Original Article

Evaluation of some Enzymatic Changes in the Liver and Kidney of Rats Following Exposure to Sublethal Concentration of Potassium Cyanide

Hasan Baghshani[1], Vahide Ghodsi* [1]

Received: 20.12.2015 *Accepted: 03.02.2016*

ABSTRACT

Background: Besides acute lethal cyanide poisoning, its chronic intoxication may also produce some pathologic effects on different tissues that precedes alterations in biochemical parameters. The present study was aimed to evaluate the effects of sublethal cyanide exposure on some tissue enzyme activities in liver and kidney of rats.

Methods: Twelve male rats were divided into two groups as follows: Group 1 rats served as control. Rats in group 2 received water containing 200 ppm inorganic cyanide. At the end of the experiment (42 days), hepatic and renal activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and rhodanese were measured.

Results: Potassium cyanide administration caused elevation of all measured liver enzymes in group 2, although the increase was only significant for AST and ALT activities as compared to control values (P<0.05). Moreover, renal AST activity in rats from group 2 was significantly higher than those from controls.

Conclusion: The altered tissue activities of some enzymes in the present study might reflect the metabolic disturbances due to cyanide intoxication in studied organs. However, further research should be focused on this issue for better understanding of the fine mechanism of cyanide effects upon metabolic enzyme activities.

Keywords: Cyanide Poisoning, Rat, Tissue Enzyme.

IJT 2016 (4): 9-12

INTRODUCTION

Cyanide (CN^-) is a potent inhibitor of aerobic metabolism, acting on cytochrome oxidase, the final component of the mitochondrial respiratory chain. It is one of the most toxic substances, affecting all classes of living cells whether they are plants, animals or microorganisms. Many natural (i.e. various species of bacteria, algae, fungi, and higher plants) as well as industrial products had contained cyanide [1]. Cyanide compounds used extensively in the mining and various industrial applications [2]. Cyanide poisoning can be caused through ingestion of cyanogenic foodstuffs, occupational exposure, or exposure to chemical warfare agents containing cyanide [1].

Deaths from acute cyanide intoxication have been documented in several species of animals [3, 4]. Besides acute lethal cyanide poisoning, its chronic intoxication may also produce some pathologic effects on different tissues that precedes alterations in biochemical parameters. The most widespread problems arising from cyanide are from chronic/sub chronic exposures. Chronic cyanide toxicity has been involved in the pathogenesis of some health problems like goiter and tropical ataxic neuropathy [5, 6]. Moreover, chronic cyanide intoxication has induced alterations in some tissue biochemical, histological and oxidative stress parameters in experimental animal models [2, 7-12].

Species differences have been reported on the organ-specific biochemical markers and the susceptibility to various toxic agents [13]. Enzymes are the catalysts of all biological and metabolic reactions in cells and their activities are considered as sensitive biochemical indicators used to investigate cellular injury, metabolic disturbances, and enzyme inactivation or induction by exogenous chemicals [14]. Alterations of the enzyme activities in functional

1. Department of Basic Sciences, Ferdowsi University of Mashhad, Mashhad, Iran.
*Corresponding Author: E-mail**:** baghishani@ferdowsi.um.ac.ir

organs may reflect the disruption of metabolic integrity [2].

The present study was aimed to assess the effects of sub-lethal cyanide exposure on tissue enzyme alterations of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase and rhodanese in liver and kidney of rats.

MATERIALS AND METHODS

Experimental Design and Sampling

Twelve male Wistar rats weighing approximately 180 g were divided randomly into two groups each of 6 cases. All these animals were acclimatized for 7 days before the beginning of experiment. The animals were housed in plastic (polypropylene) cages using paddy husk bedding at room temperature (25 ± 1°C) in a 12-h light/dark cycle with 50 ± 5% humidity. Animals received standard laboratory balanced commercial diet *ad libitum*. Group 1 rats received basal diet and tap water throughout the experiment and served as the control. Rats in group 2 received basal diet and tap water containing 200 ppm inorganic cyanide (KCN, Merck, Germany).

The experiment was approved by Research Ethics Committee of the Faculty of Veterinary Medicine of Ferdowsi University of Mashhad.

At the end of the experiment (42 days), all rats were exterminated by ether anesthesia, their liver and kidneys removed and dissected. Tissue samples cleaned free of extraneous material and washed with physiological saline. Samples from liver and kidney were frozen in liquid nitrogen and stored at −70 °C until analysis.

