ELSEVIER

Toxicology 174 (2002) 87–95

TOXICOLOGY

www.elsevier.com/locate/toxicol

Does prolonged oral exposure to cyanide promote hepatotoxicity and nephrotoxicity?☆

Altamir B. Sousa [a], Benito Soto-Blanco [a], José L. Guerra [a], Edna T. Kimura [b], Silvana L. Górniak [a,*]

[a] *Research Center for Veterinary Toxicology (CEPTOX), Department of Pathology, Faculty of Veterinary Medicine, University of São Paulo, São Paulo, Brazil*

[b] *Department of Histology and Embryology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil*

Received 22 March 2001; received in revised form 30 November 2001; accepted 27 January 2002

Abstract

Long-term exposure to cyanide and/or its main metabolite, thiocyanate, has been associated with goiter, pancreatic diabetes and several neurological disorders. However, very little is found in the literature relating the nephrotoxic and hepatotoxic effects of these substances. Thus, the objective of the present study was to verify the effects of prolonged exposure to potassium cyanide (KCN) in these organs. Forty-six male adults rats, weighing approximately 200 g at the beginning of the experiment, were distributed into five groups—four experimental and one control. Experimental groups were dosed with target doses of 0.3, 0.9, 3.0 or 9.0 mg KCN/kg per day, in the drinking water, during 15 days and the control groups received only tap water. At the end of this experiment, all rats were subjected to euthanasia and plasma samples were obtained in order to determine thiocyanate and thyroidal hormones levels and fragments of thyroid, kidney and liver were collected. Rats treated with the highest cyanide dose (9.0 mg KCN/kg per day) showed lower body weight gain. An increase in the thiocyanate levels was verified in all experimental groups. The histopathologic study revealed hydropic degeneration of the renal tubular epithelial cells in those animals, which received KCN at the dose of 3.0–9.0 mg/kg per day. This study also showed hydropic degeneration of the hepatocytes of those animals, which received KCN at a dose of 9.0 mg/kg per day, and in the thyroid gland an increase was observed in the number of reabsorption vacuoles on follicular colloid, in a dose-dependent manner, in all animals of the experimental groups. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Cassava; Cyanide; Thiocyanate; Liver; Kidneys; Thyroid gland

1. Introduction

Cyanide is a substance ubiquitous in the environment and has been associated with many intoxication episodes in humans and animals resulting from the ingestion of foods, environmental pollution, chemical war, suicide, homicide, oc-

☆ This work is part of the Master dissertation presented by Altamir Benedito de Sousa to the Department of Pathology, School of Veterinary Medicine, University of São Paulo.

* Corresponding author. Fax: +55-11-3818-7829.
E-mail address: gorniak@usp.br (S.L. Górniak).

0300-483X/02/$ - see front matter © 2002 Elsevier Science Ireland Ltd. All rights reserved.
PII: S0300-483X(02)00041-0

cupational factors and use in some drugs such as nitroprusside and laetrile (El Ghawabi et al., 1975; Schulz et al., 1979; Way, 1984; Watts, 1998). In plants, cyanide can be found mainly as cyanogenic glycosides, as found in *Manihot* sp. (cassava), *Linum* sp., *Lotus* sp., *Phaseolus lunatu*s, *Sorghum* sp. (Conn, 1978) and the content of this substance can be high as 100–800 mg/kg of the plant material (Conn, 1978; Poulton, 1983).

In acute intoxication, cyanide produces a rapid inhibition of cytochrome oxidase, resulting in an energy deficit within the target tissue. The enzyme cytochrome oxidase enables cells to utilize oxygen. By inhibiting this enzyme, cyanide causes cellular anoxia (USEPA, 1989). On the other hand, mammals have an efficient mechanism of cyanide detoxification by conversion through an intramitochondrial enzyme, rhodanese, to the less toxic compound thiocyanate (SCN) that is excreted mainly in the urine (Tylleskär et al., 1991). This enzyme is widely distributed throughout the tissues, but chiefly in the liver and the kidneys (Ong, 1989; Gusmán et al., 1977). Thus, cyanide acute toxicity occurs only when the amount of cyanide greatly exceeds the minimal concentration necessary to inhibit cytochrome oxidase.

