Research Proposal: Development of Methods for Establishing a Global Network for Aflatoxin Exposure

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Focus

Domain - Aflatoxin Region - Global

Background

Developing countries without sophisticated and regulated food systems often fail to assure safe food as happens in the USA and Europe. Aflatoxins (AFs), produced by Aspergillus flavus and A. parasiticus, are among the most potent occurring food-borne toxicants. Without careful production, storage and processing of many food commodities in peanut, various other nuts, and corn AFs may be found.

Immune suppression and nutritional interference is associated with chronic but moderate levels of AF exposure and these affects are not attributed to aflatoxicosis since they operate indirectly, and aflatoxin exposure is not commonly measured in medical examinations of sick people. The indirect promotion of infectious diseases via immune suppression and suppressed micro-nutrition means that chronic aflatoxicosis is potentially important as a factor in epidemics such as HIV, Malaria and Tuberculosis. Food contamination based systems to detect exposure have not been effective in alerting public health systems to the risk of AF and we believe that biological exposure measured by markers is more relevant as a predictor of enhanced risk.

Surveillance for exposure to aflatoxin is not occurring despite the connection to epidemiology, and for this reason there is little concern for the quality of food. While contamination by the AF- producing molds may be universal within a given geographical area, the levels or final concentration of AFs in the grain product can vary from < 1 µg/kg (1 ppb) to > 12,000 µg/kg (12ppm). For this reason, the measurement of human consumption of AF by sampling foodstuffs is extremely imprecise. Further, obvious contamination of a commodity with the fungi does not necessarily demonstrate the presence of AFs, and the appearance of a sound, uninfected sample of commodity does not preclude the existence of significant quantities of AFs. Therefore, accurate assessment of global AF exposure using the biomarker technique is urgently needed and highly justified.

AFB1 that is ingested is both detoxified and activated in the liver has a wide range of toxicities and is one of the most potent genotoxic agents in many model systems. Acute toxic effects in humans, as evidenced by a recent outbreak in Kenya, include vomiting, convulsions, coma, and death with cerebral edema and fatty accumulation in the liver, kidney, and heart. Chronic aflatoxicosis is characterized by bile duct proliferation, periportal fibrosis, icterus and cirrhosis of the liver. Prolonged exposure to low levels of AFB1 leads to liver cancer and other tumors in animals. High frequency of p53 mutations (G->T transversion at codon 249) were found to occur in HCC tissues collected from populations with high dietary AF exposure in China and

Southern Africa. Furthermore, the role of synergistic interactions between AF and hepatitis B virus (HBV) in the formation of HCC has also been confirmed. AFB1 is classified as a known human carcinogen by the IARC. Biomarkers for AF may measure chronic exposure via the albumin adduct, short term exposure via the predominant AFB1-DNA adduct which is 8,9-dihydro-8-(N7-guanyl)-9-hydroxy-AFBl (AFB-N7-gua), which results from covalent bond formation between C8 of AFB1-8,9-epoxides and N7 of guanine bases in DNA. This initial AFB-N7-gua adduct can convert to a ring-opened formamidopyrimidine derivative, AFB-FAPyr. These products are short term markers of exposure as is the commonly observed biomarker AFM1 which is a 'detoxification' product reflecting recent (24-48 h history).

The major AFB1 protein adduct is AFB1-lysine adduct in serum albumin which has a half-life in the body of about 3-4 weeks.

Technical Review

AF exposure and AF-linked adverse health effects, especially in the developing world, have been great public concerns from the liver cancer perspective. The human risk assessment has been hindered by lack of adequate dosimetry data on AF intake and metabolism and the wide variations of AF distribution in food commodities. Extensive efforts were devoted in the field over the past 25 years to develop highly sensitive and specific methods for detecting AF biomarkers based upon the knowledge of toxicology of AF gleaned from both experimental and human studies. The AF molecular biomarkers currently used in human and animal studies include AFB1 metabolites and AFB1-macromolecular adducts, such as AFM1 and AFB-N7-Gua in urine and AFB1-albumin adducts in serum. AFB1-albumin adduct as a biomarker is important because its estimated longer in vivo half-life (up to 3-4 weeks), compared to urinary AF metabolites (usually representing 24-72 h exposure), may reflect integrated exposures over longer time periods. From a practical perspective pertinent to human biomonitoring and epidemiological studies, the measurement of serum AFB1-albumin adducts offers a rapid, facile approach that can be used to screen very large numbers of people. Thus, AFB1-albumin adduct is the most reliable molecular biomarker for studying human exposures to AFs. There are four analytical techniques currently available for measuring AFB1-albumin adducts in human blood: enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunoaffinity column (IAC)-HPLC with fluorescence detection, and isotope dilution mass spectrometry (IDMS) method. Using these techniques, highly significant associations between AFB1-albumin adduct level and AFB1 intakes were found in human populations from several regions of the world. Furthermore, about 2% of the ingested AFB1 became covalently bound to serum albumin, a value

