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Cross-species and tissue variations in cyanide detoxification rates in rodents and non-human primates on protein-restricted diet

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Abstract

We sought to elucidate the impact of diet, cyanide or cyanate exposure on mammalian cyanide detoxification capabilities (CDC). Male rats (~8 weeks old) (N=52) on 75% sulfur amino acid (SAA)-deficient diet were treated with NaCN (2.5 mg/kg bw) or NaOCN (50 mg/kg bw) for 6 weeks. *Macaca fascicularis* monkeys (~12 years old) (N=12) were exclusively fed cassava for 5 weeks. CDC was assessed in plasma, or spinal cord, or brain. In rats, NaCN induced seizures under SAA-restricted diet whereas NaOCN induced motor deficits. No deficits were observed in non-human primates. Under normal diet, the CDC were up to ~80X faster in the nervous system (14 milliseconds to produce one μmol of thiocyanate from the detoxification of cyanide) relative to plasma. Spinal cord CDC was impaired by NaCN, NaOCN, or SAA deficiency. In *macaca fascicularis*, plasma CDC changed proportionally to total proteins ($r=0.43$; $p<0.001$). The plasma CDC was ~2X relative to that of rodents. The nervous system susceptibility to cyanide may result from a “multiple hit” by the toxicity of cyanide or its cyanate metabolite, the influences of dietary deficiencies, and the tissue variations in CDC. Chronic dietary reliance on cassava may cause metabolic derangement including poor CDC.

Keywords

cassava; cyanate; cyanide; konzo; neurotoxicity; rhodanese

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1. Introduction

Cyanide has remained of interest to toxicologists for its highly acute toxic (lethal) potential and ability to induce rapid and mass casualties (Borron and Baud, 2012, Greenfield et al., 2002, Jackson et al., 2014), its widespread industrial use (Amizet et al., 2011) and, reportedly, a role in a host of neurodegenerative diseases (Cliff et al., 2011, Dawson et al., 1995, Di Filippo et al., 2008, Howlett et al., 1990, Mills et al., 1999, Tshala-Katumbay et al., 2002, Tyllleskar et al., 1995). Exposure to cyanide may occur through smoke inhalation, illicit use, or diet (Jackson et al., 2014). Neurodegenerative conditions associated with the ingestion of cyanogenic cassava include the motor system disease known as konzo in central, eastern, and southern Africa, tropical ataxic neuropathy in western Africa and Asia (Banea et al., 1992, Banea-Mayambu et al., 1997, Madhusudanan et al., 2008, Mlingi et al., 1991, Oluwole et al., 2000, Tyllleskar et al., 1994); and a form of cerebellar-parkinsonism-dementia syndrome in Nigeria (Osuntokun, 1973). Other rare diseases including the amyotrophic lateral sclerosis, however, have been associated with errors in the metabolism of cyanide but through mechanisms that are still unclear (Kato et al., 1985, Wilson, 1983, Wilson et al., 1971).

The acute toxicity of cyanide is mostly due to its ability to inhibit the complex IV (cytochrome C oxidase) of the mitochondria electron transport chain thus, shutting down the production of cellular energy (Cooper and Brown, 2008, Isom et al., 1975, Isom and Way, 1974). However, the mechanisms of cyanide-associated neurodegeneration have remained unclear and factors that determine the susceptibility to cyanide poisoning have yet to be elucidated. Current knowledge suggests that cyanide detoxification pathways utilize the enzymes rhodanese (thiosulfate sulfurtransferase, TST; EC: 2.8.1.1), and mercaptopyruvate sulfurtransferase (MPST, EC 2.8.1.2) in metabolic conversions of cyanide. The TST- or MPST-mediated pathways use sulfur to convert cyanide into the reportedly less toxic thiocyanate (Aminlari et al., 1989, Cipollone et al., 2007, Kassa et al., 2011, Nagahara et al., 1995). Cyanide detoxification capabilities (CDC) appear to be dependent on the bioavailability of sulfur donors including the sulfur amino acids (SAA) cysteine and methionine suggesting that SAA- or protein-deficient diet may increase the individual susceptibility to cassava-associated neurodegeneration (Banea-Mayambu et al., 1997, Kassa et al., 2011, Tor-Agbidye et al., 1999, Tyllleskar et al., 1995). SAA deficiency may occur as a result of chronic malnutrition or dietary reliance on cassava, a carbohydrate-enriched crop with very low protein content and only 1–2% of SAA (Diasolua Ngudi et al., 2002, Nassar and Sousa, 2007). Under normal conditions, the main cassava glucoside i.e. linamarin is converted to cyanohydrins and hydrogen cyanide, which in turn is metabolized to thiocyanate (*vide supra*). However, under SAA-deficiency, oxidative pathways are favored and there is an increase in the production of cyanate, a well-known protein carbamoylating agent with neurotoxic properties (Boivin et al., 2013, Diasolua Ngudi et al., 2002, Kimani et al., 2013a, Llorens et al., 2011, Sreeja et al., 2003). Despite this current state of knowledge, the susceptibility factors and mechanisms underlying the toxicity of cyanogenic cassava have remained poorly understood partly due to the lack of an experimental model. In this study, we sought to elucidate the impact of a protein (SAA)-restricted diet, or cyanide, or cyanate on the CDC in rodents and/or non-human primates (*macaca fascicularis*). We found cross-species and tissue variations in mammalian CDC, which may be impaired by dietary deficiencies and cyanide or cyanate toxicity.