More recently, many studies have shown the risks of the toxic effects in relation to the prolonged exposure to cyanide (Okolie and Osagie, 1999). This kind of intoxication is characterized by high levels of thiocyanate in plasma, the main metabolite of cyanide. In fact, there are a number of reports on the possible cyanide and thiocyanate-related etiology of thyroid disorders (Oke, 1984; Tewe et al., 1984; Kamalu and Agharanya, 1991; Adewusi and Akindahunsi, 1994). Thus, as thiocyanate acts as a competitive inhibitor of iodide accumulation in the thyroid, it could produce goiter and/or hypothyroidism (Kamalu and Agharanya, 1991). Another pathology associated with the prolonged exposure to cyanide is the tropical ataxic neuropathy characterized by Parkinson disease, optic atrophy, deafness, and spinal ataxia. This affects people living in certain tropical areas of Africa where the staple diet consists largely of cassava that contains the cyanogenic glycoside linamarin (Oke, 1980; Spencer, 1999). A relationship has also been suggested between pancreatic diabetes and prolonged exposure to the cassava (McMillan and Geevarghese, 1979), however, a recent work showed that this disease is not caused by cyanide itself (Soto-Blanco et al., 2001a).

Although many researchers have been studying these pathologies related with prolonged exposure very little is found in the literature associating hepatotoxicity and nephrotoxicity to this kind of exposure. On these grounds, the purpose of the present study was to determine whether prolonged exposure to cyanide could also produce damage to the liver and kidneys.

2. Materials and methods

2.1. Chemicals

Potassium cyanide (Merck®), potassium thiocyanate (Farmitália Carlo Erba®), chloridric acid 1 N (Ecibra®), saturated bromine water (Haloquímica®), arsenius oxide (Sigma®) 20 g/l in 0.1 N sodium hydroxide (Merck®), sodium chloride (Synth®), pyridine (Synth®), *p*-phenylenediamine (*p*-PDA) (Sigma®), sodium perchlorate (Sigma®) were used. All chemicals were analytical-grade and the aqueous solutions were prepared with double-distilled water.

2.2. Animals and experimental design

Forty-six Wistar adult male rats (*Ratus norvegicu*s) (200–250 g), inbred in the Department of Pathology, School of Veterinary Medicine and Zootechny, University of São Paulo (São Paulo, Brazil), were used throughout the study. The animals were divided into five groups. The four experimental groups received KCN solution at different concentrations in the tap water in order to obtain the target doses of 0.3, 0.9; 3.0 and 9.0 mg/kg per day of KCN; every day the amount of KCN administered in the drinking water was adjust to the body weight and to the water consumption, and a fresh solution of cyanide provided. One group of animals (control) received only tap water. As reported by Leuschner et al. (1991) the stability of KCN in the drinking water is stable for at least 4 days after preparation, and a pilot study in our laboratory showed no signifi-

cant difference ($P > 0.05$) in cyanide concentrations in the water within 24 h period. These animals were housed in standard cages, in pairs, in a temperature-controlled environment on a 12-h light:12-h dark cycle, and fed a standard rat chow (Nuvilab).

On day 15, the rats were anaesthetized with ether and blood collected from the hepatic vein in order to determine thiocyanate and thyroidal hormones levels. After this procedure, the animals were subjected to euthanasia and samples of thyroid, liver, and kidneys were collected for the histopathological studies.

2.3. Biochemical analysis

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities and creatinine, and urea concentrations were measured in serum using reagents from Merck® (AST and ALT) and Celm® (creatinine, and urea). Thyroidal hormones, thyroxine (T4) and triiodothyronine (T3), concentrations were measured in serum by radioimunoassay using kits Count-a-Count from DPC®.

Plasma thiocyanate levels were determined by the method of Tominaga and Midio (1991) in which the colorimetric procedure of the König synthesis of pyridine dyes of *p*-phenylenediamine is used. Briefly, aliquots of 1 ml of plasma diluted with 5 ml of double-distilled water were applied to columns (6 cm of the resin in a 5.0 Pyrex burette 185×5 mm) of a weakly basic anion exchanger Amberlite® IR-4B (OH) 14–52 mesh. Columns were washed three times with 5 ml of double-distilled water before eluting thiocyanate by the addition of 15 ml of 1 M sodium perchlorate, in a 1 ml/min flow rate. Aliquots of 1 ml of eluate were acidified with 0.5 ml of 1 M HCl. Mixed after each addition of the following reagents: 100 μl of saturated bromine water, 200 μl of arsenius oxide (20 g/l in 0.1 N NaOH), 1.8 ml of pyridine (10 ml 12 N HCl, 60 ml pyridine, 40 ml deionized water) -*p*-PDA (2 g in 1-l 0.5 M HCl) reagent (3:1, prepared immediately before use). The reddish-pink complex formed was read at 505 nm, after 15 min. Simultaneously blank determinations were performed where plasma was replaced with water. Thiocyanate concentrations in the samples were read from a standard curve prepared with thiocyanate of known concentrations (20–250 μmol/l). Data was expressed as μmol thiocyanate/l.

