PRO0812

Can arsenic toxicity in mammals be reduced by feeding Saskatchewan grown lentils?

INVESTIGATORS

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STUDY SPONSORS

National Sciences and Engineering Research Council of Canada (NSERC)
Saskatchewan Pulse Growers

TYPE OF STUDY

Health Outcome: Pre-clinical trial: Mammalian model feeding trial

OBJECTIVES

1. To design and validate a mammalian model of arsenic toxicity and determine the most sensitive bioindicators (e.g., measurable biochemical, hormonal, immunological, pathological responses) of adverse health effects from this exposure.

2. To feed laboratory rodents exposed to environmentally meaningful levels of arsenic, with high and low selenium rodent diets to determine if dietary selenium can reduce damage induced by arsenic exposure.

3. In the same experimental model, to feed animals nutritionally balanced diets made from lentils with only selenium levels being different (because the lentils were grown in SK and Western USA), and measure differences in arsenic induced damage.

4. To test the potential of high-selenium lentils from the Canadian prairies as a therapeutic food to alter the outcome of arsenic-stimulated atherosclerosis (plaque development within blood vessels) using a mouse model.

WHY STUDY NEEDED

Chronic arsenic (As) toxicity (arsenicosis) causes serious health problems in humans including disfiguring skin lesions, common cancers, and severe artherosclerosis. Selenium (Se) is a micronutrient thought to play an important role in mitigating the health costs associated with arsenic toxicity. Clinical results from trials in which people have been supplemented with Se have shown increased longevity and remarkable decreases in incidence of common cancers. Selenium is an antagonist of As toxicity and has been shown to decrease As concentrations in blood and hair and to be protective against genetic damage from As toxicity in laboratory rodents.

It has been proposed that Se, supplemented in tablet form to children and adults, would have a beneficial effect on wide spread pandemic arsenic toxicity in South Asia and, although there is growing evidence to support this hypothesis, definitive proof is lacking. Such proof would entail a controlled study in which As exposed animals are treated with low Se diets, or the same diets except for supplemental Se treatment, which can both be compared with nutritionally similar diets, except that Se is naturally incorporated into the food base of the diet (i.e., SK lentil).

Current research shows that Saskatchewan grown lentils are a uniquely rich source of Se due to the selenium rich soils in the province. Thus lentils from Saskatchewan present a unique opportunity for natural Se biofortification and could provide a whole food solution to arsenic toxicity.

The aim of the study was to examine the potential benefits of selenium-rich lentils grown in Saskatchewan in counteracting arsenic toxicity.

HYPOTHESIS

Increased dietary selenium will reduce the body burdens of arsenic and decrease clinical signs of chronic arsenic toxicity.

STUDY DESIGN

In four separate experimental studies, laboratory rodents were used to investigate the effects of chronic arsenic poisoning and the potential of diets made from high selenium lentils to reduce or eliminate the damage caused by arsenic.

Study 1: Dose-dependent study
Established the dose-dependent effects of As using 3 environmentally relevant levels of arsenic (0.4, 4, and 40 ppm) by exposing rats to arsenic in their drinking water for 18 weeks.

Study 2: Compared effects of consuming high, versus low Se, otherwise nutritionally matched, rodent chow to reduce arsenic toxicity. Rats received As (40 and 80 ppm) in drinking water and challenge diets with three levels of Se (deficient: 50.01 ppm, adequate: 0.15 ppm, and fortified: 0.6 ppm) for 16 weeks.

Study 3: Compared effects of consuming diets made from high-Se SK lentils versus low-Se lentils from NW United States to reduce arsenic toxicity. Rats drank control (0 ppm As) or As (40 ppm) water while consuming SK lentils (0.3 ppm Se) or NW USA lentils (0.01 ppm Se)

diets for 14 weeks.

Study 4: Performing a study on ApoE-/- mice with our collaborators at McGill University, Montreal. Mice were exposed to 200 ppb As in their drinking water while being fed a high- or low-selenium lentil diet. After 13 weeks the atherosclerosis lesion area and plaque composition were assessed, and lipid and glutathione (GSH) concentrations in plasma measured.