2. Material and methods

2.1. Rodent studies

2.1.1. Chemicals—Sodium cyanide (NaCN CAS No. 143-33-9, 97.2% purity) and cyanate (NaOCN CAS No. 917-61-3, 96% purity) were bought from Sigma-Aldrich (St.

Louis, MO) and stored at room temperature. All other laboratory reagents were of analytical or molecular biology grades.

2.1.2. Animals—Young adult male heterozygous rats (Crl: NIH-Fox1 rnu/Fox 1+, 6–8 weeks old) (N= 52), weighing 140–210 g upon arrival were donated by Professor Neuwelt, Department of Neurology, Oregon Health & Science University (OHSU). These rats are known to have a normal phenotype (Charles River technical data sheet 2009). Animals were caged in an animal room maintained on a 12/12-h light dark cycle in the Oregon Institute of Occupational Health Sciences for the experimental studies. Food and water were given *ad libitum*. Experimental protocols were approved by the OHSU Institutional Animal Care and Use Committee (IACUC).

2.1.3. Diet and dosing regimens

2.1.3.1. Diet: Custom-synthesized isonitrogenous rodent diet with either all amino acids (AAA-diet; code TD09460) or lacking 75% of the SAA-content relative to the control diet (SAA-deficient diet; code TD09463) were purchased from Harlan (Madison, WI) and stored at 4°C until use. Previous experimental studies showed animals fed with SAA-free chow had dramatic weight loss and muscle weakness (Tor-Agbidye et al., 1999). We chose a 75%-deficient but not free SAA to allow for animal survival and cyanide detoxification.

2.1.3.2. Dosing regimens: Rats were first acclimated for a 5-day period on a diet consisting of 4:1 portions of normal rodent chow (PMI Nutrition International, NJ) and either AAA-diet (N = 28, group 1) or SAA-deficient diet (N = 24, group 2). On the 6th day, animals were assigned to experimental groups (N = 7–10/group) and treated intraperitoneally (one injection per day) for up to 6 weeks (till first occurrence of physical signs notably motor deficits) as follows: (1) AAA-diet, 2.5 mg/kg body weight (bw) NaCN; (2) AAA-diet, 50 mg/kg bw NaOCN; (3) AAA-diet, equivalent amount of vehicle (1 µl/g bw saline); (4) SAA-deficient diet, 2.5 mg/kg bw NaCN; (5) SAA-deficient diet, 50 mg/kg bw NaOCN; (6) SAA-deficient diet, equivalent amount of vehicle (1 µl/g bw saline). Dose selection for cyanide was informed by findings that indicate the existence of sublethal and/or lethal cyanide poisoning in konzo-affected areas (Banea-Mayambu et al., 1997). Cyanate was given at doses similar to those known to induce neuropathy in humans (Ohnishi et al., 1975). The rats were weighed daily to assess changes in body weight and adjust the dose of the test articles accordingly.

2.2. Primate studies

2.2.1. Animals—Adult male *macaca fascicularis* monkeys (N = 12, mean aged 12.7 years) were used and housed at the Haman Ranch (The Mannheimer Foundation, Inc., LaBelle, FL). Animal protocols were approved by the Mannheimer Foundation's Animal Care and Use Committee (IACUC) (The Mannheimer Foundation, Inc., LaBelle, FL).