2.4. Histological analysis

A significant fragment of kidney, thyroid and liver (left lobe) from three rats in of each group were fixed in 10% buffered formalin. Fragments were processed routinely, paraffin embedded, sectioned at 5 μm, and stained with hemotoxylin and eosin (HE) and periodic acid-Schiff (PAS) (a tissue section for liver and kidney fragments, and three for thyroids because of dislocation of colloid in some of them). Frozen sections (three tissue sections for each fragment) were stained with Sudan black. The slides were evaluated at light microscopy and the intensity of lesions was subjectively quantified: $-$, no lesions; $\pm$, minimal; $+$, mild; $++$, moderate; and $+++$, severe.

2.5. Statistical analysis

Variance analysis (ANOVA) followed by Dunnett test was used to analyze data from biochemical analyses, as well as for determination of water consumption and weight gain of the rats. The results were presented as the mean with their standard errors. All analyses were realized using the software GraphPad Instat v.2.01. In all experiments, $P < 0.05$ was the criterion for statistical significance.

3. Results

A significant difference ($P < 0.05$) in weight gain was found between rats from the control group and those exposed to 9.0 mg KCN/kg per day (Table 1). On the other hand, KCN treatment did not affect the consumption of water. The KCN consumption per kg of body weight is presented in Fig. 1.

Plasma thiocyanate levels in rats from the experimental groups were significantly higher than those from controls (Table 2). The activities of

ALT in rats treated with 0.9 mg/kg were reduced, and the activities of AST were increased in 0.3, 0.9, and 3.0 mg/kg groups and reduced in the 9.0 mg/kg, when compared with those activities from control group. On the other hand, no significant variation was found in the T4 and T3 levels.

Table 1
Weight gain (g) (mean ± S.D.) of rats that received different doses of KCN in the drinking water during 15 days

Group	Evaluation period (day of experiment)			
	n[a]	1–7	7–15	1–15
Control	10	29.4 ± 9.9	38.8 ± 9.7	77.1 ± 12.7
0.3 mg KCN/kg per day	10	24.9 ± 5.5	37.8 ± 9.4	70.5 ± 6.4
0.9 mg KCN/kg per day	10	27.5 ± 10.8	40.0 ± 9.2	79.6 ± 13.9
3.0 mg KCN/kg per day	10	31.8 ± 10.9	36.1 ± 5.0	78.8 ± 10.5
9.0 mg KCN/kg per day	6	5.0 ± 6.4[b]	13.2 ± 8.0[b]	23.3 ± 20.1[b]

[a] Number of animals in each group.
[b] Significant differences ($P<0.05$) were found between the experimental and control groups.

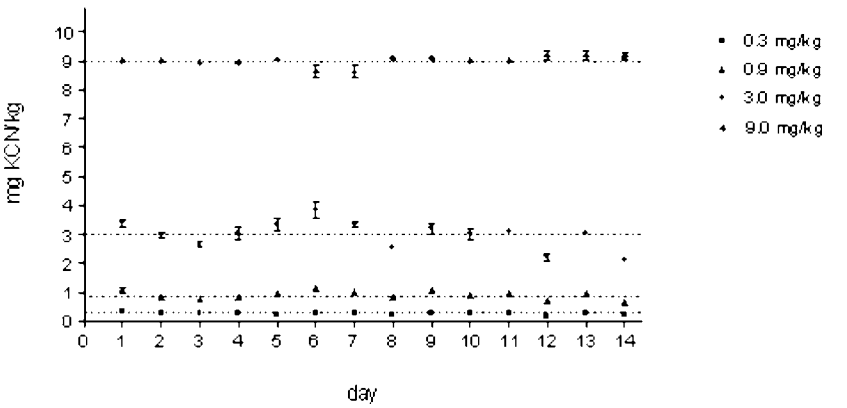

Fig. 1. Daily cyanide ingestion (in mg/kg) from each experimental group and its respectively target dose.

