Food Additives and Contaminants, March 2005; 22(3): 270–279

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Short-term safety evaluation of processed calcium montmorillonite clay (NovaSil) in humans

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(Received 25 January 2005; revised 28 February 2005; accepted 28 February 2005)

Abstract
NovaSil clay (NS) provides significant protection from the adverse effects of aflatoxins (AFs) in multiple animal species by decreasing bioavailability from the gastrointestinal tract. It is postulated that NS clay can be safely added to human diets to diminish exposure and health risks from AF contaminated food. To determine the safety and tolerance of NS in humans and establish dosimetry protocols for long-term efficacy studies, a randomized and double-blinded phase I clinical trial was conducted. Volunteers (20–45 yr in age), were clinically screened for confirmation of their health status. Fifty subjects (23 males and 27 females) were randomly divided into two groups: The low-dose group received nine capsules containing 1.5 g/day, and the high-dose group received nine capsules containing 3.0 g/day for a period of 2 wk. NS capsules were manufactured in the same color and size and were distributed to each participant three times a day at designated sites where follow-up was taken to record any side effects and complaints. Blood and urine samples were collected before and after the study for laboratory analysis. All participants completed the trial and compliance was 99.1%. Mild GI effects were reported in some participants. Symptoms included abdominal pain (6%, 3/50), bloating (4%, 2/50), constipation (2%, 1/50), diarrhea (2%, 1/50), and flatulence (8%, 4/50). No statistical significance was found between the two groups for these adverse effects ($p > 0.25$). No significant differences were shown in hematology, liver and kidney function, electrolytes, vitamins A and E, and minerals in either group. These results demonstrate the relative safety of NS clay in human subjects and will serve as a basis for long-term human trials in populations at high risk for aflatoxicosis.

Keywords: *Aflatoxins, clinical trial, NovaSil clay, safety evaluation*

Introduction

Aflatoxins (AFs), mainly produced by *Aspergillus flavus* and *A. parasiticus*, are a group of naturally occurring fungal metabolites that have long been recognized as significant food contaminants (Busby and Wogan 1984, CAST 2003). Aflatoxin B_1 (AFB_1), the most common mycotoxin found in human food and animal feed, is a potent hepatotoxic and genotoxic agent which has been listed as a known human carcinogen (Group I) (IARC 1993, 2002, Wang and Groopman 1999). Exposure to dietary AFB_1 is one of the major risk factors in the etiology of human hepatocellular carcinoma (HCC) in several regions of Africa and Southeast Asia (Wogan 1992, IARC 1993, 2002). AFB_1 has also been shown to be a potent immunotoxic agent in animals and humans (Hinton et al. 2003; Turner et al. 2003). Therefore, development and application of intervention strategies that are highly effective and economically feasible against dietary AFB_1 contamination and aflatoxicoses are critical for improving human health, especially in high-risk areas. A culturally acceptable and sustainable intervention in the human diet may involve the inclusion of selective clay minerals that possess high affinity and high capacity for AFs.

The ingestion of clay minerals (geophagy) by humans and animals for a variety of beneficial purposes has been well-documented for centuries (Carretero et al. 2002). One of these clays, NovaSil

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ISSN 0265–203X print/ISSN 1464–5122 online
DOI: 10.1080/02652030500111129

or NS, is a naturally-occurring and heat processed calcium montmorillonite that is commonly used as an anticaking additive in animal feed. Previous research has shown that NS was a selective enterosorbent of AFB_1 when included in the diet at levels up to 0.5% (weight to weight) in animal models (Phillips 1999, 2002). NS significantly protected a variety of young animals from aflatoxicoses, including chicks (Phillips et al. 1988 and 1999; Pimpukdee et al. 2004), turkey poults (Kubena et al. 1991), pigs (Lindemann et al. 1993), lambs (Harvey et al. 1991a), and rodents (Mayura et al. 1998). In addition, NS also reduced AF residues in milk from dairy cows (Harvey et al. 1991b) and goats (Smith et al. 1994), as well as molecular dosimetry biomarkers of AF exposure in chicks and rodents (Phillips 1999). Mechanistically, NS decreases the uptake of AFs in the gastrointestinal tract, leading to significantly reduced AF exposure and subsequent toxicity (Phillips 1999; Phillips et al. 2002). Information derived from equilibrium adsorption isotherms and molecular modeling studies has indicated that NS has a preference for binding to AFs that contain a planar ketolactone system (Phillips et al. 2002).

