure in humans was first proposed by Shetty and Bhat (1998). Turner et al. (1999) reported that analysis of urinary

Table 2. Reduction in FB1 levels with NovaSil treatment in the Ghanaian population.

<table>
<thead>
<tr>
<th>NovaSil treatment</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>High dose (3.0 g day⁻¹)</td>
<td>Low dose (1.5 g day⁻¹)</td>
</tr>
<tr>
<td>% detectable FB1 samples</td>
<td>39.1 (9/23)</td>
</tr>
<tr>
<td>Mean FB1 (95% CI)</td>
<td>4.05 (0.99–7.10)</td>
</tr>
<tr>
<td>Median FB1 (ng ml⁻¹ urine)</td>
<td>0.19*</td>
</tr>
<tr>
<td>Mean FB1 (95% CI)</td>
<td>1.07 (0.22–1.91)</td>
</tr>
<tr>
<td>Median FB1 (ng mg⁻¹ creatinine)</td>
<td>0.20*</td>
</tr>
</tbody>
</table>

Notes: Data represent the mean, confidence interval (CI) and median from each treatment group.
*Significantly different from placebo group, p ≤ 0.05 (Wilcoxon rank-sum test).
*aPlacebo = microcrystalline cellulose.

Figure 3. Distribution of urinary FB1 between treatment groups at weeks 8 and 10. Data represents the distribution of FB1 (ng FB1 ml⁻¹ urine) in the various treatment groups based on a NovaSil intervention in Ghana. There was a statistically significant difference among treatments (p ≤ 0.05). There was a significant difference between the median FB1 levels in the NS treated groups vs. the placebo group (p ≤ 0.05). There was not a significant difference between median FB1 levels between the high and low dose groups (p = 0.566).

Figure 4. Distribution of creatinine standardised urinary FB1 between treatment groups at weeks 8 and 10. Data represents the distribution of FB1 (ng FB1 mg⁻¹ creatinine) in the various treatment groups based on a NovaSil intervention in Ghana. There was a statistically significant difference among treatments (p ≤ 0.05). There was a significant difference between the median FB1 levels in the NS treated groups vs. the placebo group (p ≤ 0.05). There was not a significant difference between median FB1 levels between the high and low dose groups (p = 0.955).
FB₁ held significant promise as there was a need for a reliable biomarker of exposure to fumonisins. A recent study by Gong et al. (2008) inferred a dose–response relationship between consumption of tortillas containing fumonisins and human urinary FB₁. Data from the present study verified that urinary FB₁ biomarker could be quantified using a rodent model following a single dose exposure. Furthermore, data from our human study in Ghana supports the work by Gong et al. (2008) in Mexico, which suggests that urinary FB₁ is a sensitive biomarker of fumonisin exposure. The decision to use urinary FB₁ as the diagnostic marker of NS efficacy was critical to our in vivo analyses. In our rodent study, all animals dosed with FB₁ displayed the urinary FB₁ biomarker within 24 h, regardless of treatment group. Levels of urinary FB₁ were considerably reduced 48 h after gavage. By 72 h, urinary FB₁ was not detectable. This 48-h window is similar to other urinary biomarkers of mycotoxin exposure (Wang et al. 2008). NS clay has been shown to sorb both aflatoxin and FB₁ in vitro, and can decrease the bioavailability and exposure to aflatoxins in humans and animals. Thus, it is possible that NS may also confer protection against FB₁ where dual-contamination of the diet is common. It is then proposed that NS was able to bind FB₁ either at the time of gavage or during the gastrointestinal passage. However, other possibilities may exist. For instance, clays similar to NS have been previously used as mucosa protectants (Droy-Lefaix and Tateo 2006) and