2.2.3. Diet—Animals were randomly assigned to two dietary conditions (N = 6/diet), namely, control monkey diet (Teklad 2050, Teklad Diets, Madison, WI) or cassava (*Manihot dulcis*). Cassava was purchased from Sysco Foods (Ft. Myers, FL, USA) in frozen form and maintained at –10 °C till use. Animals under cassava diet first received a transition diet for a week (starting with 100% control diet for 2–3 days and then, control/cassava (1/1) diet for 2–3 days). Thereafter, they were fed the control/cassava diet in a 1/3 ratio for 2–3 days and, finally, 100% cassava; or the control diet, for 5 weeks. Animals were fed fresh daily thawed cassava and/or regular diet under the supervision of an experienced veterinarian to provide 70 kcal/kg of body weight per day while water was given *ad libitum*. Animals were weighed weekly to assess changes in body weight.

2.3. Specific protocols

2.3.1. Animal observations—Rodents were examined daily for physical signs, including tremors and the hind limb extension reflex, which is elicited when the animal is gently raised by the tail. Motor functions were assessed by animal performance on an accelerating rotating rod. Animals were individually placed on rotating rods in a software-driven rotarod apparatus (AccuScan Instruments, Inc., Columbus, OH) set in an accelerating mode. The rotation speed was gradually increased from 5 to 25 r.p.m. The apparatus had an automatic system for fall detection *via* photobeams. Animals were tested on alternate days and each session consisted of two consecutive trials of 90s. The latency to fall was recorded and compared across treatment-groups. Rotarod testing was carried out only in animals maintained on SAA diet to determine whether the dietary restrictions had an impact on the motor performance in rats treated with NaCN.

For the non-human primate studies, animals were first moved to single housing units. Physical examination and biological samples were collected (blood, feces, and food) for baseline evaluations. Thereafter, physical examination and sample collection were conducted weekly, on the same day, for 5 weeks. Blood was collected for complete blood count (CBC); liver function test (AST (aspartate transaminase), ALT (alanine transferase), ALP (alkaline phosphatase, GGTP (gamma glutamyl transpeptidase), total bilirubin), trypsin, as well as, plasma protein (total protein, globulin, and albumin); kidney function test (BUN (blood urea nitrogen), creatinine, BUN:Creatinine ratio); metabolic profiles (glucose, cholesterol and triglycerides); pancreatic function test (amylase, lipase); CPK (creatinine phosphokinase), as well as electrolytes (phosphate, Ca^{2+} , Mg^{2+} , Na^+ , K^+ , Cl^-). Animals were monitored daily for attitude, alertness, responsiveness, appetite, food consumption, and clinical signs for abnormal gait and deficits in arm and hand coordination.

2.3.2. Tissue preparation for the measurement of cyanide detoxification rates

—In the rodent studies, plasma samples were collected on the last day of the experimentation. Rats were deeply anesthetized with 4% isoflurane (1 liter oxygen/min), and the blood (1.5–3.5 ml/rat) collected via cardiac puncture in vacutainer tubes with anticoagulant and kept overnight at 4°C. Thereafter, the plasma samples were centrifuged at 15,000 rpm for 15 min at 4°C. The samples were then aliquoted in cryotubes and stored at –80°C until later for protein assay using the Pierce BCA (bicinchoninic acid) protein assay kit (Thermo Scientific, Rockford, IL, USA). Spinal cord and brain tissues were sonicated in ice-cold 25mM potassium phosphate buffer (pH 8.6) for 15 seconds repeated 3 times at 1.75 voltage. The mixture was centrifuged at 12000x g for 45 min at 4°C and the supernatant was assayed into a new tube to form the soluble protein extract. In the non-human primate study, animals were chemically restrained with intramuscular (IM) ketamine HCl (Ketaved®, Saint Joseph, MO) dosed at 10 mg/kg bw. About 3 ml of blood was obtained from the femoral vein by venipuncture using heparinized Vacutainer® tubes and blood collection sets (Franklin Lakes, NJ). Heparinized blood was centrifuged at 1000 RCF for 15 minutes. Thereafter, plasma was separated and stored at –80° C for subsequent biochemical assays.