No observable adverse effects have been reported in short-term animal studies following the addition of NS clay to the diet (Phillips 1999; Phillips et al. 2002). No maternal or fetal toxicity was found in Sprague-Dawley (S-D) rats ingesting NS clay at dietary concentrations as high as 2% throughout pregnancy (Mayura et al. 1998; Wiles et al. 2004). In addition, no significant changes in trace metal bioavailability were found in a variety of maternal or fetal tissues (Wiles et al. 2004). A recently completed study in S-D rats treated with 0.25–2% NS clay in the diet over a six-month period did not exhibit dose-dependent adverse effects on body weight gains, feed conversion ratios, relative organ weights, gross and histological appearance of major organs, hematological and serum biochemistry parameters. Also, essential nutrient levels including vitamins A and E, Fe, and Zn were unaffected (Afriyie-Gyawu et al. 2005).

Due to the demonstrated safety and efficacy of NS in multiple animal models as well as its low cost, NS inclusion may be especially beneficial in the diets of humans that are at high risk for aflatoxicosis in developing countries. As the first step towards this effort, a two-week short-term safety evaluation of NS was carried out in healthy human volunteers. The purpose of this phase I clinical study was to determine short-term safety and tolerance of NS in normal human subjects. This information will be used to design the experimental dosimetry protocol for use in long-term human intervention studies with NS.

Material and methods

Materials

NS clay is manufactured by Engelhard Chemical Corporation (Iselin, NJ). NS was examined for concentrations of various environmental contaminants, including dioxins and heavy metals to insure compliance with federal and international standards. Of the 17 USEPA priority dioxins/furans, only octachlorodibenzo-p-dioxin (OCDD) at 2.34 parts per trillion (ppt) was present above the limits of detection (LOD = 1.02 ppt) in NS. Based on this value, the toxic equivalent (TEQ) of this contaminant in NS was calculated to be 0.000234 ppt. Detectable concentrations of heavy metals in NS, such as Hg, Pb, Cd, and As, were 100- to 500-fold lower than JECFA standards (JECFA 1998; Afriyie-Gyawu et al. 2005). NS was then sterilized at 121°C and forwarded to Guangxi Pharmaceutical Company in Nanning, China for packaging into capsules. The NS capsules were produced under sterile conditions using US Good Manufacturing Practices. All other chemicals, reagents, and solvents used were obtained commercially at the highest purity available.

Study subjects

Fifty healthy adults with different ethnic/racial backgrounds were recruited through word-of-mouth, e-mail solicitation, and posted flyers at Texas Tech University Health Sciences Center and The Institute of Environmental and Human Health. All subjects were blinded to the study dose assignment and agreed to comply with all study procedures. Criteria of subject eligibility included adults aged 20–45 in good general health with normal liver and renal function as shown by blood and urine tests, no history of chronic illness, no use of prescribed medications for chronic or acute illness, and no pregnancy, lactation, or diagnosis of endometriosis for female participants.

Selection of doses

Two dose levels (1.5 grams per day and 3.0 grams per day) were extrapolated from previously published dosimetery data from animal studies (Phillips 1999; Phillips et al. 2002). The high dose was selected based on the fact that no toxic effects were demonstrated in experimental animals dosed at levels approximately ten times higher (Afriyie-Gyawu et al. 2005). The low dose was equivalent to the minimal effective concentration that reduced the effects of AF in animals.

Sample size rationale

The sample size determination of a total of 50 subjects with 25 in each treatment group was based on the standard number (18–25 subjects/dose group) required by the US NIH (2000) and 20–80 subjects required by the US FDA (1997). The selection of 50 subjects (25/group) was also determined on the basis of the null statistical hypothesis that there are no differences in the clinical parameters between treated groups with NS. To detect differences at 20% of the study participants with a standard deviation of 10% over a two-week study period, the values of n (size of each group) to achieve a 90% power to detect a difference Δ with a one-sided significance level of 5% are given by: $n = 2(0.1(Z\alpha + 1.645))^2/\Delta^2$. For Δ equal to 0.20, a minimum of 23 participants in each group would be appropriate.

Procedures of study

The primary objective of the study was to evaluate possible adverse effects resulting from daily administrations of NS to healthy human subjects. A second objective was to directly examine the adherence of study subjects to the dose protocol. The overall study design followed the guidelines for a randomized and double-blinded phase I clinical trial and the procedures are shown as a flow chart in Figure 1.

