AFRICAN JOURNAL OF MEDICINE and medical sciences

VOLUME 34 NUMBER 1 MARCH 2005

ISSN 1116—4077

Afr J Med. med. Sci. (2005) 34, 59-63

Gross morphological studies on the effect of cyanide on the developing cerebellum of wistar rat *(rattus novegicus)*

IO Imosemi[1*], AO Malomo [1,2], OW Oladejo[1], FC Osuagwu[1], OE Ekpo[4], EEU Akang[3] and MT Shokunbi[1]

Departments of Anatomy[1], Surgery[2] and Pathology,[3] College of Medicine, University of Ibadan and Department of Anatomy[4], Ladoke Akintola University of Technology, Ogbomoso, Nigeria

Summary

The teratogenic effect of maternal cyanide consumption on the gross morphology of the post-natal phase of the developing rat cerebellum was studied. Twenty pregnant female rats weighing between 170g and 190g were separated into control and experimental groups. The control animals were fed a standard diet of mice cubes, while the experimental animals were fed 500 ppm potassium cyanide, mixed with the standard diet. The diets were fed to the animals and their litters in separate cages and water provided *ad libitum* during gestation and to the offspring after birth. After birth, the offspring (five per group) of days 1, 9, 14, 21, 28 and 50 were weighed, killed by cervical dislocation and the gross parameters studied. In the experimental animals, no significant differences were observed in the studied parameters between the control and experimental animals on day 1. A significant reduction in body weight was observed on day 14 ($P < 0.05$). The brain weight was significantly reduced on day 9 ($P < 0.05$). Similarly, the cerebellar weight was significantly reduced on days 14, 21 and 28 ($P<0.05$). The maximum vermal length was significantly reduced on day 50 ($P< 0.05$), and the maximum side-to-side dimension of the cerebellum was also reduced on day 28 ($P< 0.05$). There was no reduction in the thickness (anteroposterior dimension) of the cerebellum in the experimental group ($P> 0.05$). From the result, it is inferred that maternal consumption of 500 ppm cyanide causes reduction in the cerebellar weight, vermal length and side-to-side dimension of the developing cerebellum in postnatal life in rats.

Keywords: *Cyanide, development, gross parameters and cerebellum*

Résumé

Les effet teratogeniques de la consommation du cyanure par la mere sur la morphologie nette de la phase postnatale du cervelet du rat en voie de développement a été étudié. Vingt rats féminins enceintes qui pèsent entre 170g et 190g sont séparés dans des groupes expérimentaux. Les animaux du contrôle ont été nourris d'une alimentation standarde de cubes des souris, pendant que les animaux expérimentaux ont été nourris de 500 cyanure du potassium du ppm, mélangé avec l'alimentation standarde. Les alimentations ont été donnees aux animaux alors que leurs petits etaient dans les cages séparées et l'eau etait fourni le libitum pendant la gestation et aux progénitures après la naissance. Après la naissance, les progénitures (cinq par groupe) de jours 1, 9, 14, 21, 28 et 50 ont été pesés, tués par déboîtement cervical et les paramètres ont ete étudiés. Parmis les animaux expérimentaux, aucunes différences considérables n'ont été observées dans les paramètres examines les animaux de contrôle et les animaux expérimentaux de premier jour. Une réduction considérable dans le poids du corps a été observée le 14eme jour ($P <0.05$). Le poids du cerveau a été considerablement réduit le 9eme jour ($P <0.05$). De la même façon, le poids du cerebellar a été réduit les 14 eme, 21eme et 28eme jours ($P <0.05$). La longueur maximale a été réduite 50eme jour ($P <0.05$), et la dimension maximale du côté-à-latérale du cervelet a aussi été réduite le 28eme jour ($P <0.05$). il n'y avait aucune réduction dans l'épaisseur (dimension de l'anteroposterior) du cervelet dans le groupe expérimental ($P> 0.05$). A partir du résultat, on infere que la consommation de la mere de 500ppm cyanure cause une réduction dans le poids du cerebellar dans la longueur du vermal et la dimension du côté lateral d'un cervelet en voie de développement dans la vie post-natale des rats.