2.3.4. Assay for cyanide detoxification rates—Cyanide detoxification rates were assessed using the rhodanese assay developed by Sörbo (Sorbo, 1953). Briefly, a solution of 200mM potassium phosphate buffer (pH 8.6) was mixed with 125 mM sodium thiosulfate and 250mM potassium cyanide. A 20μl of sample to be assayed was added to the solution, mixed, and incubated at 37°C for 20 minutes. The reaction was then stopped by addition of 37% formaldehyde. The end product of the reaction was thiocyanate, which formed a red precipitate on mixing with an equal volume of 410 mM ferric nitrate solution. A standard curve was made from 40mM potassium thiocyanate in deionized water and diluted accordingly. A 200μl of the blank, standard dilutions, plasma, spinal cord and brain samples

were assayed in duplicates. The absorbance of the final solution was read at or near 460 nm on a plate reader using the Epoch multivolume spectrophotometer system equipped with a Biotek Gen 5 data analysis software (Biotek instruments, Inc, USA). The concentration of thiocyanate in each sample was calculated from a calibration curve (standard) of potassium thiocyanate solution (0.05–40 mM). The enzyme activity was expressed in μmole of thiocyanate formed per minute per mg of protein at pH 8.6 and 37°C. This was later expressed as the number of milliseconds required to produce one μmol of thiocyanate per mg of protein (ms/ $\mu\text{mol}/\text{mg}$) in the tested sample.

2.4. Statistical analysis

Changes in body weight across the three treatments and two diets were analyzed in a two-stage manner. In the first stage, simple linear regression was used to estimate an intercept and slope coefficient separately for each animal based on the collection of daily weight measurements collected over the course of the study. Thus, the collection of repeated measures per animal was summarized in terms of each animal's initial bodyweight (intercept) and each animal's change in weight per day (slope). In the second stage, the intercept and slope coefficients were analyzed by ANOVA to determine whether the parameter of interest (intercept or slope) was systematically affected by treatment or diet (or the interaction between the two factors). Rotorod performance was analyzed by dichotomizing performance according to whether the animal remained on the rod for at least 60 seconds. Since each animal contributed exactly two trials, each dichotomized as described above, the final outcome (y) was the number of instances, between the two trials, that the animal lasted for at least 60 seconds ($y=\{0,1,2\}$). The collection of counted proportions over the study period was analyzed using generalized estimating equations. A secondary analysis of rotorod performance on the termination day used survival analysis to examine time until the animal fell off the rod. A regression analysis was used to determine whether rat CDC were affected by diet, treatment, or their interaction. In non-human primates, total proteins (mg/ml) and CDC were fitted using a mixed-effect model, with treatment and week as the fixed factors and subject (animal) as the random effect. All statistical analyses we conducted at the significance level of $p < 0.05$.

3. Results

3.1. Body weight changes

Initial body weight (day 1) of rodents did not significantly differ across the three treatment groups for either diet ($p=0.47$, AAA Diet; $p=0.19$, SAA Diet), although initial weight was, on average, 52.5 (95% CI: 37.3–67.7) grams lower for those animals in AAA Diet compared to SAA Diet. Differences across the three treatment groups with respect to daily changes in body weight was influenced by type of diet [$F(2,46)=3.42$, $p=0.001$; test of treatment:diet interaction]; consequently, the effect of treatment was summarized separately within each type of diet. Overall, animals placed on SAA Diet gained less body weight relative to those on the AAA diet, regardless of treatment (Table 1). Follow-up tests revealed that within the AAA Diet, animals treated with NaOCN gained weight at a significantly reduced rate relative to rates of weight gained by animals in both the NS and NaCN groups, while animals in the NS and NaCN groups gained weight at essentially the same rate (Figures 1a). However, within SAA Diet, the average daily change in body weight did not significantly differ among the three treatments [$F(2,46)=0.39$, $p=0.68$] (Figures 1b).

Non-human primates appeared to have similar weight change patterns across the two diets i.e. cassava versus control primate chow. However, their body weights significantly ($p < 0.05$) decreased over time, regardless of the type of diet. A marginal difference was observed between animals under the cassava-diet, which had lost more weight, and those

under the primate chow ($p=0.06$). Relative to the initial body weight, animals under the cassava diet lost more weight (8.22%) compared to those under the primate chow (1.63%) (Figure 2).

3.2. Physical and behavioral changes

Seizures were observed in rats treated with NaCN and maintained on the SAA-restricted diet. The events seizures occurred after the first week of the study specifically on days 8 (2 rats), 9 (3 rats), 10 (2 rats), 12 (4 rats), 13 (5 rats), 17 (4 rats), 19 (3 rats) and 1 animal per day for days 26, 27, 31, and 32, respectively. All these animals developed seizures within 45 minutes following the injection with NaCN. No Seizures were observed in animals treated with NaCN, NaOCN or vehicle under AAA diet, or NaOCN or vehicle under SAA-restricted diet.