The study was approved by Institutional Review Boards for the protection of human subjects at Texas Tech University and Texas Tech University Health Sciences Center and was carried out during the months of May through August, 2004. Initially, 81 subjects consented to the terms of the study and were screened through a series of health history questionnaires, physical examinations, and laboratory urine and blood tests. A total of 50 subjects who met the recruiting standards were voluntarily enrolled in the study. They were randomly divided into two study groups, and each subject was assigned a unique identifier that included their randomization number and initials. The low-dose group took three capsules of NS (0.5 g) three times a day for two weeks. The high-dose group took three capsules of NS (1.0 g) three times a day for two weeks. All capsules were the same color and size. The study subjects met with study personnel at designated distribution sites three times a day to receive and ingest the study compound (NS capsule). Bottled water was provided to the subjects for each ingestion and capsules were ingested about 4–6 hours apart. Study personnel witnessed the ingestion and discussed and recorded any side effects or ingestion episodes that subjects experienced. A final clinical visit was arranged for each subject at the end of the two-week study, including general physical examination and collection of blood and urine samples. All clinical parameters for blood and urinary chemistry

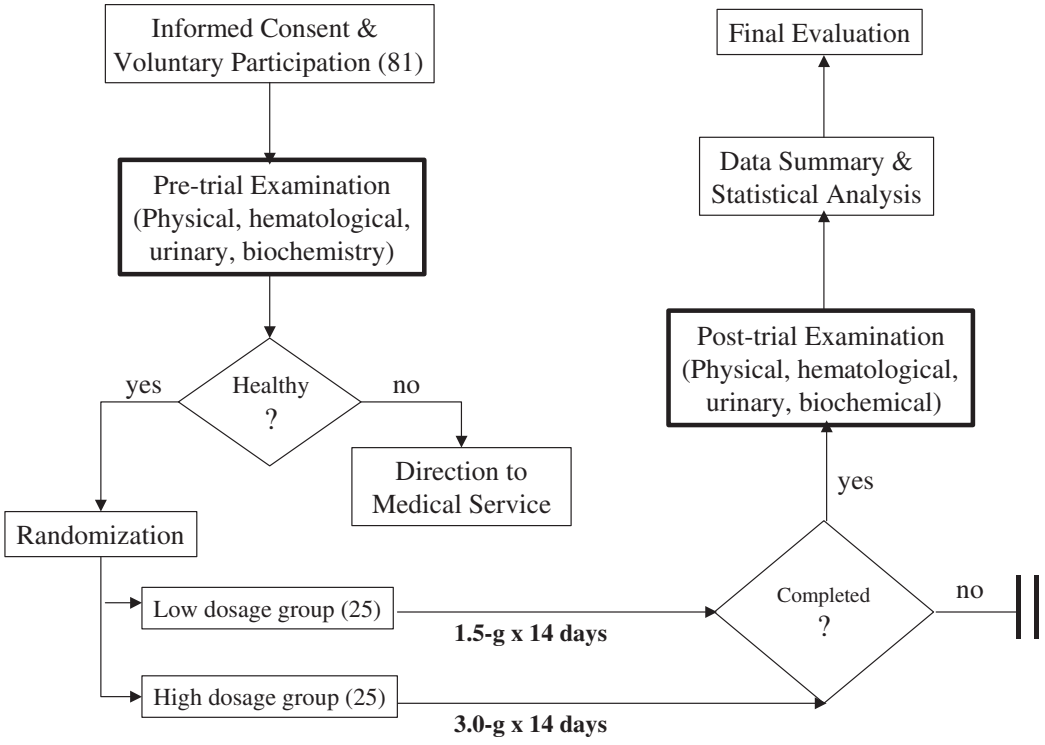

Figure 1. Flow chart of study design and procedures of phase I clinical study with NS clay.

and biochemistry, with the exception of serum level of minerals and vitamins A and E, were analyzed at the clinical diagnostic laboratory of Texas Tech University Health Sciences Center. Laboratory personnel who performed the analysis had no knowledge of status of sample sources.

Toxicity and adverse events monitoring

Although no severe toxicity was expected in the two-week period of study, a 24-hour on-call pager number manned by registered nurses was made available for participants who experienced any adverse events. Gastrointestinal symptoms were expected to be the major complaint, if any. A symptom checklist for toxicity monitoring was developed and was included with the Daily Diary Worksheet as an assessment tool for participants. Physicians and/or registered nurses on the investigative team reviewed the Daily Diary Worksheet on a weekly basis. Any symptoms were assessed according to the following criteria:

Mild (grade 1), slightly bothersome and relieved with symptomatic treatment;

Moderate (grade 2), bothersome and interfered with activities and only partially relieved with symptomatic treatment;

Severe (grade 3), prevented regular activities and not relieved with symptomatic treatment.

Physical examination and laboratory analysis were performed to verify symptoms, if necessary, in the course of the study. Any symptoms in subjects that were linked to treatment by the study physician, would result in immediate reduction or discontinuation of capsules (although this did not happen during the study period). An Adverse Event Report was also developed using US NIH Guidelines and was available for use in reporting any adverse event to the funding agency and the IRB.

Outcome measures

Measures of safety. The safety of the study was measured by the analysis of biochemical parameters for function of liver and kidney and normality of hematologic parameters, as well as physical examinations.

Measures of toxicity. Determination of any adverse effects was done by laboratory testing, interviews by study personnel, and/or physical examination in the course of the study.