Introduction

Nearly two-third of the world's population is undernourished [1]. Hence diseases due to malnutrition especially under nutrition are rife, particularly in the developing countries. It has been realized that the nervous system is susceptible to damage from dietary abnormalities [1]. The nervous system is particularly dependent on energy derived from carbohydrates of which it has no immediate store. It also has a variety of highly complicated enzyme systems, which govern and control the use of this energy [1] and is susceptible to factors that interfere with energy production and utilization.

Apart from dietary deficiencies, dietary toxins such as food substances containing cyanogenic glycosides like garri, rice and cassava are now being recognised as important factors in the aetiopathogenesis of some diseases of the nervous system [1]. Cyanide released from cyanogenic glycosides, for example, will inhibit cytochrome C oxidase and thereby impair aerobic oxidation and energy

Correspondence: Mr. I.O. Imoseni, Department of Anatomy, College of Medicine, University of Ibadan, Ibadan, Nigeria. Email: innosemi@yahoo.co.uk

release for cellular function. It is therefore important to determine and identify dietary toxins such as cyanogenic glycosides in common foodstuffs consumed by man, especially if such toxins may cause pathological effect [1].

Cyanide intoxication has long been recognised as an important problem in the field of clinical toxicology. Cyanide compounds are salts of very weak hydrocyanic acid (HCN). Cyanide has been recognised as a neurotoxicant [2], and it has been suggested that increase in the level of intraneuronal calcium and lipid peroxidation might be mechanisms by which cyanide produces nerve injury [3]. Cyanide is present in a wide variety of plants. It can be released by enzymatic and non-enzymatic hydrolysis of cyanogenic glycosides present in high concentration in most of the plant sources of food consumed in the tropics [4].

The central nervous system (CNS) has been found by [5], to be the target of harmful toxic environmental agents, which produce behavioural abnormalities in humans without any quantitatively evident neuropathological changes. This was termed microneuronal hypoplasia, which is a retardation of brain development characterized by a quantitative reduction in the normal population of late generated, short axoned neurons in specific brain regions.

Considering the important function of the cerebellum in various motor activities and the neurotoxic effect of cyanide arising from the consumption of improperly processed food containing cyanogenic glycosides, this study was designed to investigate the gross morphological effect of cyanide consumption of varying duration, on the postnatal development of the cerebellum of Wistar rats.

Materials and methods

Breeding of animals

Twenty sexually mature female Wistar albino rats, weighing between 170g and 190g were obtained from the preclinical animal house of University of Ibadan. The rats were kept in standard cages and fed with standard rat pellet cubes daily (M.O. Ladokun & Sons Ltd, Ibadan). The rats were mated, and confirmed to be pregnant by the presence of vaginal plug. The pregnant rats were then separated into control and experimental groups (ten animals in each group), kept in standard cages (one per cage) and fed with either the standard rat pellet cubes, or experimental diets: 500 ppm potassium cyanide (Hopkin and Williams), mixed with 500g of powdered mice cubes daily respectively during pre and postnatal life. The level of cyanide used above was chosen following the observation of (6), that deaths of rats occurred when they were fed diets containing 960ppm cyanide. The diets were given to the rats in glass containers and water was provided *ad libitum*. After birth, sixty litters were studied - thirty for the control and thirty for the experimental group. They were still fed with their respective diets and were killed at various stages in postnatal life. The cerebellum of the litters of days 1, 9, 14, 21, 28 and 50 were dissected out, rinsed in normal saline and fixed in 10% formol saline. The fixed tissues were blotted dry with filter paper and measured directly.

