

Research Proposal: Enterosorbent intervention therapies for populations at risk for Aflatoxin related diseases

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Geographical Location

Ghana

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Background

Interventions that reduce the dose of aflatoxins from contaminated foods include food surveillance, community education, chemoprevention strategies and a novel approach that was developed at Texas A&M University. In earlier work, we have shown that a calcium montmorillonite clay (NS) acts as an aflatoxin enterosorbent in the stomach and intestines of animals, and may provide a practical, cost-effective, culturally acceptable and sustainable solution to the problem in human populations at high risk for aflatoxicosis (Phillips et al., 2006). NS was heat processed and carefully selected to contain tolerable levels of trace metals and dioxins, and has been shown to be relatively safe for human consumption based on animal and human data (Afriyie-Gyawu et al., 2005; Wang et al., 2005). In a recent 3-month clinical intervention trial in Ghana, we have confirmed the safety of NS and its ability to significantly diminish human exposure to AF. Also, our studies have indicated that NS did not interfere with important nutrients including vitamins A and E and Fe and Zn. A potential pitfall is that NS is derived from a naturally-occurring clay material that can vary from batch-to-batch in composition, particle size, non-framework trace metal content and dioxin levels. And, due to a lack of consistency and uniformity, parent NS may not be appropriate for long-term studies in humans. To address this issue, in this project we will utilize a refined NS clay with uniform particle size (UPSN) for long-term human application. This procedure will ensure that UPSN is consistent and uniform in composition between batches and favors its use in clinical trials and as eventual therapy for human aflatoxicoses. In the proposed project, our specific aims are fourfold, and are founded on the central hypothesis that UPSN clay can be implemented in human studies to significantly diminish exposure (and risk) from aflatoxin-contaminated diets. Biomarkers of aflatoxin exposure, genetic susceptibility and health effects will be measured and correlated with the therapeutic efficacy of UPSN clay following intervention trials in Ghana, West Africa. This clay-based technology is environmentally benign and is built upon strong interdisciplinary and multi-institutional capacity. Mutually beneficial and ongoing collaborations exist among all project personnel.

Technical Review

Innovative sorption strategies for the detoxification of aflatoxins have been developed and characterized. NovaSil clay (NS) has been shown to prevent aflatoxicosis in a variety of animals when included in the diet. Results have shown that NS clay binds aflatoxins with high affinity and high capacity in the

gastrointestinal tract, resulting in a notable reduction in bioavailability of these poisons without interfering with the utilization of vitamins and other micronutrients. This strategy is being evaluated as a potential remedy for acute aflatoxicosis, and as a sustainable human intervention for aflatoxins via the diet. A Phase I Adverse Events trial in the U.S. confirmed the apparent safety of NS for further study in humans. We have now completed a Phase IIa clinical trial in Ghanaians at high risk for aflatoxicosis and this study has indicated that NS (at a dose level of 0.25%) is effective in decreasing biomarkers of aflatoxin exposure and does not interfere with nutrients. More specifically, this study involved a 3-month double-blind and placebo controlled, phase IIa clinical trial conducted in the Ejura-Sekyedumase district, Ashanti Region, Ghana (Afriyie-Gyawu et al., 2007a,b; Wang et al., 2007). The objective was to evaluate the safety, efficacy, tolerance and sorption specificity of dietary NS when administered to humans for the prevention of aflatoxin exposure and toxicity. Five hundred and seven volunteers were clinically screened to evaluate their general health, pregnancy status, and blood AFB₁-albumin adduct levels, and 177 of them were enrolled as study participants. Blood and urine samples were collected for laboratory analysis. Over 90% of the participants completed the study, and compliance rate was more than 97%. No NS-related, significant differences were shown in hematology, liver and kidney functions, and electrolytes among the three groups. In the serum biochemical analysis, isolated statistical differences in a few parameters were detected but no trends of association or dose-dependency were observed, and were all within the normal physiological ranges (Afriyie-Gyawu et al., 2007). Additionally, results from this study indicated that ingestion of NS, significantly reduced biomarkers of aflatoxin exposure in the blood and urine from study participants (Wang et al., 2007). Further studies confirmed that vitamins A and E, and nutrient minerals were not affected by NS treatment in humans. Based on our previous work, this proposal is designed to investigate refined NS clay that will maintain batch-to-batch consistency for therapy and potential inclusion in foods for humans in Ghana. Biomarkers will be correlated with aflatoxin exposure, genetic susceptibilities and health effects with and without NS therapy in ongoing analyses.