Measures of adherence. Adherence to the study was determined primarily by monitoring a daily diary completed by study personnel regarding the subject's ingestion compliance and any possible side effects of ingesting the capsules.

Measurement of serum vitamins A and E

Human serum vitamins A (VA) and E (VE) were extracted under subdued red light following procedures previously described (Ruperez et al. 2004). Briefly, human serum samples (50 µl) were mixed with 150 µl of ethanol:chloroform (3:1, v/v, containing 0.01% BHT antioxidant) to precipitate proteins and were further extracted with 300 µl of hexane in a 1.5 ml microcentrifuge tube. After centrifugation, the hexane layer was removed and dried by Centrivap (Labconco, Kansas, MS). The residue was reconstituted with 300 µl of mobile phase for HPLC analysis according to the procedures of Burri et al. (2003). Analysis was carried out using a Thermo Finnigan Liquid chromatograph with a P4000 pump, an AS3000 autosampler with a 100 µl loop, and a UV6000 LP photodiode array detector (Thermo Separation Products, Riviera Beach, FL). Chromatographic separation was achieved with a Microsorb 100-5 C_{18} column with 150 mm × 4.6 mm ID and 5 µm particle size (Varian, Palo Alto, CA) using mobile phase A (ACN:THF:MeOH:AA at 85:5:5:5, v/v/v/v) and mobile phase B (ACN:THF:MeOH:AA at 55:35:5:5, v/v/v/v) under a flow rate of 1 ml/min with an injection volume of 50 µl. The elution profile consisted of 95% A and 5% B for the first 5 min, followed by a gradient to 5% A and 95% B over 13 min. Afterwards, conditions were maintained for 2 min and then the column was washed with 95% A and 5% B for 8 min. The total run time was 28 min. Quantitation of both vitamins was based on comparison of peak areas and retention times to reference standards.

Analysis of serum minerals

Levels of trace minerals in human serum samples were measured as follows. Serum samples (approximately 0.45 g) were mixed with 200 µl of concentrated nitric acid in a 15 ml centrifuge tube and heated overnight at 90°C and cooled. Then 100 µl of 30% H_2O_2 was added and the samples were heated at 70°C for one hour and cooled; then 50 µl of concentrated hydrochloric acid was added and the samples were heated at 70°C for one hour and cooled; then the samples were brought to a final volume of 10 ml with purified water. Mercury (Hg) concentrations were determined by cold vapor atomic absorption (CVAA) using an M-7500 (Cetac Technologies, Omaha, NE) with stannous chloride as a reductant. Aluminum (Al), boron (B),

barium (Ba), beryllium (Be), calcium (Ca), cobalt (Co), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), molybdenum (Mo), sodium (Na), phosphorus (P), sulfur (S), silicon (Si), strontium (Sr), titanium (Ti), vanadium (V), and zinc (Zn) were determined with an inductively coupled plasma – optical emission spectrometer (ICP-OES) using a CirOS (Spectro Analytical Instruments, Fitchburg, MA) with axial viewing and ytterbium (Yb) as an internal standard. Silver (Ag), arsenic (As), cadmium (Cd), chromium (Cr), manganese (Mn), nickel (Ni), lead (Pb), selenium (Se), and thallium (Tl) were determined with an inductively coupled plasma – mass spectrometer (ICP-MS) using an Elan 6100 (Perkin-Elmer, Wellesley, MA) with As, Cr, Mn, and Se acquired in DRC mode, and bismuth (Bi), gallium (Ga), and rhodium (Rh) as internal standards. In addition to blanks, spiked blanks, duplicate samples, and spiked samples, standard reference materials (Seronorm, Billingstad, Norway) were prepared and analysed with each batch of samples to verify results.

Data management and statistical analysis

A specific database file was created with Microsoft Access for data management. Separate electronic forms linked by unique SUBJECT_ID were generated simulating the original questionnaire, clinical report, and daily diary for ingestion and toxicity monitoring to facilitate data input. All data recorded were double-checked by two personnel. Since the ultimate goal of this study was to determine if the ingestion of NS capsules was safe in normal human volunteers, the statistical evaluation focused on comparisons between the two dose levels and before or after the treatment for all clinical and biochemical parameters. Paired *t*-test and two-factorial ANOVA for qualitative measurement of data and Chi-square test for analysis of adherence and rate of side-effect/toxicity data were designated using the SPSS 12.0 software (SPSS, Inc., Chicago, IL).

Results

Study participant characteristics

Demographic distribution of 50 human subjects enrolled in the study is shown in Table I. Because the process of randomization was based on the eligibility criteria, slight gender and ethnic background differences were noticed in the two groups, however, no statistically significant difference between the groups was found for gender, age, or ethnic background ($p > 0.10$). No significant changes were found in general physical parameters, such as body weight and blood pressure before and after the study.