very similar to that observed when rats were administered AFB1. Using these techniques, AFB1-albumin adduct was detectable in almost 100% of adults' sera and in 12-100% of children's sera in China and various African countries. In addition to studying AF exposure, AFB1-albumin adduct has been used as a biological response indicator for acute and chronic human diseases, such as aflatoxicosis in Africa, risk of HCC in Taiwan, China, and Africa, and infectious disease linked immune suppression. Moreover, AFB1-albumin adduct has been used as the surrogate efficacy biomarker for assessment of several human intervention trials, including a recently completed 3-month study in Ghana with NovaSil clay. However, high cost of these antibody-based or MS-based methods limits their wide applications in developing countries; therefore, development of a highly sensitive, non-antibody, non-radioactive, and non-MS based analytical method for rapidly measuring serum AFB1-albumin adduct is required for establishing the global network of AF exposure.

Obtaining biological samples for surveillance is also a challenge in developing countries where clinic facilities and sample collection/preservation can present a problem. While blood samples provide the best biomarkers for chronic aflatoxicosis urine samples are much easier to collect.

Another unknown issue that needs attention is the identification of benchmark or representative populations for surveillance.

Problem Statement

Aflatoxins (AFs) represent a group of naturally occurring mycotoxins that are hazardous contaminants of food, especially in peanuts and corn. Acute exposure to high levels of AFB1 via the diet causes disease (aflatoxicosis) and death in humans, as evidenced by numerous reports, including the recent outbreak in Kenya, are relatively rare. Much more serious for health is chronic sub-clinical symptom inducing levels exposure AFs which in addition to being a major risk factor in the etiology of human hepatocellular carcinoma (HCC) is also shown to be an anti- nutritional agent that reduces vitamins and proteins in animals and humans. Furthermore, AFB1 is a potent immunotoxic agent, which may aggravate infectious diseases in the developing world thus AF acts to promote more easily recognized diseases while being undiagnosed as an environment health risk. Therefore, accurate assessment of global AF exposure is urgently needed. In this proposal, we propose to design, enable and pilot a global surveillance system to study AF exposure using serum AFB1-albumin adduct as a biomarker.

Vision and Approach

Goals

The long-term goal of this project is to enable and establish a global network for assessment of AF exposure in high-risk populations and to investigate the efficacy of NovaSil for diminishing incidence and mortality from human aflatoxicoses. The establishment of such a network will allow epidemiological evaluation of the linkage between AF exposure, nutritional deficiency, and infectious diseases and also allow public health agencies to be proactive in responding to food quality issues. Our working hypothesis for this project is that levels of AFB1-albumin adducts in human serum are highly stable and related to AF exposures, and will be a reliable effective biological response indicator for human aflatoxicosis in high-risk populations.

The immediate goals of this research are:

1. To develop a highly sensitive, non-antibody, non-radioactive, and non-mass spectrometry (MS) based analytical method for rapidly measuring serum AFB1-albumin adduct. The method will be validated in animals treated with a single or repeat dose of AFB1 and in archived blood samples previously collected from high risk populations in developing countries.
2. To develop and validate a sampling method for surveillance based on existing clinic infrastructures and capacities.
3. To pilot test/demonstrate a surveillance network in selected partner countries. Workshops will be held at Institutions in Africa, Latin America, and Asia in order to train participants to apply the validated method for human biomonitoring studies and to establish a global network for AF exposure using AFB1-albumin adduct as the biomarker.

Objectives

1. Measurement of aflatoxin adducts and fumonisin biomarkers on samples to be collected in Ghana and other West Africa sites. The work in this objective is in support of work conducted in Projects UAB148-Jolly and TAM149-Phillips.
2. Complete analysis of 1500 blood samples collected in West Africa in support of UAB148-Jolly and TAM149-Phillips.

3. To hold training courses in Institutions of Africa, Latin America, and Asia

to familiar with the validated method for network participating members.