Problem Statement

Aflatoxins (AFs) are harmful by-products of mold growth that can be fatal to humans and animals. The problem is longstanding, unavoidable and seemingly inextricable. A recent outbreak of AF poisoning in Kenya resulted in a 39% case fatality rate and was linked to consumption of foods containing AF levels as high as 8,000 ppb (CDC 2004). The poorest people in developing countries, who are most likely to consume foods contaminated with AFs, suffer the most severe effects including disease (aflatoxicosis) and even death following acute exposure (Lewis et al. 2005). Additionally, it is estimated that more than 80% of all hepatocellular cancer cases occur in developing countries (Wild and Hall 2000). Studies also suggest that low level exposure to AFs can cause immunosuppression and increased susceptibility to disease (Turner et al. 2003; Jiang et al. 2005). Other consequences of exposure

include severe growth faltering and anti-nutritional effects (Hinton et al. 2003; Gong et al. 2002, 2004; Turner et al. 2007). Thus, feasible interventions and therapies to diminish human exposure to AFs are imperative. In this proposal, studies will provide an innovative and culturally acceptable strategy that will improve aflatoxicosis prevention and management in high risk populations (Ghana, etc.) by utilizing NS clay (i.e., capsules, tablets, powder, or food additives) to reduce human exposure and disease.

Vision and Approach

Goals

We have recently established the safety and efficacy of parent NovaSil (NS) clay in Phase I and Phase IIa clinical intervention trials. Our results confirmed the high affinity and capacity of NS as an enterosorbent of aflatoxins in humans and validated its therapeutic potential for the treatment of acute aflatoxin poisoning such as the recent outbreak in Kenya. In the proposed project, our specific aims are fourfold, and are founded on the central hypothesis that refined NS clay (UPSN) can be implemented in human studies to diminish exposure (and risk) from aflatoxin-contaminated diets. Biomarkers of aflatoxin exposure, genetic susceptibility and health effects will be measured and correlated with the therapeutic efficacy of UPSN following human intervention trials in Ghana, West Africa. Eventually, the preferred long-term delivery of NS may be through its inclusion in salt (like iodine), or as an additive in common peanut and maize- containing foods. Upon completion of this project, our results are expected to be of particular significance and relevance to rural and border communities in the U.S. and developing countries in Africa and in China, where the incidence and health impacts of aflatoxin exposures are often elevated. The ultimate goal of this project will be to provide a practical clay-based strategy and scientific capacity in Ghana for the improvement of health and well-being of humans.

Objectives

1. Molecular characterization of refined NS clay and other potential binding agents.
2. Safety and efficacy of refined NS clay for aflatoxin and other mycotoxins utilizing other animal model studies.
3. Safety and efficacy of refined NS (Pilot study and clinical intervention trial)
4. Biomarker analysis and correlation with refined NS intervention.
5. Training, capacity development and technology transfer.

Research Approach

In the proposed project, our specific aims are fourfold, and are founded on the central hypothesis that UPSN clay can be implemented in human studies to significantly diminish exposure (and risk) from aflatoxin-contaminated diets. Based on earlier in vitro isothermal evidence in our lab, we also postulate that UPSN (with restricted delamination and uniform particle size)

will be similar to parent NS and not interfere with the sorption of lipophilic and water soluble vitamins. In Objective 1 of this proposal, the thermodynamics of ligand adsorption (e.g., enthalpy of sorption), specificity, isothermal shape, capacity, affinity, and optimal binding characteristics of aflatoxins to UPSN surfaces will be determined. X-ray diffraction analysis and molecular modeling of UPSN interactions with important ligands (e.g. AFB1, vitamins A and E and riboflavin), will also provide valuable insight into the mechanism and site of ligand sorption to UPSN surfaces. Additionally, these findings will help to confirm binding characteristics and other similarities to parent NS. In Objective 2, our goal is to compare UPSN to parent NS clay and other sorbents regarding safety and selectivity, which will serve as a rationale for our proposed clinical intervention trials in humans in Ghana. Our research approach will utilize male and female Sprague-Dawley rats fed rations containing 0, 0.25, and 2.0% levels of either clay or placebo ad libitum over a 3-month period. Parameters studied will include: Body weight gain, feed conversion efficiency, relative organ weight, gross and histological appearance of major organs, hematological and serum biochemistry parameters, and essential nutrients including vitamins A and E, riboflavin, Fe, Zn, Se and other minerals. In Objective 3, we will determine the effectiveness of UPSN for the remediation of aflatoxin-contaminated human diets in a well-defined, high risk population for aflatoxicosis in Ghana. The research approach will consist of randomized, double-blind, placebo controlled, intervention trials. Samples of food from the marketplace and households, food intake questionnaires, and human body fluids will be collected (before, during and after the interventions). In Objective 4, analytical biomarkers will be utilized to delineate the consequences of UPSN intervention on blood micronutrient status (vitamins and minerals), hematological and serum biochemistry, urinalysis, body weights, and selected clinical outcomes (e.g., diarrhea, incidence of infectious disease and cancer). Biomarkers of exposure in study participants may include aflatoxin metabolites in urine (short-term) and aflatoxin albumin adducts in peripheral blood (long-term), selected genotypes for glutathione S-transferase, microsomal epoxide hydrolase, X-ray cross complementing group 1 protein, metabolites of polycyclic aromatic hydrocarbons and fumonisins.

Training & Capacity Development Approach

The training/capacity development approach of this project will be to focus on allowing scientists and technicians from the host country to learn these advanced techniques for measuring aflatoxin exposure in human subjects. Also, emphasis will be placed on ways in which they can be applied to influence health and health policy. This will be done by having scientists visit the Phillips laboratories (Texas A&M University, TAMU) and train in advanced analytical techniques for exposure assessment.