Table I. Demographic information and physical parameters of study subjects.

	Group		
	Low-dose	High-dose	Total
Participant	25	25	50
Gender			
Male	14	9	23
Female	11	16	27
Ethnic			
Caucasian	6	4	10
Hispanic	2	7	9
Asian	14	13	27
African	3	1	4
Age (year)	29.4 ± 5.2*	31.7 ± 8.3	30.5 ± 6.9
Weight (kg)			
Before	80.1 ± 22.9	72.6 ± 22.3	76.4 ± 22.7
After	79.9 ± 22.0	72.9 ± 21.9	76.4 ± 22.0
Blood Pressure			
Systolic (mmHg)			
Before	125 ± 15	119 ± 12	121 ± 14
After	123 ± 15	123 ± 16	122 ± 15
Diastolic (mmHg)			
Before	79 ± 9	77 ± 9	77.3 ± 10
After	76 ± 9	76 ± 11	75.9 ± 10

*mean ± SD.

Table II. Compliance of study protocol.

	Group		
	Low-dose	High-dose	Sum
Total (person*time)	1050	1050	2100
Taken (person*time)	1042	1039	2081
Missed (person*time)	8	11	19
Adherence (%)	99.2	99.0	99.1

Compliance and completeness of the study

Adherence to the dose protocol for this two-week study was excellent as demonstrated in Table II. All 50 study subjects completed the study. According to the person time (14 days × 3 times/day × 25 people/group), greater than 99% compliance were reached in both study groups. Five subjects missed taking capsules eight times during the two-week study in the low-dose group and six subjects missed taking capsules 11 times during the two-week study in the high-dose group. No statistically significant differences in the rate of compliance were found among study groups ($p > 0.50$).

Adverse events and side effects

Both doses of NS used in this study (1.5- or 3.0-gram/day) were tolerable for the all study participants. Adverse symptoms were reported in

the two-week study period based on subject reporting and follow-up interviews and are summarized in Table III. Gastrointestinal adverse effects were noticed in some subjects, 24% (6/25) in the 1.5-g group and 28% (7/25) in the 3.0-g group. Symptoms included bloating, constipation, diarrhea, flatulence, and abdominal discomfort. A neurological adverse symptom (i.e., dizziness), was only recorded in two subjects in the 1.5-g NS group. No statistically significant difference was observed for these adverse symptoms between low- and high-dose groups ($p > 0.25$). All symptoms described were recorded in the first two days after taking the NS capsules and no symptoms (or complaints) were recorded thereafter. All side-effects reported, except from one subject, were assessed as mild, and no significant difference between two treatments groups was observed ($p > 0.55$).

Table III. Side-effects recorded from study subjects.

	Group		
	Low-dose	High-dose	Sum
Symptom reported			
Bloating	1	1	2
Constipation	0	1	1
Diarrhea	0	1	1
Flatulence	1	3	4
Abdominal discomfort	2	1	3
Dizzy	2	0	2
Total (%)	6 (24)	7 (28)	13 (26)
Side-effect grade			
Mild	6	6	12
Moderate	0	1	1
Severe	0	0	0

Hematological analysis of blood samples showed no significant differences in parameters representing complete blood cell counts and differential counts (Table IV), with the exception of red blood cell count and hemoglobin levels. These were somewhat lower but statistically significant in the low-dose group ($p < 0.05$) compared to values before the study. This reduction was not dose dependent, as it was not observed in the high-dose group.

Blood chemistry analysis, as shown in Table V, revealed a reduced concentration of total protein ($p < 0.01$), albumin ($p < 0.05$), and alanine aminotransferase (ALT) ($p < 0.01$) in the low-dose group, but not the high-dose group. Alkaline phosphatase (AP) activity was increased in the high-dose group compared to values before the study. No significant differences were found in other parameters, such as blood electrolytes (Na^+, K^+, and Cl^-), glucose, blood urea nitrogen (BUN), creatinine, total bilirubin, gamma-glutamyltranspeptidase (gamma-GT), aspartate aminotransferase (AST), and triglycerides.

Urinalysis, as shown in Table VI, did not show significant differences in parameters such as specific gravity, pH, leukocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, and blood (erythrocytes and hemoglobin) in both groups.

Changes of serum minerals and vitamins A and E

Concentrations of minerals and vitamins A and E were measured in serum samples of study subjects and results are shown in Tables VII and VIII. No statistically significant differences were found in serum vitamins A and E concentrations after the

Table IV. Hematological parameters of study subjects.