Table 2
Plasma levels of thiocyanate (in μmol/l), serum levels of T3 (in μg/dl), T4 (in ng/dl), creatinine, and urea (in mg/dl) and serum activities of AST and ALT (in U/l) (mean ± S.D.) of rats that received different doses of KCN in the drinking water during 15 days

	Control ($n=10$)	KCN (mg/kg per day)			
		0.3 ($n=10$)	0.9 ($n=10$)	3.0 ($n=10$)	9.0 ($n=6$)
Thiocyanate	56.8 ± 5.9	75.7 ± 7.7[a]	114.2 ± 17.2[a]	153.9 ± 11.1[a]	133.2 ± 11.0[a]
T3	81.4 ± 8.2	80.6 ± 8.6	84.8 ± 11.6	83.0 ± 12.6	76.6 ± 9.8
T4	3.32 ± 0.37	3.33 ± 0.71	3.56 ± 0.84	3.97 ± 0.82	3.32 ± 0.94
ALT	41.2 ± 7.2	36.4 ± 3.6	34.2 ± 4.8[a]	39.4 ± 5.9	47.2 ± 7.3
AST	60.3 ± 10.5	80.1 ± 7.7[a]	79.9 ± 6.2[a]	73.0 ± 11.1[a]	43.2 ± 4.4[a]
Creatinine	0.42 ± 0.04	0.44 ± 0.06	0.49 ± 0.06	0.43 ± 0.04	0.38 ± 0.02
Urea	33.7 ± 1.08	34.5 ± 3.31	35.6 ± 4.23	31.4 ± 1.77	32.9 ± 3.29

[a] Significant differences ($P<0.05$) were found between the experimental and control groups.

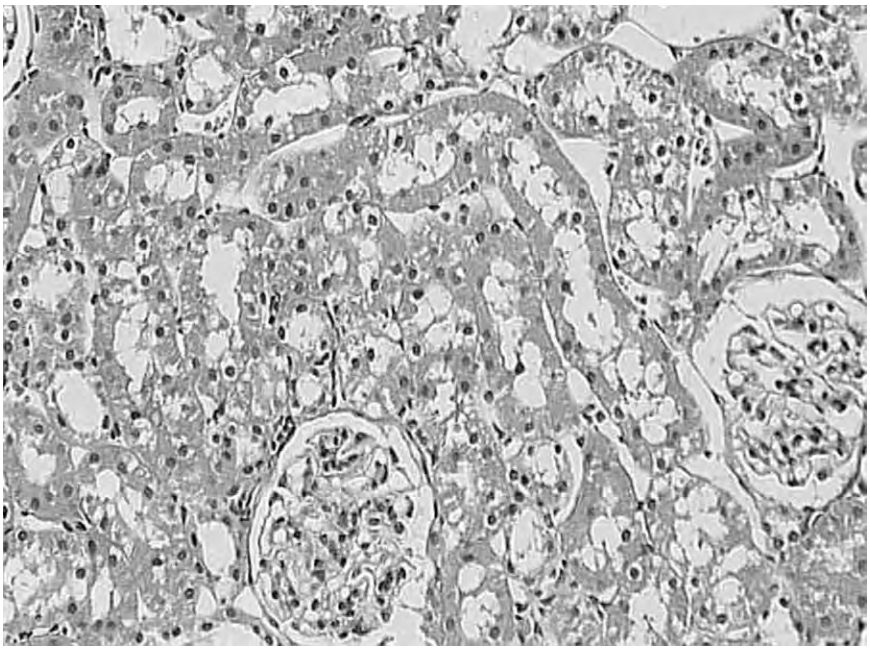

Fig. 2. Kidney from a rat that received 9.0 mg KCN/kg per day showing congestion, cytoplasmic vacuolization, swelling and rupture of the epithelial cells of the proximal tubules (H and E, 360 ×).