Animals treated with NaOCN/SAA-restricted diet developed motor deficits relative to their NaCN and vehicle-treated counterparts. Odds of remaining on the rotorod for at least 60 seconds (1 minute; a convenient and interpretable choice) did not significantly change over time for NS- and NaCN-treated rats, with the odds decreasing by 4% for each additional day of testing ($p \sim 0.10$ for each group). By contrast, the odds of remaining on the rod decreased 14% (95% CI: 6–21% decrease) per day of testing ($p<0.001$) for animals on NaOCN. A secondary analysis examined motor performance at study termination. An overall (log-rank) test showed the time needed to fall off the rotorod significantly differed across the three groups ($p=0.040$), but the same comparison restricted to NaCN and NS animals showed no difference ($p=0.16$; log-rank test), suggesting the difference in performance is limited to NaOCN. A description of how the odds of remaining on the rod changed over time (per each additional day of treatment) is shown in Table 2 and Figure 3. Regardless of the type of the diet, *macaca fascicularis* remained alert and responsive. No abnormality in gait, arms or hand coordination, were observed during the course of the study. One animal developed a metabolic derangement as a result of hyperglycemia, which required treatment with insulin.

3.3. Total plasma proteins

Neither the diet nor the treatment had a significant effect on total plasma proteins in rodents. In non-human primates, there was no indication that differences between diet changed over the six weeks [$F(5,50)=1.57$, $p=0.18$; test of diet:week interaction]. Consequently, the effects of diet and time were summarized as separate main effects. Average protein level didn't significantly differ between the two diets [$t(10)=0.90$, $p=0.39$]. There were significant changes over time, however [$F(5,55)=9.50$, $p<0.001$], relative to week 1, with a significant decreased in proteins during the last two weeks (Table 3).

3.4. Tissue variations in rodents cyanide detoxification rates

We observed a differential pattern of CDC across tissues. The highest (faster) detoxification rate was observed in brain, followed by the spinal cord, and lastly, plasma. Overall, the time required to produce one μmol of thiocyanate per mg protein was $\sim 80\times$ more in plasma relative to brain and spinal cord regardless of the diet (Table 4). Plasma rates were not influenced by diet or treatment (Figure 4a).

With regard to rodent nervous system, CDC was influenced by diet or chemical treatment, in the spinal cord, but only by chemical treatment in the brain. We found a significant effect of diet-treatment interaction on the detoxification activity in the spinal cord ($\chi^2=13.2$; $df=2$; $p<0.001$). The detoxification rates in the NaCN group were slower ($p<0.001$) under SAA-restricted diet relative to the normal AAA diet (up to 60.15 ms vs. 15.37 ms, respectively). Within the SAA-fed group, the detoxification rates in the NaCN-treated animals were slower compared to those in animals treated with saline ($p<0.001$) or NaOCN ($p<0.05$),

respectively. Within the AAA normal diet, however, the time required to produce one μmol of thiocyanate was not significantly different for either NaCN relative to saline ($p=0.74$) or between NaOCN compared to saline ($p=0.065$). The rates were, however, marginally slower for NaOCN compared to NaCN ($p=0.048$) (Figure 4b).

The CDC of rodent brain was significantly ($p<0.05$) influenced by chemical treatment only. We found no effect of diet, regardless of treatment ($p=0.50$). With regards to chemical treatment, rates averaged 5.4 (95% CI: 0.4–10.4) ms slower in animals treated NaCN relative to saline ($p<0.05$) or 6.7 (95% CI: 2.2–11.2) ms slower in NaOCN treated rats compared to saline ($p<0.05$) regardless of the diet (Figure 4c). The two NaCN and NaOCN-induced changes were not significantly different from each other ($p=0.64$).

3.5. Plasma cyanide detoxification rates in *macaca fascicularis*

There was no indication that differences between diets changed over the study period [$F(5,50)=0.39$, $p=0.85$; test of diet:week interaction]. Consequently, the effects of diet and time were summarized as separate main effects. Average CDC didn't significantly differ between the two diets [$t(10)=1.68$, $p=0.12$]. There were significant changes over time, however [$F(5,55)=6.84$, $p<0.001$], and those differences, relative to Week 1, are shown in Table 5. We found a positive correlation between changes in total proteins and CDC in both the control (Spearman $r=0.35$, $p=0.04$) and cassava (Spearman $r=0.36$, $p=0.03$) groups (overall Spearman $r=0.43$, $p<0.01$).

