Influence of dietary cyanide on immunoglobulin and thiocyanate levels in the serum of Liberian adults

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INFLUENCE OF DIETARY CYANIDE ON IMMUNOGLOBULIN AND THIOCYANATE LEVELS IN THE SERUM OF LIBERIAN ADULTS

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Serum thiocyanate, antibody titers to thiocyanates, and serum immunoglobulins (IgM, IgG, IgA) were measured in 73 Liberian adults normally consuming diets of low, moderate, high, or no (control) cassava-derived cyanide (CN⁻). When control and low groups (n = 40; daily intake less than 0.60 mg CN⁻ per kg body weight) were contrasted with moderate and high groups (n = 33; daily intake greater than or equal to 0.60 mg CN⁻ per kg body weight), the authors observed that (1) one-time serum thiocyanate measurements were not sensitive to long-term cyanide intake; however, (2) antibody titers to thiocyanates were positively correlated with cassava-based cyanide intakes (r = .22, P = 0.05); and (3) serum IgM, IgG, and IgA levels were elevated in individuals regularly consuming moderate and high levels of dietary cyanide. Possible responsible mechanisms and health implications are discussed.

In the humid tropics and subtropics, an assortment of microbial pathogens are endemic and host immunological status represents a major determinant of overall health. Under environmental conditions of high pathogen prevalence, any compromise in immunoglobulin levels may result in an increased susceptibility to disease.

Serum immunoglobulins (IgM, IgG, IgA) generally play a protective role in the response to and in the eventual clearance of particulate and soluble antigens in the vertebrate immune system. For example, an individual's humoral immune system may be compromised as the result of (1) the failure

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of light and heavy chains of immunoglobulins to assemble,1,2 (2) defects in the heavy chains of immunoglobulins,3,4 (3) excess production of immunoglobulins without any apparent antibody activity to a given antigen,5,6 (4) decreased production of a single class of an immunoglobulin,7 and (5) hypoproduction of all five classes of immunoglobulins.8

Additionally, malnutrition characterized by the absence of specific amino acids9 can diminish immunoglobulin production and function, making one at risk to infections by agents that are normally well controlled in the healthy population.

For the more than 300 million people of the tropics and subtropics who regularly consume cassava (Manihot esculenta) as a staple, the chronic ingestion of sublethal levels of cyanide (CN−) is inevitable. Cassava (also known as manioc, tapioca, yuca), the seventh most important world crop10 and the major root and tuber crop in developing countries,11 contains cyanogenic glucosides, biological precursors of cyanide. Generally, cyanide is liberated in minute quantities when the roots and leaves of the plant are prepared for consumption.

Sublethal cyanide ingestion requires sulfur for its \((in\ vivo)\) detoxification to thiocyanate \((SCN^-)\) with sulfane sulfur playing a critical role. Sulfane sulfur appears to be formed from cysteine via transamination and subsequent reactions catalyzed by mercaptopyruvate sulfurtransferase.12 Cysteine, however, is also a vital amino acid involved in the structure and function of immunoglobulins.13,14 During detoxification, cyanide may deplete the sulfane pool15 and subsequently impair all the normal functions of this pool, including providing sulfur for the disulfide bond bridges of IgM, IgG, and IgA.

As stated by Westley,15 the detoxification of dietary cyanide increases the demand for cysteine, which may drastically lower cysteine availability for antibody synthesis, structure, and function. The authors’ interest in this study was to determine if chronic cassava consumption, as a major source of cyanide, could adversely influence the levels and possibly the function of serum immunoglobulins in adults.

Seventy-three adult men and women of varying ages, indigenous to the Republic of Liberia and consuming different levels of cassava-derived cyanide were studied. The Republic of Liberia was selected because it is a small country, located almost entirely within the humid tropics zone, yet displays a northwest-to-southeast regional gradient in cassava consumption.16

In this report, the authors examine the relationships between the dietary cyanide intakes, serum thiocyanate levels, and specific serum immunoglobulins of these adults with the aim of identifying and evaluating changes in immune status associated with indigenous patterns of cassava ingestion. The results reported here are part of a larger study on the health consequences of dietary cyanide17 and represent the authors’ initial investigations of the impact of sublethal cyanide consumption on the immune system.

METHODS AND MATERIALS

The Sample

Seventy-three adult Liberians attending the central government hospital as outpatients or residents in the surgical ward were studied. Heavy smokers and individuals exhibiting any overt infections were excluded from the study. With the informed consent of participants, evaluations were made of their cassava-derived dietary cyanide intakes, general health status, serum thiocyanate, serum antibody titers to thiocyanates, and serum IgM, IgG, and IgA levels. Male subjects comprised 66 percent of the sample \((n = 48)\) and female subjects comprised 34 percent \((n = 25)\). The mean age of the patients was \(36.8 \pm 2.2\) years, with ages ranging from 15 to 80 years. The people in this study represented the 17 major indigenous ethnic groups of Liberia.