	Group			
	Low-dose		High-dose	
	Before	After	Before	After
WBC ($\times 10^9$/L)	6.7 ± 1.2[a]	6.4 ± 0.8	6.6 ± 1.8	6.8 ± 2.1
RBC (million/mm^3)	5.0 ± 0.5	4.8 ± 0.6*	4.9 ± 0.5	4.8 ± 0.5
Hemoglobin (g/dL)	15.1 ± 1.4	14.8 ± 1.5*	14.7 ± 1.6	14.7 ± 1.5
Hematocrit (%)	42.1 ± 6.8	42.4 ± 4.3	42.4 ± 4.4	42.3 ± 3.8
MCV (fL)	87.8 ± 3.4	87.8 ± 3.4	87.4 ± 4.3	87.6 ± 4.2
MCH (pg)	30.5 ± 1.3	30.6 ± 1.3	30.3 ± 1.8	30.4 ± 1.9
MCHC (g/dL)	34.8 ± 0.5	34.8 ± 0.5	34.7 ± 0.5	34.7 ± 0.8
Platelet ($\times 10^9$/L)	273.5 ± 54.9	267.9 ± 46.6	279.5 ± 80.5	280.8 ± 62.9
MPV (fL)	8.3 ± 0.9	8.2 ± 0.8	8.3 ± 0.8	8.4 ± 0.8
RDW (%)	12.8 ± 0.6	12.6 ± 0.6	13.3 ± 1.1	13.2 ± 1.1
Neutrophils (%)	57.4 ± 8.8	59.6 ± 7.7	55.4 ± 9.9	58.0 ± 7.7
Lymphocytes (%)	32.1 ± 8.7	29.8 ± 7.5	33.9 ± 9.6	31.6 ± 6.9
Monocytes (%)	7.0 ± 2.0	7.3 ± 1.5	7.4 ± 2.0	7.6 ± 1.6
Eosinophils (%)	2.9 ± 1.6	2.7 ± 1.6	2.5 ± 1.9	2.3 ± 1.8
Basophils (%)	0.6 ± 0.5	0.6 ± 0.6	0.6 ± 0.6	0.7 ± 0.5
PT (second)	11.8 ± 0.5	12.1 ± 0.9	12.0 ± 0.6	12.0 ± 0.6

[a]mean ± SD; * $p < 0.05$ as compared to values before the study.

Table V. Blood chemistry of study subjects.

	Group			
	Low-dose		High-dose	
	Before	After	Before	After
Sodium (mmol/L)	143.4 ± 1.8	143.4 ± 2.8	142.7 ± 1.8	142.7 ± 2.4
Potassium (mmol/L)	4.2 ± 0.4	4.2 ± 0.4	4.1 ± 0.3	4.1 ± 0.2
Chloride (mmol/L)	105.6 ± 2.7	106.1 ± 2.3	105.5 ± 2.2	106.4 ± 2.0
CO_2 (mmol/L)	25.4 ± 1.9	24.9 ± 1.8	24.6 ± 1.8	24.7 ± 1.6
Glucose (mg/dL)	94.2 ± 16.0	102.5 ± 27.3	89.6 ± 13.1	92.0 ± 10.6
Bun (mg/dL)	12.2 ± 2.7	12.3 ± 3.4	11.4 ± 2.8	11.3 ± 3.0
Creatinine (mg/dL)	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.2	0.9 ± 0.2
Calcium (mg/dL)	9.4 ± 0.3	9.3 ± 0.4	9.3 ± 0.4	9.3 ± 0.3
Total Protein (g/dL)	7.6 ± 0.5	7.4 ± 0.4**	7.4 ± 0.4	7.4 ± 0.5
Albumin (g/dL)	4.2 ± 0.3	4.1 ± 0.3*	4.1 ± 0.4	4.1 ± 0.3
Triglycerides (mg/dL)	152.1 ± 88.2	151.6 ± 100.6	135.0 ± 73.0	155.4 ± 91.3
Total Bili (mg/dL)	0.5 ± 0.3	0.5 ± 0.3	0.6 ± 0.3	0.6 ± 0.4
Alk Phos (IU/L)	72.3 ± 15.2	75.4 ± 12.3	79.3 ± 17.6	85.7 ± 17.7*
ALT (IU/L)	29.8 ± 18.4	26.2 ± 20.5**	29.2 ± 21.8	22.6 ± 7.0
AST (IU/L)	24.0 ± 10.2	22.4 ± 9.4	25.2 ± 21.4	20.9 ± 4.8
GGT (U/L)	23.1 ± 18.0	23.1 ± 16.5	21.5 ± 16.8	19.8 ± 9.8

[a]mean ± SD; *$p < 0.05$, **$p < 0.01$ as compared to values before the study.

Table VI. Urinary analysis of study participants.