3.6. Metabolic panel in *macaca fascicularis*

The metabolic panel (CBC, CPK, electrolytes, liver, kidney, and pancreas functions) appeared normal and was not influenced by the type of diet except for an increase in blood glucose in one of the animal, and changes in BUN and BUN/creatinine ratio over time. A significant decrease in BUN and BUN/creatinine ratio (Figure 5), with a significant increase in creatinine levels, were observed in animals fed cassava relative to those under the normal diet ($p<0.05$).

4. Discussion

We report cross-species and tissue variations in CDC of rodents and *macaca fascicularis* maintained on SAA- or cassava protein-restricted diets, respectively. In rodents, we found a differential pattern of CDC across tissues with higher detoxification rates in the brain followed by the spinal cord and, lastly, plasma. *Macaca fascicularis* display plasma CDC ~ twice faster than those measured in rodents. These findings are consistent with previous studies that showed that CDC may vary across species, tissues, and cellular compartments within the same tissue (Al-Qarawi et al., 2001, Aminlari et al., 1989, Cipollone et al., 2007, Drawbaugh and Marrs, 1987, Mimori et al., 1984, Sylvester and Sander, 1990). Higher CDC in the nervous system tissues of rodents, i.e. ~ 80X faster (14 milliseconds to produce one μmol of thiocyanate from the detoxification of cyanide) relative to plasma, may reflect an ontogenetic adaptation of organs that prominently rely on aerobic energy metabolism (Cooper and Brown, 2008, Isom et al., 1982, Isom and Way, 1974).

The nervous system CDC were impaired by SAA dietary deficiency and/or chemical treatment with NaCN or NaOCN in the spinal cord, but only chemical treatment in the brain. Whether this differential pattern of susceptibility reflects the existence of a more robust sulfur- buffering system in the brain relative to the spinal cord has to be determined. The same proposal may also hold true for the plasma in which the CDC were not impaired by SAA deficiency possibly due to a compensatory effect by the existence of abundant sulfur donors such as albumin (Drawbaugh and Marrs, 1987, Wrobel et al., 2004). The lack of

chemical impact on plasma CDC may be explained by the fact that plasma CDC mostly depends, conceivably, on the bioavailability of sulfane sulfur whereas in the brain, the CDC also depends on the activities of sulfurtransferases; of which the enzyme activities may be inhibited upon oxidative damage and/or protein carbamoylation associated with treatment with NaCN and/or NaOCN, respectively (Kassa et al., 2011, Kraus and Kraus, 2001, Nagahara, 2013). Changes in primate plasma CDC in proportion to total plasma proteins indicate that total pool of sulfur donors may be critical for the detoxification capabilities of cyanide in plasma.

Physical signs associated with the toxicity of NaCN or NaOCN included seizures in the SAA-deficient group, poor gain in body weight in the SAA-deficient or NaOCN-treated animals, and motor deficits in animals treated with NaOCN and fed the SAA-deficient diet. *Macaca fascicularis* also tend to lose weight while exclusively fed cassava. While comparisons across dietary groups require caution (different experimental groups and species), our results do suggest that dietary deficiency in SAA and/or proteins may be accompanied by growth retardation. This finding is consistent with previous studies that showed dramatic weight loss in rodents fed SAA-deficient diet or evidence of stunting in children who rely on cassava as staple food (Banea-Mayambu et al., 2000, Stephenson et al., 2010, Tor-Agbidye et al., 1998). Poor gain in body weight in animals treated with NaOCN is consistent with previous findings (Alter et al., 1974, Kimani et al., 2013b). Seizures were observed only in rodents fed SAA and treated with NaCN, and mostly after the first week of the experimentation suggesting that SAA dietary deficiency may modulate the acute toxicity of cyanide. This proposal, however, may not hold true in light of differences in body weight and possibly age and/or nervous system maturation levels between the two experimental groups of rodents. However, it is worth pointing out that cyanide may also induce seizures through its 2-imino-4-thiazolidine-carboxylic acid metabolite (Spencer, 1999). Poor rotorod performance of NaOCN-treated animals may be attributed to the well-known motor system toxicity of NaOCN (Ohnishi et al., 1975, Tellez et al., 1979, Tellez-Nagel et al., 1977). *Macaca fascicularis* showed no physical abnormalities with the exception of a metabolic derangement and hyperglycemia observed in one animal. This animal had normal pancreatic function throughout the study period. Such isolated finding may also not be related to the exclusive dietary intake of the rich-carbohydrate cassava (Sandhyamani et al., 1999; Yessoufou et al., 2006). Absence of acute toxicity may be attributed to the presumably low cyanogenic content of the sweet cassava (*manihot dulcis*) (Sarkiyayi and Agar, 2010). The time-dependent decrease in BUN, BUN/Cr ratio along with the increase in creatinine in *Macaca fascicularis* fed cassava may be explained by a metabolic adjustment to a protein-restricted (cassava) diet (Kumar et al., 1972). These changes were reminiscent of cyanide metabolism in humans (Mukherjee et al., 2009) suggesting that feeding and toxicological studies using *macaca fascicularis* may help modeling the biochemical changes associated with cassava cyanogenesis and neurotoxicity.