Dietary Cyanide

The free and bound cyanide contents of 106 samples of 15 different traditional cassava foodstuffs were quantified by enzymatic assay.18
and the dietary pattern of each adult was determined by dietary recall. Dietary data were frequently supplemented with direct observations of food preparation and consumption methods. Mean daily cyanide ingestion levels were calculated by (1) determining the daily, weekly, or monthly frequency of consumption of various cassava-based food products, (2) multiplying the assayed per gram cyanide content of each cassava foodstuff cited by the approximate quantity consumed, (3) adjusting this value to its daily equivalent, and (4) dividing this by the individual's body weight. These values were then stratified into four patterns of chronic daily sublethal cyanide ingestion (Figure 1).

**Specimen Collection**

Under the supervision of local clinicians, 5 mL of venous blood was collected in heparinized tubes, separated via centrifugation, and the serum was removed and stored at −70° C in a mechanical freezer for subsequent thiocyanate and immunological determinations.

**Thiocyanate Levels**

Daily serum SCN− levels were quantified using a sensitive gas chromatographic method. As described, a total volume of 1.2 mL of mixture per sample was obtained by mixing in a 10:1:1 ratio, saturated NaCl in 0.2N NaOH, 20 μM of SeCN− in H2O, and 20 mg/mL of tributylsulfonium perchlorate (the derivatizing agent), respectively. For each analysis, 100 μL of serum was added to each 1.2 mL of mixture, and an additional 1.2 mL of ethyl acetate was added for the extraction of the thiocyanate. The within-day reproducibility (as indicated by the coefficient of variation) ranged between 5.9 percent of 20 mmol/L and 1.9 percent for 100 mmol/L. The between-day reproducibility was 7.2 percent for 100 mmol/L.

**Thiocyanate Sensitive Antibody**

To perform the passive hemagglutination test, 200 mg of potassium thiocyanate was added to 10 mL of a 10 percent suspension of sheep red blood cells; after mixing, 1 mL of 25 percent gluteral-
dehydrate was added to the tubes containing the red blood cells and the KSCN, and rotated at room temperature for 15 minutes. The 10 mL of coated cells were dispensed in 2.5 mL amounts in 50 mL centrifuged tubes and washed with approximately 37.5 mL of saline. Three such washings were done.

The coated cells were then adjusted to a concentration of 0.5 percent and used in 100 μL amounts in a U-shaped microtiter plate to which was previously added 100 μL of a given dilution of the patient’s serum. Serial tenfold dilutions were run from 1/20 to 1/5,120. The titer was read as the reciprocal of the highest dilution of the patient’s serum that produced agglutination of the coated red blood cells.

Humoral Immunoglobulins

Serum IgM, IgG, and IgA levels were assayed using the Mancini technique of single radial immunodiffusion (RID).20 Commercial immunoplates and reagents for RID determinations were obtained from Meloy Laboratories (Springfield, Va).

Statistical Methods

Standard statistical procedures were used in data analysis, including simple linear regression.21 To increase subsample sizes and improve the reliability of the analyses, the control and low-cyanide groups were combined (n = 40), and the moderate and high cyanide groups were also combined (n = 33). Means are presented plus or minus their standard errors (SEM). Values of P less than or equal to .05 are considered significant.

RESULTS

In this study serum thiocyanate did not appear to be sensitive to dietary cyanide intake. The combined means were similar (control + low = 21.81 ± 7.52, while moderate + high = 18.09 ± 5.20), and the correlation coefficient between these two variables was not significantly different from zero (r = .05, P > 0.05). However, antibody titers to thiocyanates were significantly correlated with dietary cyanide intakes (r = .22, P = 0.05), and titers increased as the amount of ingested cassava-derived cyanide increased.

Serum IgM, IgG, and IgA were also affected by dietary cyanide levels. As shown in Table 1, combined group means suggest a general pattern of elevated immunoglobulin levels for the combined moderate + high group compared with the control + low group. Further analysis of this data suggests that IgM appears most susceptible to dietary modification, particularly among women.

DISCUSSION

The results of this initial study suggest that cross-sectional serum thiocyanate levels are of limited use in evaluating long-term dietary cyanide ingestion. The lack of correlation between these measurements emphasizes the difficulty of using a cross-sectional parameter (one-time serum SCN− levels) to reflect a longitudinal process (decades of chronic CN− intake).

Diurnal variation in serum SCN− levels, physiological or genetic adaptations in SCN− clearance rates, are some factors that may have obscured the expected positive association of serum metabolite levels with precursor intake loads. Both intake levels and titers were longitudinal assessments of exposure and response, respectively, and were thus more complementary measures. Chronically ingested sublethal cyanide is converted to thiocyanate, presumably at levels proportional to intake, and eventually induces production of SCN−-sensitive antibody titers in response to these long-term serum SCN− levels.