Measurement of gross parameters

The gross parameters measured and the instruments used include:

i). Weight of the animals before sacrifice using a Micro-analytical balance (Microwa 7720 witzerland).
ii). Weight of the brain using the same instrument as in (i) above.
iii). Weight of the cerebellum using a Microanalytical balance (Microwa 5540 Switzerland).
iv). Maximum length of the vermis using Vernier calipers and measuring on a ruler.
v). Maximum side-to-side dimension of the cerebellum using the same instrument as in (iv) above.
vi). Maximum thickness (anteroposterior dimension) of the cerebellum using the same instrument as in (iv) above.

Statistical analysis

The data obtained were subjected to statistical analysis using a computer software package (SPSS for Window). The mean, standard deviation and level of significance were calculated.

Results

In the course of the experiment, it was observed that while the mothers of the control animals were calm, those of the experimental group were aggressive and restless and tended to bite handlers, and the only mortality (rat) recorded occurred on day 49 postpartum among the experimental group. At the end of the experiment, the following observations were made.

Gross observations

Body Weight: A significantly greater average body weight was recorded for the control group (27.96± 1.43g) than for the experimental group (26.28± 1.54g) on day 14 postpartum ($P<0.05$) (Table 1).

Brain weight: A significantly greater average brain weight was observed in the control group (0.73± 0.055g) as compared with the experimental group (0.59± 0.056g) on day 9 postpartum. ($P<0.05$) (Table 2).

Cerebellar weight: The weight of the cerebellum was significantly greater in the control group (252.40±4.62mg) compared with that of the experimental group (237.80±10.72mg) on day 14 postpartum, (277.80± 5.12mg) for control group and (265.00± 5.34mg) for the experimental group on day 21 postpartum, and (297.80± 6.72mg) for the control group and (276.80± 11.37mg) for the experimental group on day 28 postpartum. These were significantly different ($P<0.05$) (Table 3).

Maximum length of the vermis: The vermal length was longer in the control group (8.62± 0.13mm) than in the experimental group (8.52± 0.15mm) on day 50 postpartum and this was significantly different ($P<0.05$) (Table 4).

Maximum side-to-side dimension of the cerebellar hemisphere: A significant reduction in the side-to-side dimension of the cerebellum was observed in the experimental group on day 28 postpartum ($P<0.05$), 12.06± 0.34mm in the control group and 11.84± 0.26mm in the experimental group (Table 5).

Table 1: Mean weight of animals in grams (g) on days 1, 9,14,21,28 and 50 postpartum.

	Mean weight in g ± SD					
Treatment	Day 1	Day 9	Day 14	Day 21	Day 28	Day 50
Control	7.46±0.26	17.12±0.71	27.96±1.43	43.40±3.52	49.48±2.18	82.78±3.74
Cyanide treated	7.28±0.43	17.18±0.83	26.28±1.54	42.60±2.49	48.58±1.12	88.28±2.05
P – value	0.476	0.795	0.017	0.752	0.384	0.051

Table 2: Mean weight of the brain of animals in grams (g) on days 1, 9,14,21,28 and 50 postpartum.

	Mean weight in g ± SD					
Treatment	Day 1	Day 9	Day 14	Day 21	Day 28	Day 50
Control	0.19±0.02	0.73±0.06	0.94±0.04	1.37±0.06	1.51±0.06	1.69±0.03
Cyanide treated	0.21±0.03	0.59±0.06	0.93±0.03	1.34±0.06	1.46±0.05	1.66±0.04
P – value	0.399	0.006	0.587	0.486	0.322	0.135

Table 3: Mean weight of the cerebellum in milligrams (mg) on days 1, 9,14,21,28 and 50 postpartum.

	Mean weight in mg ± SD					
Treatment	Day 1	Day 9	Day 14	Day 21	Day 28	Day 50
Control	56.2±1.48	167.4±5.81	252.4±4.62	277.8±5.12	297.8±6.72	312.6±5.98
Cyanide treated	53.0±3.32	166.4±5.13	237.8±10.7	265.0±5.34	276.8±11.4	306.0±9.06
P – value	0.173	0.817	0.018	0.046	0.007	0.190

Table 4: Mean length of the vermis in millimetres (mm) on days 1, 9,14,21,28 and 50 postpartum.