The histopathological study revealed alterations in kidney tissue, characterized by congestion and cytoplasmic vacuolization of the epithelial cells of the proximal tubules, in rats from 3.0 and 9.0 mg/kg groups (Fig. 2). The liver of the rats from the 9.0 mg/kg group showed degeneration of the hepatocytes (Fig. 3). The content of the cytoplasmic vacuoles were PAS and Sudan black negative; therefore, the content of these vacuoles were neither glycogen nor lipid, characterizing this lesion as hydropic degeneration. No evidence of inflammatory infiltrate or apoptotic cells was found. Microscopic lesion in the thyroid gland was observed in all treated rats. It consisted of an increase in number of the reabsorption vacuoles in a dose-dependent manner (Fig. 4), but follicular cell hyperplasia was not evidenced. The histopathological study revealed no lesions in the organs of the rats from the control group. The intensity of the observed lesions is presented in Table 3.

4. Discussion

Iyayi (1991) verified that the administration of rationed cyanide accompanies a bitter taste and consequently reduces the consumption of such foods. Furthermore, the administration of KCN intraperitoneally or by gavage could introduce stress to the animals and thereby interfere with the results. In the present experiment the KCN was administered via tap water, which did not alter the palatability once the water consumption did not differ between the control and experimental groups.

Reduction of weight gain in chronic cyanide exposure, was found in several animal species, such as broilers (Panigrahi et al., 1992), dogs (Ibebunjo et al., 1992), pigs (Tewe et al., 1984), sheep (Onwuka et al., 1992) and goats (Onwuka, 1992; Onwuka et al., 1992; Soto-Blanco et al., 2001b), as well as rats (Philbrick et al., 1979; Tor-Agbidye et al., 1999). In the present study,

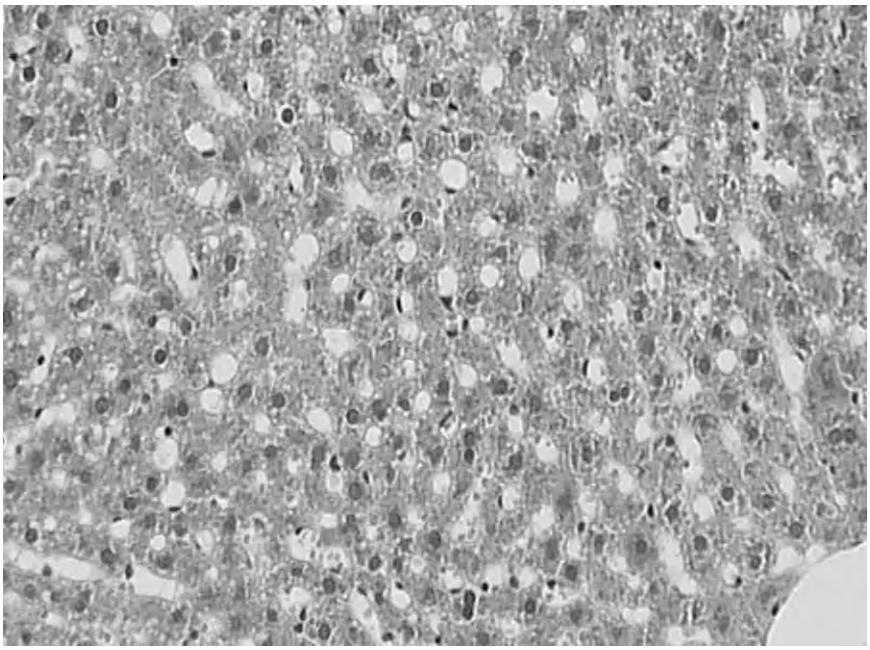

Fig. 3. Liver from a rat of the 9.0 mg KCN/kg per day group. Note vacuolar degeneration of hepatocytes (H and E, 360 ×).

reduction of weight gain was present only in those animals that received the highest dose of KCN (9.0 mg/kg per day). Soto-Blanco et al. (2001b) associated impaired corporal development in goats receiving KCN to reduced T3 levels, since hypothyroidism may result in impaired secretion of growth hormone (Lloyd et al., 1990) and in a reduced number of growth hormone receptors (Koenig et al., 1987). Therefore, this can be excluded in the present study once no interference on thyroidal hormone levels was found. One hypothesis for reduced body weight gain is the mobilization of endogenous sulfur from the breakdown of body proteins of sulfur amino acids utilized in cyanide detoxification, thus reducing the quality of the proteins that are used in the growth promoting process of the body. In fact, Onwuka et al. (1992) verified that small ruminants under prolonged cyanide exposure presented sulfur amino acid deficiency and growth impairment. Another assumption is that the KCN promotes hypoxia, inhibiting mitochondrial oxidative phosphorylation, so could affect the growth through impaired cellular energy metabolism. Nevertheless, one should not discharge the possibility that disturbance on hepatic and renal metabolism could affect body growth.