As this pattern of cyanide exposure requires sulfur for its detoxification to thiocyanate, the authors had originally hypothesized that the ingestion of moderate to high levels of dietary cyanide might deplete body reserves of sulfur-containing amino acids (especialy cysteine), exhaust sulfane
TABLE 1. SUMMARY OF IMMUNOGLOBULIN AND TITER LEVELS ASSOCIATED WITH DIFFERENT DAILY CYANIDE (CN⁻) INTAKES

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>Male Subjects</th>
<th>Female Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM (mg/100 mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control + Low*</td>
<td>112.50 ± 14.05</td>
<td>110.84 ± 17.91</td>
<td>115.27 ± 23.42</td>
</tr>
<tr>
<td>Moderate + High**</td>
<td>142.21 ± 21.66</td>
<td>118.35 ± 13.47</td>
<td>197.10 ± 63.26</td>
</tr>
<tr>
<td>IgG (mg/100 mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control + Low*</td>
<td>1552.75 ± 139.69</td>
<td>1606.40 ± 136.36</td>
<td>1463.33 ± 301.53</td>
</tr>
<tr>
<td>Moderate + High**</td>
<td>1670.61 ± 224.34</td>
<td>1763.26 ± 154.91</td>
<td>1286.50 ± 153.81</td>
</tr>
<tr>
<td>IgA (mg/100 mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control + Low*</td>
<td>155.48 ± 25.73</td>
<td>156.80 ± 17.40</td>
<td>153.27 ± 31.20</td>
</tr>
<tr>
<td>Moderate + High**</td>
<td>153.39 ± 10.74</td>
<td>150.92 ± 12.35</td>
<td>159.10 ± 22.16</td>
</tr>
<tr>
<td>Titer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control + Low*</td>
<td>1536.75 ± 186.26</td>
<td>1902.40 ± 220.37</td>
<td>927.33 ± 276.62</td>
</tr>
<tr>
<td>Moderate + High**</td>
<td>1944.24 ± 182.08</td>
<td>1870.87 ± 228.46</td>
<td>2113.00 ± 301.68</td>
</tr>
</tbody>
</table>

*Control + Low refers to 0 mg to 0.6 mg CN⁻; n = 40: male subjects = 25, female subjects = 15.
**Moderate + High refers to 0.61 mg CN⁻ and above; n = 33: male subjects = 23, female subjects = 10.

sulfur pools, and alter the stability and concentration of serum immunoglobulins. This hypothesis was based on the assumption that cysteine needed for intrachain and interchain disulfide bonds would be less available for immunoglobulin structure and function during periods of in vivo dietary cyanide detoxification.

Chronic dietary cyanide, however, at moderate to high levels appears to raise levels of serum immunoglobulins in adult Liberians. Several direct and indirect mechanisms may be responsible for these effects. Dietary cyanide may stimulate immunoglobulin production by acting as an "immunogen." Cyanide by itself would probably be a weak immunogen due to its low molecular weight. However, a stronger antigenic potential may be produced by the binding of cyanide or cyanide derivatives to macromolecular proteins (eg, carboxymethylated hemoglobin). In addition, bound forms of cyanide in the diet may also have antigenic potential.

Immunoglobulin levels may also be elevated as an indirect result of certain sociocultural practices. Traditional cassava-processing methods (eg, prolonged fermentation of peeled roots and subsequent fungal contamination, oral contact with intermediate products during fufu preparation, use of undisinfected, rough-surfaced wooden mortars and pestles for the protracted beating of intermediate and final cassava food products) and the usual consumption patterns (ie, food swallowed in lumps without prior mastication) may expose cassava eaters to higher levels of microbial contamination than they would otherwise encounter. This contamination could result in an increased number of infections, which would in turn stimulate host-humoral immunity, particularly IgM levels. Elevated IgM in male and female subjects consuming moderate or high levels of dietary cyanide is of possible clinical importance, since this antibody has strong antibacterial capabilities, such as agglutination and activation of the complement system. In a region where such bacterial diseases as Hanson's disease (leprosy), louse-borne relapsing fever, yaws, and cholera are endemic, high levels of specific IgM are biologically significant. Furthermore, high IgG levels in men consuming moderate to high levels of cassava-derived cyanide in contrast to the low level of IgG in the moderate to high female group cannot be readily explained. This observation and the observation that serum IgA levels are essentially the same for both male and female subjects in the control + low groups and in the moderate + high groups requires additional study.

This phase of the study concerned itself with
the influence of dietary-derived cyanide on serum thiocyanate, SCN⁻-sensitive antibody titer, and serum IgM, IgG, and IgA levels. Other aspects of this study will include the use of a larger and more defined sample size to establish the range and average values of serum immunoglobulins in young, adult, and elderly Liberians.

Also, a study of the microflora of Liberians, consuming moderate to high levels of dietary cyanide vs those consuming no or low quantities of cyanide, could reveal reduced susceptibility to specific endemic agents as a result of the stimulation of the humoral immune system.

The final phase of this study will involve a close scrutiny of immunoglobulin structure in individuals normally consuming low, moderate, or high levels of dietary cyanide to determine if cyanide detoxification via cysteine and the sulfane-sulfur pool alters cysteine availability for immunoglobulin structure and function.

Acknowledgments

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