In general, animals were exposed for 3+ or 4 months to the arsenic water and respective diets. Food and water consumption, growth rate and behavior were assessed every 1 to 2 days. Samples (blood, urine, feces) were collected to examine biological / health responses in the experimental animals on a monthly basis. When animals were humanely euthanized at the end of each of the studies, detailed necropsies (autopsies) were conducted. Gross and histopathology examination of major organs (liver, lung, kidney, spleen, thyroid glands, lungs, heart, blood vessels in extremities) was carried out. Tissue samples (liver and blood) and excreta (urine and feces) were analyzed to determine responses to the selenium treatments.

All the studies and the animals used were carried out under the auspices and with approved experimental protocols from the Canadian Council on Animal Care and University of Calgary or University of Saskatchewan Animal Care Committees.

The diets were all nutritionally balanced and formulated by Harlan Laboratories, a commercial rodent diet manufacturer. Only the selenium levels were different. Lentils for all the diets were supplied by Dr A. Vandenberg, University of Saskatchewan. The same types of lentil were used, but they had been grown in regions with high (Saskatchewan) or low (Idaho) selenium soil, which is reflected in lentils grown on those soils.

FINDINGS

Arsenic–exposed animals on high selenium diets compared to those on selenium deficient diets showed the following:

- [35][17] Higher blood stores of the major, protective antioxidant, glutathione (GSH)
- [35][17] Decreased liver damage as measured by oxidative damage in liver tissue (TBARS assay)
- [35][17] Recovery of the antibody mediated immune response which is suppressed by arsenic exposure
- [35][17] High Se lentil diets, and body burdens of As were reduced, as was evident through lower arsenic residues in the kidneys, and higher fecal and urinary arsenic excretion in animals on the high selenium diets.
- [35][17] Arsenic-exacerbated plaque formation was reduced or completely abolished in specific areas of the aorta of mice on the Se-fortified lentil diet, whereas vascular plaques were increased in As-exposed mice on the Se-deficient and -adequate diets. Additionally, Se deficiency contributed to pro-atherogenic composition of serum lipids in As-exposed mice as indicated by HDL:LDL. This study was the first to show the potential of high-Se lentils to protect against As-triggered atherosclerosis

SIGNIFICANCE OF STUDY

In a rodent model, the key achievements of this study were the following:

1. Determined the dose-dependent health effects of arsenic (As) exposure in a rodent model

2. Showed it was possible to reverse these health effects with commercial diets fortified with selenium.
3. Showed that selenium-rich Saskatchewan grown lentils diets also increased arsenic detoxification from the body and decrease damage from As exposure.
4. Through using a mouse model of atherosclerosis, we showed that high Se lentil diets reduced the plaque formation in the aorta and improved the blood cholesterol profile in favour of higher beneficial High Density Lipoproteins compared with the detrimental Low Density Liporoteins.

PUBLICATIONS, PRESENTATIONS, EDUCATIONAL MATERIALS PRODUCED

1. Krohn RM., M Lemaire, Negro Silva LF, Mann KK, Smits JEG. August 2015. High-selenium lentil diet protects against arsenic-induced atherosclerosis in a mouse model. *In Press -Journal of Nutritional Biochemistry*

2. Sah S., A. Vandenberg, J.E. Smits. 2013. Treating chronic arsenic toxicity with high selenium lentil diets. *Toxicology and Applied Pharmacology* 272: 256-262 doi: 10.1016/j.taap.2013.06.008

3. Sah S., Judit E. G Smits. 2012. Dietary selenium fortification: A potential solution to chronic arsenic toxicity. *Toxicology & Environmental Chemistry* 94(7): 1453-1465 DOI:10.1080/02772248.2012.701104

4. Nain S., Smits, J.E.G. 2012. Pathological, immunological and biochemical biomarkers of sub-chronic arsenic toxicity in experimental rats. *Environmental Toxicology* 27(4): 244–254

VALUE TO PRODUCERS/PULSE INDUSTRY

Increased imports of high selenium lentils from Canada may provide a whole food solution to the people affected with arsenicosis in severely affected countries (Bangladesh, northeastern India and the IndoGangetic plains region).
High selenium lentils may confer cardiovascular health benefits to other mammals, including humans, based on findings in experimental animals, but this requires further research.