	Mean length in mm ± SD					
Treatment	Day 1	Day 9	Day 14	Day 21	Day 28	Day 50
Control	2.92±0.13	5.80±0.19	7.42±0.034	7.82±0.19	8.04±0.11	8.68±0.13
Cyanide treated	2.82±0.11	5.66±0.34	7.34±0.055	7.76± 0.26	7.92±0.13	8.52±0.15
P – value	0.298	0.338	0.242	0.769	0.109	0.035

Table 5: Mean side-to-side dimension of the cerebellum in mm on days 1, 9,14,21,28 and 50 postpartum.

	Mean dimension in mm ± SD					
Treatment	Day 1	Day 9	Day 14	Day 21	Day 28	Day 50
Control	5.76±0.25	8.56±0.15	9.98±0.35	11.50±0.38	12.06±0.34	13.04±0.32
Cyanide treated	5.60±0.28	8.46±0.32	9.74± 0.15	10.96±0.62	11.84±0.26	12.80±0.19
P – value	0.078	0.631	0.201	0.204	0.004	0.342

Table 6: Mean thickness of the cerebellum in mm on days 1, 9,14,21,28 and 50 postpartum.

	Mean thickness in mm ± SD					
Treatment	Day 1	Day 9	Day 14	Day 21	Day 28	Day 50
Control	3.76±0.17	5.06±0.22	5.66±0.11	5.76±0.15	6.16±0.15	6.34±0.05
Cyanide treated	3.88±0.11	5.24±0.29	5.66±0.13	5.90±0.10	6.10±0.07	6.20±0.16
P – value	0.208	0.121	1.000	0.245	0.468	0.080

Maximum thickness of the cerebellum: The anteroposterior dimension of the cerebellum was not significantly different among the groups (P>0.05) (Table 6).

Discussion

Anomalies of the developing central nervous system have been induced in experimental animals by a variety of experimental factors such as nutritional deficiency [7], cytotoxic agents [8] and X- irradiation [9].

The minimum lethal dose of cyanide depends on its rate of absorption into the body; the more rapidly the tissue level builds up, the more acute are the signs and symptoms of the poisoning and the smaller is the total absorbed dose which is required to produce a given effect. The cell membranes of animal tissues have been demonstrated to be very permeable to cyanide suggesting that permeability may play an important role in cyanide toxicity [10]. In this present study, the litter size, body weight, brain weight and cerebellar weight, vermal length, side-to-side dimension and the thickness of the cerebellum were not significantly affected at birth. The explanation of this is not completely clear, but it implies that as cyanide is known not to cross the placental barrier effectively [11], consumption of cyanide at 500 ppm during gestation did not cause sufficient metabolic disturbances in the mother, to detectably affect intrauterine cerebellar development at the level of the present studies.

A study carried out on feeding trials with gestating rats demonstrated that little if any thiocyanate was transferred to the fetus through the placenta when fresh and dried cassava (173ppm and 92ppm) or diets containing potassium cyanide were fed [11]. This probably explains our findings, which contrast with those of some workers who used other agents. For instance, [12] reported that trichlorfor (metrifonate) administered to guinea pigs subcutaneously produced teratogenic effects, as there was reduction in cerebellar weight. The mechanism behind this teratogenic effect is not known, but alkylation of DNA or impairment on its repair mechanism is possible explanation. Also alcohol exposure during brain development has been reported to produce neuron attrition in multiple ways including, disruption of membrane integrity, inhibition of protein synthesis, alterations of lipid solubility, or disruption of cytoskeletal elements leading to reduction in weight of almost all the regions of the developing brain [13].