4. A new rapid and less expensive method for measuring blood serum using lysine adducts rather than the slower and more expensive method of using albumin adducts was developed. Under this objective, individuals in the host country and possibly other countries (through workshops) will be trained in the new methodology.
5. To conduct pilot human biomonitoring studies in network participating countries and collect data for the final establishment of the global network of AF exposure using AFB1- albumin adduct as the biomarker.

Research Approach

Tech approaches in Phase 1 method development will include step-wise procedures for release of AFB1-lysine adduct from serum albumin using enzyme digestion; solid-phase enrichment of the adduct using chemically selected polymer-based cartridge; HPLC separation, and followed by fluorescence detection. The preliminary data shows a recovery of 88% and the limit of detection at 0.1 pg/mL serum. The method will be validated with blood samples of F344 rats treated with either a single dose (25, 50, 100, and 200 g/kg body weight) or repeated doses (5, 10, and 20 g/kg body weight for 5 weeks) of AFB1 and with archived human blood samples previously collected from high risk populations in Ghana and China. The tech approaches in phase 2 will include training courses in network participating countries in Africa, Latin America, and Asia. These courses will use standard materials, standard operating procedures, and good laboratory practice guidelines to train network participating members for use of the analytical method and for the overall study design on a longitudinal human biomonitoring study. The biomonitoring study will be firstly conducted in 1 country in each continent for developing standard protocols including Institutional Review Board Approval, sample collection, separation, storage, transportation, analysis, and final data entry and report, which will then be distributed to all participating team members. The tech approaches in phase 3 will include establish network databases using Microsoft Access. Data sorting and statistical evaluation will be performed with focuses on identifying high risk populations and developing intervention strategies. Association analysis will be made on finding susceptibility factors in determination of human aflatoxicoses, studying the linkage between AF exposure and human nutrition deficiency, and examining roles of AF exposure in infectious diseases. Specifically, ANOVA-simultaneous component analysis (ASCA), a statistical method recently developed, will be used to deal with complex datasets collected from the network. This method provides a generalization of an ANOVA setting and allows for the characterization of variability within the overall population profile as a

consequence of both among and within subjects factors. Thus, it will provide an estimate of the percentage of variation in the dataset that is due to AF exposure dose, time, host susceptibility factors, and their interaction. Furthermore, it provides axis scores for variables that can be used to identify AFB1-albumin adduct that is related to specific factors. ASCA will be conducted in Matlab using code freely distributed at http://www.bdagroup.nl. As a verification of ASCA, redundancy analysis (RDA) will be used. RDA is a constrained ordination technique that will be used to explain variation in the overall dataset with exposure and time being constraints on the ordination.

Training & Capacity Development Approach

Dr. Jia-Sheng Wang is professor of molecular toxicology and the leader for Division of Human Health Sciences at The Institute of Environmental and Human Health, Texas Tech University System. Dr. Wang received his M.D. in preventive medicine from the Shanghai First Medical College, China with the major in food-borne diseases. He received a Ph.D. in Pathology and Immunology from the Boston University School of Medicine and did his postdoctoral training as a NIEHS fellow in molecular epidemiology at the Johns Hopkins University School of Public Health. For more than 25 years, he has focused on studying human health effects of exposure to mycotoxins, especially for AFs. He worked at Dr. G. N. Wogan's research group in MIT from 1986-1992 to develop a monoclonal antibody based immunoaffinity method for detecting AFs and other mycotoxins in food samples. He was the key research scientist at J. D. Groopman's research group in the Johns Hopkins University to develop and apply AFB1 monoclonal antibody based immunoaffinity-HPLC-fluorescent and immunoaffinity-LC-MS methods for AF-related human epidemiological studies in China, Mexico, and The Gambia, and chemoprvention studies in China. Mouse monoclonal antibody he developed against a synthetic AFB1-lysine- cationized bovine serum albumin conjugate is one of the most sensitive antibodies for AF and associated biomarkers. Over the past 5 years, with USAID Peanut CRSP support (LAG-G-00-96-90013-00) Dr. Wang has worked closely with Dr. Timothy D. Phillips at Texas A&M University, Dr. Pauline Jolly at the University of Alabama at Birmingham, and Drs. David Ofori- Adjei, Nii-Ayi Ankrah, and William Ellis in Ghana on a NovaSil intervention study and AF- related immunosuppression study in Ghana. This group has published 8 papers. This team has attended several international mycotoxin meetings including Mycoglobe in Ghana and IUPAC in

Istanbul, Turkey. They have developed a list of participants who are interested in the global network study at the IUPAC meeting in Istanbul. These three groups in the US have the necessary experience to conduct international studies in China, Vietnam, Mexico, Gambia, and Ghana by Dr.