	Group			
	Low-dose		High-dose	
	Before	After	Before	After
Glucose (+/−)*	0/25	1/24	0/25	0/25
Bilirubin (+/−)	0/25	0/25	0/25	1/24
Ketones (+/−)	0/25	0/25	0/25	1/24
Blood (+/−)	2/23	2/23	5/20	5/20
pH	5.8 ± 0.6[a]	5.9 ± 0.7	6.0 ± 0.6	6.1 ± 0.6
Protein (+/−)	0/25	1/24	1/24	2/23
Urobilinogen (Ehrlich units/dL)	0.3 ± 0.3	0.3 ± 0.2	0.4 ± 0.4	0.3 ± 0.3
Nitrite (+/−)	0/25	1/24	0/25	1/24
Leukocyte Ester (+/−)	3/22	2/23	3/22	4/21
SPEC Gravity	1.02 ± 0.01	1.02 ± 0.01	1.02 ± 0.01	1.02 ± 0.01
Hyaline Cast (+/−)	2/23	0/25	1/24	0/25
RBC/HPF (+/−)	2/23	2/23	5/20	5/20

*Positive/negative; [a]mean ± SD.

treatment with either dose of NS ($p > 0.25$). No significant differences were found in levels of the majority of minerals analysed, with two exceptions: lower inorganic sulfur concentration ($p < 0.05$) in the low-dose group and higher strontium concentrations ($p < 0.01$) in both groups.

Discussion

The strategy of reducing food-borne exposure to AFs through the inclusion of various binding agents or ''detoxifying clays'' in the diet has been given considerable attention in the scientific field. NS clay is one of the best characterized in terms of its ability to decrease the toxicity of AFs in animals. Additionally, no observable adverse effects in animals have been reported in short-term and long-term studies following the ingestion of NS clay from the diet (Phillips et al. 1988; Phillips 1999; Phillips et al. 2002; Afriyie-Gyawu et al. 2005). Although NS is commonly used as an anticaking agent in animal feeds throughout the world, its safety and efficacy have never been determined in humans. Due to the demonstrated safety and efficacy of NS in multiple animal models, as well as its low cost, NS inclusion may be especially beneficial in the diets of humans who are at high risk for aflatoxicosis in developing countries. This randomized and double-blinded phase I clinical study is the first report of NS clay safety in humans and will serve as the first step towards intervention in

Table VII. Serum minerals analysis of study subjects.

	Group			
	Low-dose		High-dose	
	Before	After	Before	After
Ca (mg/L)	90.8 ± 4.8[a]	86.6 ± 11.1	87.6 ± 12.5	90.9 ± 3.6
Cu (mg/L)	1.01 ± 0.24	0.93 ± 0.21	1.18 ± 0.57	1.17 ± 0.52
Fe (mg/L)	1.05 ± 0.31	1.06 ± 0.44	1.05 ± 0.46	1.10 ± 0.40
K (Mg/L)	167.6 ± 15.5	164.4 ± 22.0	159.2 ± 25.4	167.3 ± 11.3
Mg (mg/L)	19.6 ± 1.4	18.6 ± 2.6	19.3 ± 2.7	19.6 ± 1.3
Mn (mg/L)	0.0039 ± 0.0021	0.0032 ± 0.0009	0.0044 ± 0.0054	0.0031 ± 0.0004
Na (mg/L)	3120.0 ± 100.0	3030.0 ± 353.2	3030.8 ± 403.8	3151.7 ± 83.6
Ni (mg/L)	0.0068 ± 0.003	0.0068 ± 0.003	0.0093 ± 0.017	0.0082 ± 0.055
P (mg/L)	121.5 ± 13.8	110.8 ± 18.0	121.1 ± 25.3	127.6 ± 17.6
Pb (mg/L)	0.0034 ± 0.0051	0.0038 ± 0.0033	0.0018 ± 0.0014	0.0019 ± 0.0013
S (mg/L)	1102.1 ± 75.8	1008.9 ± 137.5*	1028.2 ± 152.5	1054.0 ± 47.2
Se (mg/L)	0.11 ± 0.01	0.10 ± 0.02	0.11 ± 0.01	0.11 ± 0.01
Si (mg/L)	2.74 ± 0.31	2.83 ± 0.57	3.26 ± 0.84	3.38 ± 0.75
Sr (mg/L)	0.055 ± 0.018	0.074 ± 0.017**	0.054 ± 0.024	0.096 ± 0.024**
Zn (mg/L)	1.2 ± 0.2	1.3 ± 0.5	1.1 ± 0.3	1.2 ± 0.3

[a]mean ± SD; *$p < 0.05$, **$p < 0.01$ as compared to values before the study.

Table VIII. Serum vitamins A and E analysis of study subjects.

