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In Vivo Metabolism in Rats of Cyanide -Containing Compounds From Cassava Leaves

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Abstract
Qualitative analysis of ethanol extracts of cassava by double dimensional thin-layer chromatography indicated the presence of novel cyanide-containing compounds, which were shown chemically to be cyanohydrin *(Rf,* 0.25) ands nitrile *(Rf,* 0.645). Administration of the eluates of those chromatographically identified CN- entities to rats by stomach intubation resulted in statically significant differences ($p < 0.01$) in blood and urine thiocyanate (SCN-) and cyanide (CN). The increased SCN-fonnation in blood and urine provided definite evidence that the cyanogens were metabolized either partially or wholly. This was further confined by non -detection of the novel cyanide compounds when two dimensional t.l.c examination of rat urine was conducted in ethylacetate: methanol: diethylether (8:.1: 1. v/v). The toxicological implication of these findings are discussed.

Keywords: novel cyanide-compounds, cassava leaves, metabolism, rats.

Introduction

Cyanogenic glycosides are present in cassava roots and leaves, which are important food sources in the tropics, funning the staple diet of hundreds of millions of people in Africa, Asia and Latin America (De Bruijn and Fresco, 1980). Hydrolysis of cyanogenic glycosides during processing of cassava plant material leads to the release of hydrogen cyanide (HCN), which is a deadly poison (Conn, 1969), and it has been generally believed that cassava toxicity is due mainly to this hydrocyanic acid. Cassava cyanide has been implicated in health problems like acute toxic effects (Mlingi et al; 1992), tropical ataxic neuropathy (Osuntokun, 1981), iodine deficiency disorder (Ermans, et al. 1983) and paralytic "Kouzo" (Tylleskar, et al. 1992). There is general agreement that a causal relationship exists between dietary cyanide exposure from cassava and acute poisoning and aggravation of goiter. But

the knowledge about the dose and degrees of susceptibility at which cyanide exposure from cassava will induce or aggravate these diseases is still uncertain (Rosling, 1994.). In this context, to study whether the cyanogens content in cassava products causes a certain disease in humans or animals, it is necessary fur the relevant forms of cyanogens (i.e, the forms in which cyanide exists) in food to be known and measured.

Following this, recent investigation from our laboratory has indicated the presence of chromatographically detectable novel cyanide-containing compounds other than the known glycosides and hydrocyanic acid from ethanolic extract of cassava leaves (Okafor and Maduagwu 1999). Chemical identities of these cyanide compounds from cassava leaves showed one of them with *Rf* value 0.645 to be nitrile while the other with *Rf* 0.25 is a cyanohydrin.

The metabolism of these novel cyanide-containing compounds need to be studied in order to establish the total toxicity caused by cassava cyanide. The appearance and disappearance of such cyanide derivatives in urine and blood when ingested could serve as metabolic indicators. This forms the basis of this present study.

Materials and Methods

Animals

Twenty five (25) male albino Wistar weighing 110g on the average and breed rats bred in the pre-clinical animal house of University of Ibadan were used. The animals were kept in Technoplast metabolic cages for one week for environmental acclimatization and had free access to their feed (mouse cubes from Ladokun Feeds Ltd, Ibadan) and drinking water. that contained no detectable amounts of cyanide.

Dosing of Animals

There were five groups of animals, each comprising 5 rats. Animals in group I, were dosed (by stomach intubation) with physiological saline and served as control. Animals in groups 2,3,4, and5 received varying concentrations of the chromatographically identified cyanide containing compounds having *Rf* values, 0.645 and 0.25 and designated fractions Ls and L3 respectively. Each animals in groups 2 and3 received 0.18mg and 0.12 mg HCN/CN equivalent respectively ofL5 fraction while each ones in groups 4 and 5 received L3 fraction containing 0.08 and 0.06 mg CN equivalent respectively

Collection of urine and blood samples of animals for cyanide and thiocyanate analysis

Twenty-four hours urine of both the control and test animals were collected, their volumes measured and their cyanide and thiocyanate contents determined using the methods of Cooke (1978) and Sorbo (1953) respectively. The rats were sacrificed

by decapitation and blood samples were drawn from the heart before death for SCN- and CN- analysis.