The main pathway of cyanide detoxification is its conversion to thiocyanate, which is catalyzed by rhodanese. Cyanide is irreversibly bound with sulfur from endogenous compounds such as thiosulphate and polythionates. Although rhodanese is recycled and large amounts of cyanide can be

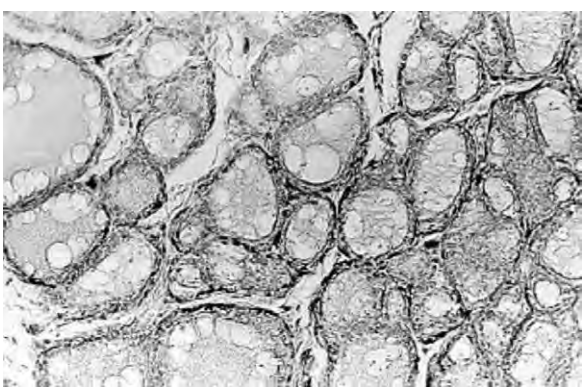

Fig. 4. Thyroid gland from a rat that received 9.0 mg KCN/kg per day with increased number of reabsorption vacuoles in the thyroid follicles (H and E, 140 ×).

Table 3
Average severity of histologic lesions in rats that received different doses of KCN in the drinking water during 15 days

	Control	KCN (mg/kg per day)			
		0.3	0.9	3.0	9.0
Kidney proximal tubules Cytoplasmatic vacuolization of epithelial cells	−	−	+	++	+++
Hepatocytes Cytoplasmatic vacuolization	−	−	+	+	+++
Thyroid follicular cells Reabsorption vacuoles	+	++	+++	+++	+++

* and −, no lesions; +, minimal; ++, moderate; and +++, severe.

metabolized, both cyanide and sulphite can inhibit rhodanese activity in the absence of thiosulphate (Bhatt and Linnell, 1987). The plasma thiocyanate levels in treated rats in this study did not present a dose-dependent increase in the two largest KCN doses, probably because cyanide doses could deplete endogenous sulfur pools and partially impair cyanide detoxification. This observation agrees with increased cyanide sensitivity observed in goats treated with KCN (Soto-Blanco et al., 2001a,b) and in sheep treated with the cyanogenic glucoside amygdalin (J.J. Villalba and F. D. Provenza, personal communication). Therefore, the highest KCN dose (9.0 mg/kg per day) probably was superior to the detoxification capacity of the treated rats, and the amount of produced thiocyanate was independent of KCN dose.

The goitrogenic effects produced by the prolonged exposure of cassava in man (Adewusi and Akindahunsi, 1994) and animals (Oke, 1984; Ong, 1989; Kamalu and Agharanya, 1991) are well defined and it is largely known that this effect is produced by thiocyanate (Poulton, 1983). Despite the lack of hyperplasia and hypertrophy of the thyroidal follicles and unaffected thyroid hormones levels, the present study revealed an increase in the number of the reabsorption vacuoles that could indicate higher activity of the captation of colloid by the follicular cells in the thyroid gland. Thus, the competitive action of the thiocyanate with iodide in the Na^+I^- symporter could decrease the synthesis of iodate compounds and, in this manner, induce an increase in the uptake of the colloid to the follicular cell that occurs at a higher degree than the production. Consequently, these vacuoles could represent the earliest step in the development of goiter. This supposition is reinforced by earlier studies showing reabsorption vacuoles in the thyroid gland from goats with high plasmatic levels of thiocyanate (Bahri, 1986; Soto-Blanco et al., 2001b).

While chronic cyanide toxicity has been linked to the etiology of goiter and other diseases such as tropical ataxic neuropathy (Osuntokun, 1981) and epidemic spastic paraparesis (Tylleskär et al., 1991), little is known about the possible effects of cyanide on the kidneys and liver. Nephrosis was associated with cassava consumption in rats (Ononogbu and Emole, 1978), dogs (Kamalu, 1993) and humans (Clark, 1936). Kamalu (1993) demonstrated periportal vacuolation of the liver from dogs treated with cassava diet and concluded that linamarin was responsible for these alterations. In the present study, hydropic degeneration was verified in the kidneys and liver. Changes in AST activities of rats from all groups that received KCN, which could be related to hepatic lesion was also verified. The no increase in ALT activities in treated animals is agreeable with the absence of necrosis in liver tissue. Furthermore, the no significance on serum levels of urea and creatinine suggests that the morphological lesions were not linked to functional alteration of the kidneys. As in this experiment the KCN was used directly, it can be suggested the nephrosis and liver injury found in chronic cassava administration could be produced by cyanide and/or some of its metabolites, and not by the cyanogenic glycosides, linamarin and lotraustralin, or any other compound from cassava.

