Food and Chemical Toxicology 66 (2014) 203–209

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

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

S. Kimani [a,c], V. Moterroso [b], P. Morales [d], J. Wagner [d], S. Kipruto [c], F. Bukachi [e], C. Maitai [c], D. Tshala-Katumbay [f,g,*]

[a] School of Nursing Sciences, University of Nairobi, Kenya
[b] Department of Comparative Medicine, Oregon Health & Science University (OHSU), USA
[c] Department of Pharmacology and Pharmacognosy, University of Nairobi, Kenya
[d] Mannheimer Foundation, Inc., Homestead, FL, USA
[e] Department of Medical Physiology, University of Nairobi, Kenya
[f] Oregon Institute of Occupational Health Sciences, OHSU, USA
[g] Department of Neurology, OHSU, USA

ARTICLE INFO

Article history:
Received 9 June 2013
Accepted 23 January 2014
Available online 2 February 2014

Keywords:
Cassava
Cyanate
Cyanide
Konzo
Neurotoxicity
Rhodanese

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 ~80× faster in the nervous system (14 ms 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 *M. fascicularis*, plasma CDC changed proportionally to total proteins ($r = 0.43$; $p < 0.001$). The plasma CDC was ~2× 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.

© 2014 Elsevier Ltd. All rights reserved.

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; Tylleskar 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; Tylleskar 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

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.

* Corresponding author at: Oregon Institute of Occupational Health Sciences & Department of Neurology, Oregon Health & Science University, 3181 Sam Jackson Park Road, Mail Code L606, Portland, OR 97239, USA. Tel.: +1 503 494 0999; fax: +1 503 494 6831.

E-mail address: tshalad@ohsu.edu (D. Tshala-Katumbay).

http://dx.doi.org/10.1016/j.fct.2014.01.047
0278-6915/© 2014 Elsevier Ltd. All rights reserved.

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) cystein 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; Tylleskar et al., 1995). SAA deficiency may occur 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 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. Materials 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 *M. 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).

2.2.2. 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, cholesterols 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% isofluorane (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 25 mM potassium phosphate buffer (pH 8.6) for 15 s repeated 3 times at 1.75 V. The mixture was centrifuged at 12000×*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 min. Thereafter, plasma was separated and stored at −80 °C for subsequent biochemical assays.

Download English Version:

https://daneshyari.com/en/article/5850745

Download Persian Version:

https://daneshyari.com/article/5850745

Daneshyari.com