Statistical analysis: Test of significance was by Student's t-test

Results and Discussion

The administration of the eluates of these identified CN entities to rats by stomach intubation resulted in statistically significant ($P < 0.01$) increases in blood and urine SCN- and CN (Tables 1 and2). Increased thiocyanate fonnation when these cyanide entities were given provided definite evidence that the cyanogens were metabolized *in-vivo* either partially or wholly. Significant increases in blood and urine thiocyanate (SCN) fonnation and cyanide concentrations indicated attempt by the animals to detoxify these compounds. Moreover, non detection of these compounds in the animals urine when their urine samples were subjected to two dimensional t.l.c in ethylacetate: methanol: diethylether (8: I: 1 v/v) lends support to their *in-vivo* metabolism. Thus rats liver and gastrointestinal walls posses relevant enzymes and micro flora for the degradation of these novel cyanide compounds. These results are consistent with reports that the known cyanogens are metabolized in animals and man (Barrett et al; 1977 ; Maduagwu and Umoh, 1986; Frakes et al; 1985; Rosling, et al; 1992).

Table 1. Excretion of cyanide and its derivatives in 24hr urine samples of rats administered oral doses of cyanide- containing bands of thin layer chromatogram of cassava extracts.

Cyanide adjunct dose level mg/kg body wt	No. of Animals	Total cyanide (glucosidic + non-glucosidic) excreted (g)		Boundcyanide (glucosidic) excreted (g)		Free cyanide (non-glucosidic) excreted (g)		Thiocyanate excreted (g)	
		Mean	s.d	Mean	s.d	Mean	s.d	Mean	s.d
L_5 1.8	5	37.04	0.37	10.27	0.24	26.77	0.46	23.12	0.5
1.2	5	20.12	0.19	4.18	0.05	15.94	0.28	11.28	0.3
L_3 0.8	5	29.04	0.34	ND		29.04	0.34	15.32	0.7
0.6	5	13.82	0.17	ND		13.82	0.17	9.09	0.2
Control	5	ND		ND		0.74	±0.01	1.07	±0.1

ND-*Non detectable
Ls-* Leaf extract with Rf value 0.645
L3-* Leaf extract with Rf value 0.25

Table 2: Blood SCN and CN of rats given doses of detectable cyanide entities on thin-layer chromatogram of cassava leaves extract.

Cyanide adjunct dose level mg/kg body wt	No. of Animals	Total cyanide (glucosidic + non-glucosidic) excreted (g)		Boundcyanide (glucosidic) excreted (g)		Free cyanide (non-glucosidic) excreted (g)		Thiocyanate excreted (g)	
		Mean	s.d	Mean	s.d	Mean	s.d	Mean	s.d
L_5 1.8	5	12.1	0.14	5.18	0	6.95	0.17	35.2	0.37
1.2	5	8.06	0.1	3.27	0.38	4.79	0.12	21.2	0.45
L_3 0.8	5	10.2	0.38	ND		10.2	0.38	27.1	0.84
0.6	5	6.13	0.14	ND		6.13	0.14	16.3	0.34
Control	5	ND		ND		ND		2.03	±0.2

ND-*Non detectable
Ls-* Leaf extract with Rf value 0.645
L3-* Leaf extract with Rf value 0.25

The fate of these novel cyanide compounds like that of other cyanogens (Rosling 1994) will differ during digestion in the gut and metabolism in the body and so will the factors determining the proportion and location of cyanide release from these compounds. Non detection of bound cyanide in the blood and urine of the animals dosed with LJ fraction is consistent with the chemical nature of this compound (cyanohydrin). Tylleskar et al. (1992) have suggested that cyanohydrins can be a major source of dietary cyanide exposure from cassava as they withstand boiling but alkaline environment of the gut will break them down to cyanide. On the other hand bound cyanide was detected in both the blood and urine of animals dosed with L5 fraction (Tables 1 and 2). However we could not confirm this bound cyanide to be the same as the glucosidic cyanide (nitrile) in L5 fraction. The *Rf* values of these bound cyanide on thin-layer chromatogram were different. It has been shown that in animals (Okafor and Maduagwu, 1999b) and humans (Brimer and Rosling, 1993), a substantial part of linamarin (glucosidic cyanide) in cassava products will be absorbed and excreted intact in urine. The blood levels of cyanide reflect the balance between the rate of absorption and the rate of enzymatic detoxification to the main metabolite thiocyanate (SCN.) Due to a kidney threshold SCN levels reflect the load during the last few days; low loads are best measured by serum and high loads by

urine levels. One of the obvious implications of the existence of various cyanide-containing compounds in cassava and their different rates and routes of metabolism is that, these explain in part why the toxic effects of residual cyanides in cassava products are rare in humans.