Post graduate study opportunities (MSc or PHD) will be determined in consultation with the HC participating institutions. How many students can participate will be determined by the funding level available and other sources of funding that are attracted to the TAMU lab.

Intended Benefits & Impact Responsiveness

Development Benefits

The sharing of unique research support core facilities at Texas A&M University (College Station), the University of Georgia, (Athens), Noguchi Memorial Institute for Medical Research (Accra, Ghana), Kwame Nkrumah University of Science and Technology (Kumasi, Ghana), the University of Alabama (Birmingham) and the University of Georgia (Griffin), will continue to add capability, capacity and dimension to the overall effort of our team. Working relationships and collaborations among team members are ongoing, and will help to ensure the success of this research as well as strengthen the scientific merit and developing country benefits of this project. Significant interactions with host country scientists will ensure the training of key individuals who will facilitate the application of NS clay technology to their various countries. Notable concerns about food safety have evoked a growing awareness of the significant hazards and risks associated with aflatoxins and its association with cancer, immunosuppression, infectious disease, and malnutrition in humans. Appropriate chemical interventions that can block or diminish exposure and prevent aflatoxin induced disease are international priorities. NS clay is already having a major impact on International Agriculture and Animal Production. This project will expedite the delivery of NS intervention strategies (and capacity) to developing countries, which will greatly impact health and benefit humans at high risk for aflatoxicosis.

US Benefits

Well-established, interdisciplinary research collaborations in the U.S. and Ghana and interactions between participating scientists associated with this project are ongoing. The sharing of unique research support core facilities at Texas A&M University (College Station), University of Georgia (Athens), Noguchi Memorial Institute for Medical Research (Accra, Ghana), Kwame Nkrumah University of Science and Technology (Kumasi, Ghana), the University of Alabama (Birmingham) and the University of Georgia (Griffin) will continue to add capability, capacity and dimension to the overall effort. This teamwork will greatly enhance the technical competence of our research and the prospects for the transfer of novel aflatoxin intervention technologies and therapies for the treatment of aflatoxicosis in animals and humans. Moreover, the project to field pathway for NS clay technology developed during the course of our study is firmly established and has been realized by the recent identification of a U.S. company that will provide a uniform particle size NS product for use in human trials. This company has the capacity to provide a variety of dose forms for animal feed and human food, which will benefit both the U.S. and developing countries. Viable working relationships and collaborations among team members will help to ensure the success of this research and strengthen the benefits of the project.

Potential Impacts

NS clay, which is commonly included in animal feeds, has significant benefits to the animal industry. These are realized particularly by the swine and poultry industries in developed and developing countries. This is due to the susceptibility of piglets, turkey poults, young chicks, quail, etc. to aflatoxins and the frequent contamination of commodities used to formulate their feeds. Based on numerous studies, it has been shown that NS clay improves growth rates, feed conversions and general health in animals. Moreover, NS inclusion in feeds for lactating dairy animals results in diminished aflatoxin residues in human foods of animal origin including milk and dairy products. Our recent findings from clinical intervention trials with NS are of particular relevance to populations in developing countries where the incidence of aflatoxicosis and infectious disease are often elevated. It is anticipated that refined NS therapy will be useful for protection of humans who are at high risk for aflatoxicosis. Eventually, the preferred delivery of UPSN may be through its inclusion in salt (like iodine), taking advantage of its anticaking properties, or as an additive in common groundnut and maize-based foods such as peanut butter and infant formula. These novel UPSN dose forms will facilitate the prospects of technology transfer and serve to protect both adults and the young at the village level.

Equipment

Shimadzu UV/visible scanning spectrophotometer (\$8k)

Why: To facilitate isothermal and biomarker analyses outlined in Objective 1

When: Year One of the project

Where: T.D. Phillips laboratory (TAMU)

Project Timeline

YEAR 1

Objective 1 (In vitro work to confirm UPSNS characteristics)

YEAR 2

Objective 2 (Animal study to confirm safety of UPSNS) and initiate Objective 3 (Screening participants for the phase IIb trial; biomarker analysis)

YEAR 3

Objective 3 (Intervention trial in Ghana)

YEAR 4

Objectives 3, 4 (Continuation of trial in Ghana; Biomarker analysis)

YEAR 5

Objective 4 (Data collection, data entry, and statistical analysis; manuscript preparation and publications).

USAID Mandate Responsiveness

MDGs

Poverty/Hunger: Improved Health: Raised Rural Incomes: Sustainable Development

Foreign Assistance Framework

Governance: Human Capacity: Economic Structure: Persistent Dire Poverty: Global Issues (HIV and Infectious Diseases, climate change, biodiversity)

IEHA

Science and Tech Applications: Increased demand for peanuts: Market Access: Increased Trade

USAID Focal Areas

Greater incomes: Greater value and market demand: Public Health: Food Security: Sustainable Value Chain: Improved Human Capacity