In sum, we have shown that mammals CDC may vary across species and tissues. Both NaCN and NaOCN toxicities, and SAA dietary restrictions, may exert inhibitory effects on CDC maintaining a vicious cycle of increased susceptibility of the nervous system to NaCN and/or NaOCN. This level of complex interactions and tissue variations of CDC may dictate the phenotypic expression and molecular signatures of cyanide-associated neurological diseases.

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Abbreviations

AAA	all amino acid diet
ALP	alkaline phosphatase
ALT	alanine transferase
AST	aspartate transaminase
BCA	bicinchoninic acid
BUN	blood urea nitrogen
CBC	complete blood count
CDC	cyanide detoxification capabilities
CPK	creatinine phosphokinase
Cr	creatinine
GGTP	gamma glutamyl transpeptidase
NaCN	sodium cyanide
NaOCN	sodium cyanate
SAA	sulfur amino acid deficient diet
MPST	mercaptopyruvate sulfurtransferase
TST	thiosulfate sulfurtransferase

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Research Highlights

- Mammals display a differential pattern of cyanide detoxification capabilities (CDC) across species and tissues.
- Plasma CDC decreases in proportion to total plasma proteins in non-human primates.
- Nervous system CDC may be impaired by dietary deficiency in sulfur amino acids and/or exposure to cyanide or cyanate.
- Susceptibility to cyanide poisoning may be modified by diet and endogenous tissue variations in CDC.

Figure 1a. **Figure 1b.** **Figure 1.**

Figure 1a. Rats treated with NaOCN, gained weight slowly compared to control or their NaCN counterparts ($p < 0.05$). Those treated with NaCN and control gained weight almost at the same rate.

Figure 1b. All animals of the SAA group gained weight essentially at the same rate regardless of treatment.

Figure 2.
Animals fed on cassava tend to lose more weight compared to the group on the normal chow, which appeared to have animals maintaining a stable weight over time

Figure 3.

The fall time from the rotating rod significantly ($p < 0.001$) decreased among rats treated with NaOCN compared to control and NaCN overtime. The fall time was also significantly ($p = 0.040$) shorter on the termination day in rats treated with NaOCN relative to control and NaCN group.

Figure 4a.

Figure 4b.

Figure 4c.

Figure 4.

Figure 4a. Rodent plasma cyanide detoxification rates (mean $\pm$ S.E.M ms/[μ mol/mg] time to produce one μ mol thiocyanate) were not influenced neither by the diet nor the treatment.

Figure 4b. Rodent cyanide detoxification rates (mean $\pm$ S.E.M ms/[μ mol/mg] time to produce one μ mol thiocyanate) in spinal cord were influenced by diet or treatment. Animals treated with NaCN and fed with SAA-deficient diet needed longer time (** $p < 0.001$) relative to their counterparts under normal AAA diet, or those on SAA diet but under saline or NaOCN. Under the AAA diet, animals treated with NaOCN (*) required more detoxification time relative to those on NaCN.

Figure 4c. Rodent cyanide detoxification rate (mean $\pm$ S.E.M ms/[μ mol/mg] time to produce one μ mol thiocyanate) in the brain was significantly ($p < 0.05$) affected by treatment. Rates were ~ 5.4 and 7.4 ms slower ($p < 0.05$) in rats treated with NaCN or NaOCN relative to their respective controls, regardless of the diet.

Figure 5.

Blood urea nitrogen (BUN) (mean $\pm$ S.E.M mg/dl) and BUN/Creatinine (CR) levels in non-human primates fed on cassava were significantly ($p < 0.05$) lower compared to control group. Additionally, these parameters significantly ($p < 0.05$) decreased over time in animals fed on cassava relative to those on the control diet.

Daily weight (g) changes in rodents treated with normal saline, NaCN and NaOCN under AAA or SAA -restricted diets

Table 1

Rodents fed on SAA diet gained weight at a slower rate compare to those under normal AAA diet. Among those on normal AAA diet, animals treated with NaOCN slowly gained weight in comparison to those on saline or NaCN (aptryx) (P<0.05).