Finally, these results have shown that rat liver (site of xenobiotic detoxification) and intestinal flora (Winkler, 1958) can effectively degrade these cyanide entities by a mechanism that involves hydrolysis to give HCN and therefore contributes to the total cassava cyanide toxicity .

References

Barrett, M.D; Hill, D.C; Alexender, J.C; Zintnak, A. (1977) Fate of orally dosed linamarin in rats. Can. J. Physio. Pharmacol.55: 134-135

Brimer, Land Rosling, H. (1993). Micro diffusion method with solid state detection of cyanogenic glucosides from cassava in human urine. Food chem. and Toxicology, 31; 599-603.

Conn, E.E (1969) Cyanogenic glycosides. J. Agric Fd. Chem 17:519

Cooke, R.D (1978) An enzymatic ássay for the total cyanide content of cassava (Manihot seculenta Crantz). J. Sci. Fd. Agric 29:345-352

De Bruijn G.H and Fresco, L.O (1980). The importance of cassava in the world food production. Netherland Journal of Agricultural Science, 37; 21- 34.

Ermans, A.M., Bourdoux, P. Kind heart, J; Lagasse, R; Luvivila, K; Mafuta, M; Thilly, L.B.; Delange, F (1983) Role of cassava in the etiology of endemic goiter and cretinism In cassava toxicity and thyroid. Research and public health issues. Proceedings of a workshop held in Ottawa, Canada, 31st May-2nd June (1982) .ed. Delange, F and Ahiluwalia, R. IDRC-207 e, 9-16. Ottawa Canada .

Frakes, R.A; Raghubir, P .S; Willwhite C.C (1985). Developmental toxicity of cyanogenic glycosides linamarin in the golden hamster Teratoloty .31: 241-246

Maduagwu, E.N and Umoh, I.B. (1986). Billiary excretion of linamarin in Wister rats. Biochem Pharmacol 35: 303.

Mllingi. N. Poulter, N.H. and Rosling, H (1992) An out break of acute intoxication from consumption of insufficiently processed cassava in Tanzania. Nutrition Research, 12:677-687.

Okafor, P.N; and Maduagwu, E.N. (1999). Chemical characterization of novel cyanide compounds in cassava (Manihot esculenta Crantz) other than the glycosides and hydrocyanic acid. W .A .1. BioI. Appl. Chem. 44:28-32.

Okafor , P .N .and Maduagwu, E.N. (1999b). Relationship between the ingestion

of cyanogenic glycosides and their excretion in urine. J. Natn. Sci. Foundation Sri-Lanka 27(3) : 203-208.

Osuntokun, B.O. (1981). Cassava diet, chronic cyanide intoxication and neuropathy in Nigeria Africans. World Review of Nutrition and Dietetics. 36: 141-173.

Rosling, H; Milingi, .N; Tyllesker, T and Banea, M. (1992). Causal mechanisms behind human diseases induced by cyanide exposure from cassava. Proceedings of first international scientific meeting. Cassava Biotechnology Network. Cartagenada India, Colombia. Pp366-374

Rosling H (1994). Measuring effects in humans of dietary cyanide exposure from cassava. Acta Horticulture 375: 271 -283.

Sorbo, B.H (1953) On the substrate specificity of rhodanese. Acta Chem.Scand. 7:1137-45

Tylleskar, T. Banea ,M; Bikangi, N; Cooke, R; Poulter, N; Rosling H; (1992) Cassava cyanogens and Konzo, an upper motor neuron disease found in Africa. Lancet, 339: 208 -221.

Winkler, W.O (1958) Report on methods for glucosidal HCN in lima beans. J Ass. Agric Chem. 41 :282.