To summarize, we have demonstrated an impairment of the weight gain in rats with cyanide exposure. The histopathologic study revealed the presence of reabsorption vacuoles in the thyroid follicles, which could indicate the earlier phase of the goitrogenic effect of thiocyanate, and hydropic degeneration in hepatocytes and epithelial cells of the renal proximal tubules. This information is important for the evaluation of the pathophysiology of long-term cyanide toxicity. As the mechanisms for this remain unclear, further investigation should be designed to better understand the role of thiocyanate in this toxicity.

References

Adewusi, S.R.A., Akindahunsi, A.A., 1994. Cassava processing, consumption, and cyanide toxicity. J. Toxicol. Environ. Health 43, 13–23.

Bahri, S., 1986. Chronic effect of cassava leaves on the plasma thiocyanate and on the performance of the goat thyroid gland. Penyakit Hewan 19, 88–90.

Bhatt, H.R., Linnell, J.C., 1987. The role of rhodanese in cyanide detoxification: its possible use in acute cyanide poisoning in man. In: Ballantyne, B., Marrs, T.C. (Eds.), Clinical and Experimental Toxicology of Cyanides. Wright, Bristol, pp. 440–450.

Clark, A., 1936. Report on effects of certain poisons contained in food plants of West Africa upon the health of the natives races. J. Trop. Med. Hyg. 39, 269–276.

Conn, E.E., 1978. Cyanogenesis, the production of cyanide, by plants. In: Keeler, R.F., Van Kampen, K.R., James, L.F. (Eds.), Effects of Poisons Plants on Livestock. Academic Press, San Diego, pp. 301–310.

El Ghawabi, S.H., Gaafar, M.A., El Saharti, A.A., Ahmed, S.H., Malash, K.K., Fares, R., 1975. Chronic cyanide exposure: a clinical, radioisotope, and laboratory study. Br. J. Ind. Med. 32, 215–219.

Gusmán, V., Ochoa, R., Gil, A., 1977. Intoxicacion cronica por acido cianhidrico en ratas y su interacion con la proteina y el tiosulfato de sodio de la dieta. Revista ICA 12, 241–252.

Ibebunjo, C., Kamalu, B.P., Ihemelandu, E.C., 1992. Comparison of the effects of cassava (Manihot esculenta Crantz), organic cyanide and inorganic cyanide on muscle and bone development in a Nigerian breed of dog. Br. J. Nutr. 68, 483–491.

Iyayi, E.A., 1991. Dietary cyanide effect on performance and serum testosterone of growing male pigs. Beitr. Trop. Landwirtsch Vet. Med. 29, 347–352.

Kamalu, B.P., 1993. Pathological changes in growing dogs fed on a balanced cassava (Manihot esculenta Crantz). Br. J. Nutr. 69, 921–934.

Kamalu, B.P., Agharanya, J.C., 1991. The effect of a nutritionally-balanced cassava (Manihot esculenta Crantz) diet on endocrine function using the dog as a model. 2-Thyroid. Br. J. Nutr. 65, 373–379.

Koenig, R.J., Brent, G.A., Warne, R.L., Larsen, P.R., Moore, D.D., 1987. Thyroid hormone receptor binds to a site in the rat growth hormone promoter required for induction by thyroid hormone. Proc. Natl. Acad. Sci. USA 84, 5670–5674.

Leuschner, J., Winkler, A., Leuschner, F., 1991. Toxicokinetic aspects of chronic cyanide exposure in the rat. Toxicol. Lett. 57, 195–201.

Lloyd, R.V., Jin, L., Song, J., Terry, L.C., Horvath, E., Kovacs, K., 1990. Effects of propylthiouracil on growth hormone and prolactin messenger ribonucleic acids in the rat pituitary. Lab. Invest. 62, 347–354.