Diet	Treatment	Weight change per day		
		Range	Median (IQR)	Mean SD
AAA	Saline (n=9)	2.90–4.17	3.73 (3.22–3.94)	3.59 (0.46)
AAA	NaCN (n=10)	2.84–4.53	3.32 (3.09–3.57)	3.45 (0.54)
AAA	NaOCN (n=9)	1.67–3.27	2.23* (2.01–2.60)	2.29 * (0.49)
SAA	Vehicle (n =7)	0.23–1.71	0.84 (0.53–1.21)	0.89 (0.53)
SAA	NaCN (n=9)	0.48–1.59	0.88 (0.72–1.40)	1.00 (0.41)
SAA	NaOCN (n=8)	0.32–1.19	0.81 (0.68–1.04)	0.80 (0.31)

Table 2
Changes in estimated odds of remaining on rotorod for at least 60 seconds for each additional day (SAA diet, separated by treatment)

The odd of remaining on the rod significantly decreased over time in animals treated with NaOCN ($p=0.001$). The differences were significant when comparing with saline (†) or NaCN (‡). No difference was seen between saline and NaCN-treated groups ($*$).

Treatment	OR (95% CI)	P value for trends	P value for comparisons
Saline (n =7)	0.96 (0.96–1.01)	0.099	0.87*
NaCN (n=9)	0.96 (0.92–1.01)	0.096	0.046 †
NaOCN (n=8)	0.86 (0.79–0.94)	0.001	0.029 ‡

Table 3
Plasma proteins (mg/ml) in non-human primates across diets and weeks

Plasma proteins (mg/ml) in non human primates markedly ($p < 0.05$) declined during the last two weeks (5 & 6) of the study period regardless of the diet.

	Diet		
Week	Control (S.E.M)	Cassava (S.E.M)	Differences (95% CI) relative to Week 1; p value
1	69.05 (1.98)	71.94 (2.26)	None
2	68.64 (2.40)	71.53 (2.74)	-0.42 (-2.54-1.70); $p=0.70$
3	69.89 (1.86)	72.78 (2.79)	0.83 (-1.29-2.96); $p=0.44$
4	72.14 (2.80)	75.03 (3.08)	3.08 (0.96-5.21); $p=0.005$
5	65.81 (1.74)	68.69 (2.78)	-3.25 (- 5.38—1.12); $p=0.003$
6	66.47 (2.29)	69.36 (2.32)	- 2.58 (- 4.71— 0.46); $p=0.02$

Table 4
Cyanide detoxification time (mean $\pm$ S.E.M ms/[μ mol/mg] to produce one μ mol thiocyanate) in plasma and nervous system tissues of rodents

Cyanide detoxification time required to produce one μ mol thiocyanate in brain or spinal cord were $\sim 80X$ lower than that required for the same process in rodent plasma i.e. brain and spinal cord detoxify $\sim 80X$ faster than plasma.

		Diet	
Tissue	Treatment	AAA (S.E.M)	SAA (S.E.M)
Brain	NS	13.97 (1.35)	12.65 (1.98)
	NaCN	19.38 (2.72)	18.05 (1.94)
	NaOCN	20.68 (2.35)	19.35 (1.79)
Spinal cord	NS	18.95 (6.77)	17.63 (5.57)
	NaCN	15.37 (8.30)	60.15 (7.89)
	NaOCN	35.54 (5.89)	28.25 (9.71)
Plasma	NS	1078.51 (125)	1117.92 (38)
	NaCN	1134.89 (26)	1180.89 (55)
	NaOCN	1130.69 (79)	968.14 (46)

Table 5
Cyanide detoxification time (mean $\pm$ S.E.M ms/[μ mol/mg] to produce one μ mol thiocyanate) in plasma of non-human primates across diets and weeks

Cyanide detoxification time required to produce one μ mol thiocyanate in plasma of non human primates inconsistently changed over time. However, time significantly increased during the last two weeks (5 & 6) of the study period regardless of the diet.

	Diet		
Week	Control (S.E.M)	Cassava (S.E.M)	P value (comparisons relative to Week 1)
1	563.54 (7.45)	629.66 (4.40)	-
2	459.27 (10.80)	491.89 (8.49)	0.002
3	553.35 (6.37)	689.89 (9.29)	p=0.69
4	573.92 (6.77)	584.01(7.11)	p=0.73
5	688.38 (9.82)	701.26 (5.41)	p=0.08
6	669.34 (6.63)	736.11 (7.26)	p=0.06