	Group			
	Low-dose		High-dose	
	Before	After	Before	After
Vit. A (μmol/L)	2.33 ± 0.42[a]	2.35 ± 0.47	2.48 ± 0.81	2.59 ± 0.63
Vit. E (μmol/L)	20.66 ± 6.67	19.23 ± 5.87	21.96 ± 6.75	22.50 ± 5.87

[a]mean ± SD.

the human diet. Results of this study showed that administration of NS capsules at 1.5–3.0 g/day to healthy human subjects for 14 days was relatively safe, as demonstrated by the analysis of biochemical and hematologic parameters, as well as physical examinations. No dose-dependent acute toxic effects or severe dose-related clinical significant side-effect symptoms were observed in this study, which are consistent with many previous reported animal studies (Phillips et al. 2002).

In addition to the randomization and double-blinded design, this study took more restricted procedures than the regular clinical studies to guarantee the quality and accuracy of the outcomes. For example, prior to encapsulation, NS clay was analysed for potentially toxic metal and dioxin contaminants to ensure compliance with international and federal standards. During the study, subjects were required to take capsules at distribution sites three times a day and investigators witnessed the capsule ingestion and recorded all side-effects and complaints. Laboratory personnel carrying out analyses of biochemical and hematological parameters and personnel performing data input and statistical analysis were totally blinded. As shown in Table III, excellent compliance (>99%) to the dose protocol was achieved. This dose protocol seems suitable for future long-term intervention studies in larger populations.

Several parameters, such as RBCs, hemoglobin, total protein, albumin, ALT, and sulfur, showed statistically significant decreases in blood samples collected after treatment in the low-dose group (Table IX), however, none of these parameters were significantly different in blood samples from the high-dose group.

Moreover, all of these changes were within the normal range of clinical references. It has been postulated that some clay minerals may sorb vitamins; however, in this study no statistical differences were observed in the levels of serum vitamins A and E after treatment with either dose of NS ($p > 0.25$) further confirming its specificity and lack of interaction with vitamins A and E. In this study, we did not evaluate the water soluble vitamins, such as B_2 (riboflavin). However, earlier work with NovaSil (HSCAS) in poultry diets indicated that a level of 0.5% w/w did not impair phytate, inorganic phosphorus (Chung and Baker 1990), riboflavin, vitamin A or manganese utilization

Table IX. Comparison of statistical and clinical significance.

	Group				
	Low-dose		High-dose		
	Before	After	Before	After	Clinical reference
RBC (million/mm^3)	5.0 ± 0.5[a]	4.8 ± 0.6*	4.9 ± 0.5	4.8 ± 0.5	4.2–6.1
Hemoglobin (g/dL)	15.1 ± 1.4	14.8 ± 1.5*	14.7 ± 1.6	14.7 ± 1.5	12–18
Total Protein (g/dL)	7.6 ± 0.5	7.4 ± 0.4**	7.4 ± 0.4	7.4 ± 0.5	6.4–8.3
Albumin (g/dL)	4.2 ± 0.3	4.1 ± 0.3*	4.1 ± 0.4	4.1 ± 0.3	3.5–5.0
Alk Phos (IU/L)	72.3 ± 15.2	75.4 ± 12.3	79.3 ± 17.6	85.7 ± 17.7*	42–128
ALT (IU/L)	29.8 ± 18.4	26.2 ± 20.5**	29.2 ± 21.8	22.6 ± 7.0	5–65
S (mg/L)	1102.1 ± 75.8	1008.9 ± 137.5*	1028.2 ± 152.5	1054.0 ± 47.2	NRA
Sr (mg/L)	0.055 ± 0.02	0.074 ± 0.02**	0.054 ± 0.02	0.096 ± 0.02**	NRA

[a]mean ± SD; * $p < 0.05$; ** $p < 0.01$; NRA, No reference available.

(Chung et al. 1990). Our future efficacy trials will address the issue.

Serum strontium was the only parameter significantly increased in both dose treatment groups and was apparently dose-dependent. It is difficult to evaluate the elevation of this divalent cation because no clinical reference is available. Strontium is a mineral naturally present in food and water, and foods such as cereals, grains and seafood may contain up to 25 mg/kg of strontium (Cabrera et al. 1999). Strontium and calcium are remarkably similar in the way that the body handles them; they are absorbed in the GI tract, concentrated in the bone and excreted primarily in the urine (Cohen-Solal 2002). Toxicologically, naturally-occurring strontium, other than its radioactive isotope, is non-toxic and a daily dose up to 680 mg did not cause significant side-effects in clinical trials for treatment of osteoporosis (Meunier 2004). Nevertheless, all of these significant parameters will be closely monitored in future long-term studies.

In conclusion, results of this study support the prospect of using NS clay in the diet of humans to block, or significantly diminish exposure to AFs and to prevent the adverse effects of AFs in humans consuming AFs-contaminated grains.

Acknowledgements

This work was supported by research grant from the United States Agency for International Development (USAID LAG-G-00-96-90013-00).

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