McMillan, D.E., Geevarghese, P.J., 1979. Dietary cyanide and tropical malnutrition diabetes. Diabetes Care 2, 202–208.

Oke, O.L., 1980. Toxicity of cyanogenic glycosides. Food Chem. 6, 97–109.

Oke, O.L., 1984. The use of cassava as pig feed. Nutr. Abs. Rev. B 54, 301–314.

Okolie, N.P., Osagie, A.U., 1999. Liver and kidney lesions and associated enzyme changes induced in rabbits by chronic cyanide exposure. Food Chem. Toxicol. 37, 745–750.

Ong, H.K., 1989. Cyanogenic and goitrogenic factors in pig nutrition. Pig News and Information 10, 19–23.

Ononogbu, I.C., Emole, I., 1978. The effect of garri on rat plasma cholesterol. Atherosclerosis 31, 101–104.

Onwuka, C.F.I., 1992. Hydrocyanic acid contents of tropical browse and their influence on performance of goats. Food Chem. 45, 5–10.

Onwuka, C.F.I., Akinsoyinu, A.O., Tewe, O.O., 1992. Role of sulphur in cyanide detoxification in ruminants. Small Ruminant Res. 8, 277–284.

Osuntokun, B.O., 1981. Cassava diet, chronic cyanide intoxication and neuropathy in the Nigerian Africans. World Rev. Nutr. Diet. 36, 141–173.

Panigrahi, S., Rickard, J., O'Brien, G.M., Gay, C., 1992. Effects of different rates of drying cassava root on its toxicity to broiler chicks. Br. Poultry Sci. 33, 1025–1041.

Philbrick, D.J., Hopkins, J.B., Hill, D.C., Alexander, J.C., Thomson, R.G., 1979. Effects of prolonged cyanide and thiocyanate feeding in rats. J. Toxicol. Environ. Health 5, 579–592.

Poulton, J.E., 1983. Cyanogenic compounds in plants and their toxic effects. In: Keeler, R.F., Tu, A.T. (Eds.), Handbook of Natural Toxins. Marcel Dekker, New York, pp. 117–157.

Schulz, V., Bonn, R., Kindler, J., 1979. Kinetics of elimination of thiocyanate in 7 healthy subjects and in 8 subjects with renal failure. Klinische Wochenschrift 57, 243–247.

Soto-Blanco, B., Sousa, A.B., Manzano, H., Guerra, J.L., Górniak, S.L., 2001a. Could prolonged cyanide exposure have a diabetogenic effect? Vet. Hum. Toxicol. 43, 106–108.

Soto-Blanco, B., Górniak, S.L., Kimura, E.T., 2001b. Physiopathological effects of chronic cyanide administration to growing goats—a model for cyanogenic plants ingestion. Vet. Res. Commun. 25, 379–389.

Spencer, P.S., 1999. Food toxins, AMPA receptors, and motor neuron diseases. Drug Metab. Rev. 31, 561–587].

Tewe, O.O., Afolabi, A.O., Grissom, F.E.G., Littleton, G.K., Oke, O.L., 1984. Effect of varying dietary cyanide levels on serum thyroxine and protein metabolites in pigs. Nutr. Rep. Int. 30, 1249–1253.

Tominaga, M.Y., Midio, A.F., 1991. Modified method for the determination of thiocyanate in urine by ion-exchange chromatography and spectrophotometry. Rev. Farm. Bioq. USP 27, 100–105.

Tor-Agbidye, J., Palmer, V.S., Lasarev, M.R., Craig, A.M., Blythe, L.L., Sabri, M.I., Spencer, P.S., 1999. Bioactivation of cyanide to cyanate in sulfur amino acid deficiency: relevance to neurological disease in humans subsisting on cassava. Tox. Sci. 50, 228–235.

Tylleskär, T., Banea, M., Bikangi, N., Fresco, L., Persson, L.Å., Rosling, H., 1991. Epidemiological evidence from Zaire for a dietary etiology of konzo, an upper motor neuron disease. Bull. WHO 69, 581–589.

United States Environmental Protection Agency (USEPA), 1989. Office of Drinking Water Health Advisories. Rev. Environ. Contam. Toxicol. 107, 53–64.

Watts, J., 1998. Japanese fear over festival cyanide poisoning. Lancet 352, 379.

Way, J.L., 1984. Cyanide intoxication and its mechanism of antagonism. Ann. Rev. Pharmacol. Toxicol. 24, 451–481.