Wang; in Ghana, Mexico, India, and Thailand by Dr. Phillips; and Ghana, Kenya, and Jamaica by Dr. Jolly. They also serve as guest professors for many international institutions and participate in training courses organized by WHO, FAO, and IARC in different countries. Therefore, in collaboration with team members from network participating countries, they will have full capacity to conduct and complete the proposed method development and global network establishment.

Intended Benefits & Impact Responsiveness

Development Benefits

AF exposure and AF-linked adverse health effects, especially in the developing world, have been great public health concerns. AFB1 is acutely toxic to all species of animals and humans. Acute aflatoxicoses occurs frequently in developing countries, as evidenced by a recent outbreak in Kenya. Chronic exposure to low levels of AFB1 may lead to high incidence of HCC (liver cancer) and other tumors in human populations of developing countries. AFB1 has an anti- nutritional effect and reduces vitamin and protein levels in animals and humans. In addition, AFB1 has a potent immunotoxic effect, which may aggravate infectious disease in the developing world. Therefore, accurate assessment of global AF exposure using the biomarker technique is highly significant for developing countries. This project will provide methods to overcome the limitation of high cost of currently available antibody-based or MS-based methods. It will also enhance the development of a highly sensitive, non-antibody, non-radioactive, and non-MS based analytical method for rapidly measuring serum AFB1-albumin adduct, which will greatly benefit developing countries.

US Benefits

AFs are among the most potent and commonly occurring food-borne toxicants. Commodities produced in the US are also frequently contaminated by AFs, including peanut, cottonseed, and corn. Preliminary data shows that certain populations in certain region of the US, such as southeast Texas, are exposed to AFs. However, potential adverse effect of AFs has never been studied in the US populations. The US Center for Disease Control and Prevention (CDC) has recognized the problem and proposed to conduct human biomonitoring studies using AFB1- albumin adduct as the biomarker in next five years. The LC/MS method for measuring the adduct has been developed at the CDC core laboratory with Dr. Jia-Sheng Wang as a consultant. Establishment of the global network for AF exposure in developing countries as proposed by this research will provide the database for comparisons with US data, and therefore, will benefit US public health.

Potential Impacts

AFs are ubiquitous food-borne toxicants, and human populations in many parts of the developing world frequently are exposed to these chemicals through contaminated food. These important mycotoxins have been strongly associated with human aflatoxicosis as well as many types of primary cancers. Thus, the contamination of these mycotoxins in human foods represents a significant public health concern. In this proposal, we will develop a highly sensitive, non- antibody, non-radioactive, and non-MS based analytical method for rapidly measuring serum AFB1-albumin adduct. The method will be hold training courses in Institutions of Africa, Latin America, and Asia to familiarize participants with the validated method for network members and to conduct human biomonitoring studies in participating countries and collect data to facilitate the establishment of a global network for AF exposure using AFB1-albumin adduct as the biomarker. Upon completion, this project will generate sufficient data for understanding the relationship between AF exposure and susceptibility factors that contribute to human aflatoxicoses in the developing world. This will have significant benefits for the risk assessment of human health effects as a result of long-term exposure to AFs.

Equipment

No special equipment is requested for method development and validation; however, two -80 C freezer ($10K/each) may be needed to archive the network blood samples, which will cost $20K.

Project Timeline

Phase 1 (Year 1)

1. To develop a highly sensitive, non-antibody, non-radioactive, and non-mass spectrometry (MS) based analytical method for rapidly measuring serum AFB1-albumin adduct at Texas Tech University;
2. To validate the method in animals (rats) treated with a single dose (25, 50, 100, and 200 g/kg body weight) or repeated doses (5, 10, and 20 g/kg body weight for 5 weeks) of AFB1 and in archived human blood samples previously collected from high risk populations in developing countries, e.g., Ghana, China, and Mexico;

Phase 2 (Year 2-4)

1. To hold training courses in Institutions of Africa, Latin America, and Asia to familiarize participants with the validated biomarker method for network members;
2. To conduct human biomonitoring studies in participating countries and

collect data to facilitate the establishment of a global network for AF exposure using AFB1-albumin adduct as the biomarker.

Phase 3 (Year 5)

1. To use the network database for developing intervention strategies, assessing susceptibility factors in determination of human aflatoxicoses, studying the linkage between AF exposure and human nutrition deficiency, and examining roles of AF exposure in infectious diseases.

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