Figure 5. MALDI-TOF mass spectra (MS). MS are based on relative intensity (y-axis) of depicted peaks (highest peak = 100%). (A) MS of underivatised standard FB₁ in water (723 m/z). (B) MS of NDA derivatised FB₁ from spiked urine sample (≈906.1 m/z) and NDA derivative + Na ion (≈928 m/z). (C) MS of a representative FB₁ positive participant urine sample verifying presence of FB₁.
NS may have reduced the urine levels of this myco-
toxin by limiting or delaying the contact of FB1 with the
intestinal cell barrier. Our study has two important
limitations. One of them is that faecal levels of FB1
were not measured and this would have been a better
verification of our proposed mechanism of action of
NS onto FB1. Another limitation is that our study
design does not allow to completely determine if FB1
was bound to gavaged NS or if dietary NS was
responsible for the reduction of FB1 in urine. Nonethe-
less, in our rodent model, NovaSil (either by
gavage or dietary) was able to significantly reduce
urinary FB1 by 20% at 24 h and by 50% at 48 h post-
gavage. These data suggest that NS may be a suitable
enterosorbent for the reduction of fumonisin exposure,
confirming previous reports in our laboratory
(Lemke 2000).

In our Ghanaian study, 60.9 and 72.1% of analysed
samples from the placebo group contained detectable
levels of urinary FB1, compared to 75% detected in a
Mexican population consuming a high intake of maize-
based tortillas (Gong et al. 2008). The range of FB1 in
urine samples from our study varied from non-
detectable to 33.12 ng FB1 ml⁻¹ urine, higher than
values reported by Gong et al. (2008) (non-detectable
to 9.31 ng FB1 ml⁻¹ urine). The week 8 and 10 mean
FB1 levels measured in participants of the placebo
group in our study (9.36 and 5.03 ng FB1 ml⁻¹ urine,
respectively) were higher than mean urinary FB1
concentration reported in Mexico (0.147 ng FB1 ml⁻¹
urine) (Gong et al. 2008). Based on ~1% urinary excretion, an estimated metabolic rate of 1500 ml urine
day⁻¹ and the average size of an adult (70 kg), it can be
estimated that the exposure to FB1 at week 8 was
20.06 µg kg⁻¹ bw day⁻¹ and 10.78 µg kg⁻¹ bw day⁻¹ at
week 10 in our placebo groups (Shephard et al. 1994;
Fodor et al. 2008). More importantly, recent studies in
a South African population consuming corn naturally
contaminated with FBs have estimated an average of
0.075% urinary excretion rate of FB1 (Van der
Westhuizen et al. 2011). If this is the case, our
Ghanaian population, may have been exposed to
10 times more than what we have previously estimated.
These values are higher than the FB1 levels that could
be extrapolated from the Mexican study (0.368 µg kg⁻¹
bw day⁻¹) (Gong et al. 2008). These differences
between Mexican and Ghanaian population biomarker
levels could be attributed to variations in maize storage,
processing, and/or consumption. A market survey in
Ghana detected levels of FB1 between 70 and
2621 (mean 608.8) µg kg⁻¹ in maize intended for
human consumption (Jolly et al. 2006). Based on an
average maize intake of 369.5g day⁻¹ (MacIntyre et al.
2002), a 1% carryover of FB1 from the diet, and an
average urinary excretion (1500 ml), Ghanaians from
our site could potentially excrete urinary FB1 levels
between 0.172 to 6.46 ng ml⁻¹ urine. Actual mean
biomarker levels measured in the placebo group of the
present study were similar to this estimate, with
measured values of 9.36 and 5.03 ng FB1 ml⁻¹ urine
in the week 8 and week 10 groups, respectively.

In the previous clinical trial in Ghana, significant
decreases in median urinary aflatoxin M1 levels were
observed at 12 weeks after NS intervention (p = 0.0445)
(Wang et al. 2008). Since samples that were collected at
12 weeks were depleted for aflatoxin biomarker anal-
ysis, we utilised samples at weeks 8 and 10, to assess the
effect of NS treatment on urinary FB1 levels. With NS,
the week 8 samples had mean values of 4.05 and
5.61 ng ml⁻¹ urine in the high and low dose treatment
groups, respectively, compared with 9.36 ng ml⁻¹ in the
placebo group. At week 10, samples had lower mean
values of 0.81 and 0.73 ng FB1 ml⁻¹ urine in the high
and low dose treatment groups, respectively, versus
5.03 ng FB1 ml⁻¹ urine in the placebo group. While the
lower levels of FB1 measured in the participants of low
dose and high dose NS are consistent with the effects of
NS in reduction of FB1 bioavailability, the possibility
that the groups (placebo, low dose NS and high dose
NS) were consuming different levels of FB1 in the diets,
represents a valid question. Nonetheless, the diets
ingested by participants were likely to be uniform
throughout the study period since the sources of maize
tend to be constrained community wide. Furthermore,
the effect of NS or placebo on FB1 urinary levels was
observed at two different time points.