A reduction in the weight of the cerebellum during the late and post weaned phases (3rd and 4th week after birth respectively), and the reduction in the maximum vermal length and the maximum side-to-side dimension during the post weaned phase could probably be due to gradual cumulative effect of cyanide fed to rats during gestation and transmitted to their offspring in the growing phase. [11] reported that a gestational carry over effect was observed in the weight of the liver of the offspring during the postnatal phase as cyanide fed during gestation caused a significant reduction in the weight of the liver in growing rats [11]. However, thiocyanate has been found to be concentrated in the milk during the later part of lactation [14]. It will be useful in future, to determine whether or not a similar carry over effect occurs with respect to cerebellar development.

Cyanide and its detoxified products inhibit the metabolic processes of nutrients such as proteins, vitamins and minerals. This raises the question of their possible interference with nutrient transportation to the foetus during gestation and postnatally during breast-feeding. No such effect was observed in this study.

In conclusion therefore, maternal consumption of 500 ppm KCN causes mild changes in postnatal cerebellar development as evidenced by the reduction in the weight of the cerebellum, vermal length and the side-to-side dimension of the cerebellum.

References

1. Osuntokun, B. O.: Chronic cyanide intoxication and a degenerative neuropathy in Nigeria. 2 Vols. Ph. D Thesis. University of Ibadan, 1969.
2. Klaassen, C. D. and Eaton, D. L.: Principles of toxicology: In M. O. Amdur J. Doull and C. K. Klaassen (Ed), Toxicology the basic science of poisons 4th Edition, Pergamon, U. S. A. 1990; 276.
3. Johnson, J.D., Meisenheimer, T.L. and Isom, G.E: Cyanide induced neurotoxicity: Role of neuronal calcium. Toxicol. Appl. Pharmacol. 1986; 84 - 464.
4. Sinclair, H. M. and Jelliffe, D. B. In: Nicholls L (ED) Tropical Nutrition and Dietetics (4th Edition, London, Balliere, Tindal and Cox), 1961.
5. Altman, J. Morphological and behavioural markers of environmentally induced retardation of brain development: An animal model. Environmental Health Perspectives, 1987; 74: 153-169.
6. Maner, J.H. and Gomez,G. Implication of cyanide toxicity in animal feeding studies using high cassava rations, 113 – 129. On chronic cassava toxicity. Proceedings of an Interdisciplinary workshop, London, England, 29th – 30th January 1973. Int. Develop. Res. Centre. Monogro IDRC OIOC. 1973.
7. Clark, G.M., Zahemhof, S., Van Marthers, E. Granel, L. and Kragger, L.: The effect of prenatal malnutrition dimensions of cerebral cortex. *Brain Res.* 1973; (54): 397 - 402.
8. Singh, S. and Sanyal, A.K.: Prenatal retardation of brain growth of chick embryos following cyclophosphamide administration. *Neurology India.* 1978; (3): 104 – 109.
9. Takennuchi, I. K. and Murakami, U.: Two types of congenital hydrocephalus induced in rats by X - irradiation in utero. Electron microscopic study of the telencephalic wall. *J. Anat.* 1979; 128(1): 693 - 703.

10. Tompsett, S. L.. A note on the determination and identification of cyanide in biological materials. *Clin. Chem.* 1959; 55: 587.
11. Tewe, O. O.: Implications of the cyanogenic glucoside fraction of cassava in the growth and reproductive performance of rats and pigs. Ph. D Thesis. University of Ibadan, 1975.
12. Hjelde, T., Mehl, A., Schanke, T. M. and Fonnum, F.: Teratogenic effects of trichlofon (Metrifonate) on the guinea - pig brain. Determination of the effective dose and the sensitive periods. Neurochemistry International. 1998; 32(5 - 6): 469 - 477.
13. West, J. R., Goodlett, C. R., Bonthius, D. J., Hamre, K. M. and Marcussen, B. L. Cell population depletion associated with fetal alcohol brain damage: Mechanisms of BAC- dependent cell loss. Alcoholism. *Clinical and Experimental Research.* 1990; 14 (6): 813 – 818.
14. Funderburk, C. F.. Studies on the physiological occurrence and metabolism of thiocyanate. Thesis University of Tennessee. 1966.

Received: 9/02/04
Accepted: 11/01/05