When evaluating the means between the placebo
(humans) or FB1 control group (rats) and NS treated
groups the reduction in urinary biomarker levels due to
NS was more evident in the human study (a 49% reduction in week 8 and an 85% reduction in week 10)
than in the rat study (a 19% reduction after 24 h and a
50% reduction after 48 h). This disparity between the
mean reductions in FB1 excretion (rats vs. humans) is
not unexpected. In the clinical trial, Ghanaians were
chronically exposed to FB1 through the diet and
treatment with NS (by capsules) occurred for a
period of 3 months, whereas, in the rat study, group
C was given FB1 as a onetime dose and NS treatment
only lasted for 2 weeks. Additionally, it is known that
gastrointestinal transit times vary according to size and
weight of different species. Thus, it is possible that, in
the humans, NS and FB1 had a longer time to interact
resulting in a larger reduction in FB1 excretion.
Nevertheless, in the rat and human studies, NS
significantly reduced the excretion of FB1 biomarker
in the urine. Further studies are warranted to establish
whether a time-related effect exists allowing NS treat-
ment to exert more effect at later time points. Based on
the 10-week mean urine values in the NS treated
groups, exposure could be estimated at 1.72 and
1.54 µg kg⁻¹ bw day⁻¹ for the high and low dose
participants, respectively. Importantly, following con-
sumption with NS, participant exposure to FB1 was
reduced below the JECFA provisional maximum tolerable daily intake for FB$_1$ of 2 µg kg$^{-1}$ bw day$^{-1}$ (JECFA 2001). Based on our previous research, NS clay has displayed: (1) favourable thermodynamic characteristics of aflatoxin sorption; (2) tolerable levels of priority metals, dioxins/furans and other hazardous contaminants; (3) safety and efficacy in multiple animal species and humans; (4) safety and efficacy in long-term animal studies; and (5) negligible interactions with vitamins, iron and zinc and other micronutrients. Additionally, recent work in our laboratory has shown that non-framework micronutrients associated with NS (i.e. Sr$^{2+}$) are bioavailable suggesting that NS has the potential to act as a supplement for these micronutrients as well as a mycotoxin enterosorbent in humans and animals (Afriyie-Gyawu et al. 2008). Further work is warranted to delineate the specific molecular mechanism(s) and surface chemistry involved in the sorption process of FB$_1$ onto NS and to confirm the efficacy and safety of NS clay as a multifunctional intervention for aflatoxins and fumonisins in animals and humans.

Mycotoxins have been linked to death and disease, with an estimated 4.5 billion people facing uncontrolled exposure in developing countries (Williams et al. 2004). The mycotoxin problem to public health is longstanding, unavoidable, and seemingly inextricable. Frequent and concurrent exposure to aflatoxins and fumonisins may enhance/synergise the toxic, carcinogenic, and teratogenic effects of these foodborne contaminants. This study is the first to demonstrate the efficacy of an enterosorbent intervention for FB$_1$ exposure using fumonisin biomarkers in human urine. Importantly, it also illustrates that a susceptible population in Ghana co-exposed to aflatoxins and fumonisins may be protected from the adverse effects of these mycotoxins using NS clay. As an intervention, capsules or other dose forms of NS could be used in clinical practice for “acute emergencies” and dietary practice for “chronic exposures.” Additional studies are planned to confirm these findings in long-term clinical intervention trials in Ghana and to look at the nutritional implications of NS supplementation of the diet. Clay-based enterosorbent strategies represent a novel approach to the problem in populations that are at high risk for mycotoxicosis. A similar clinical intervention with NS in a population at risk for hepatocellular carcinoma (HCC) in the USA is ongoing, and will examine dietary and environmental risk factors for HCC including aflatoxins and fumonisins and the effect of NS intervention.

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