Analysis and Measurement

Tissue samples were rapidly thawed and homogenized in 10 volumes (w/v) of ice-cold 0.05 M phosphate buffer (pH 7.4) for 5 min, and centrifuged at 4,000×g for 15 min at 4 °C and the supernatant was kept in ice until assayed. The activities of aspartate aminotransferase (EC 2.6.1.1, AST) and alanine aminotransferase (EC 2.6.1.2, ALT) were determined by the colorimetric method of Reitman and Frankel [15]. Activities of lactate dehydrogenase (EC 1.1.1.28, LDH) and alkaline phosphatase (EC 3.1.3.1, ALP) were determined colorimetrically [16]. Rhodanese was assayed based Sorbo [17] as described previously [18], in which its activity as followed by the production of thiocyanate from thiosulfate and cyanide. All the enzyme activities were presented in units per gram tissue wet weight.

Statistical Analysis

All data have been represented as mean ± standard error of mean (SEM). The obtained values were analyzed using Student's *t* test. The level of significance was set at $P < 0.05$. All calculations were performed using SPSS/PC software (Chicago, IL, USA).

RESULTS

Mortality was not observed in any experimental group throughout the study. Values of measured enzymes activities in liver and kidney of experimental groups are shown in Table 1 and 2. KCN administration caused elevation of all measured liver enzymes in group 2, although the increase was only significant for AST and ALT activities as compared to control values (Table 1). Moreover, renal AST activity in rats from group 2 was significantly higher than those from controls ($P<0.05$) (Table 2).

Table 1. Values of liver enzyme activities (U/g tissue) in experimental groups (n=6 in each group).

Parameters	Control	Cyanide
AST	256.55±29.46[a]	449.38±48.02[b]
ALT	71.23±7.86[a]	119.94±13.24[b]
ALP	185.90±38.72	234.24±68.27
LDH	808.27±176.72	1217.84±96.58
Rhodanese	42.29±12.33	57.96±13.58

Mean±SEM in each column with no common superscript differ significantly ($P<0.05$)

Table 2. Values of kidney enzyme activities (U/g tissue) in experimental groups (n=6 in each group).

Parameters	Control	Cyanide
AST	164.70±20.05 [a]	279.95±23.24 [b]
ALT	54.69±9.15	91.07±13.26
ALP	260.27±75.85	350.97±82.01
LDH	658.12±101.22	693.19±30.03
Rhodanese	29.60±8.63	23.49±4.89

Mean±SEM in each column with no common superscript differ significantly ($P<0.05$)

DISCUSSION

Studies on the tissue enzyme alterations might reflect the metabolic abnormalities and

cellular injuries in some organs. The liver and kidney have extremely important function in detoxification and excretion of metabolic wastes and xenobiotics [13]. Exposure to toxic chemicals cause alterations in some tissue enzyme activities [7, 12, 19, 20]. AST and ALT are distributed extensively in several different organs and have important roles in carbohydrate and amino acid metabolic pathways and their activities is established to change under several physiological and pathological circumstances [21].

In the present study, hepatic AST and ALT and renal ALT levels were raised in cyanide exposed animals. Elevated serum activities of ALT following sublethal cyanide poisoning have been described in rat [22] and pig [23]. Despite the present results, significant decrease of liver ALT activity has been reported in rabbits subsequent to chronic cyanide intoxication [9]. Indeed, findings of Okolie and Osagie [24] in rabbits showed no statistically significant differences in the serum and heart activities of AST following chronic cyanide exposure.

Based on the present findings, renal and hepatic LDH activities were increased no significantly subsequent to chronic exposure of rats to KCN. In line with the current finding, Okolie and Osagie [9] and Okolie and Iroanya [7] noted that cyanide feeding in rabbits led to increment of LDH in kidney, liver, and lung which may be connected to the function of LDH in anaerobic glycolytic pathway which is augmented in cyanide intoxication [7, 9]. Cyanide, known as a strong metabolic toxicant, can inhibits the terminal step in respiratory chain, resulting in cellular hypoxia and a shift from aerobic to anaerobic metabolism. In this poisoning, increased anaerobic glycolytic pathway helps refund for less ATP from oxidative phosphorylation; however the surplus pyruvate formed is converted into lactate.

Rhodanese (thiosulphate: cyanide sulfurtransferase; EC 2.8.1.1) is a pervasive enzyme that is active in all living organisms and is supposed to be involved in cyanide detoxification [1, 18]. Rhodanese-catalyzed conversion of cyanide to thiocyanate (SCN^-) is one of the particular in vivo metabolic reactions for cyanide detoxification [1]. The role of rhodanese in cyanide detoxification is supported by the high activities of it in some mammalian tissues (e.g., the hepatic tissue) exposed to cyanide [25]. As the preset results show, cyanide administartion caused no significant alteration in rhodanese activity of liver and kidney that is different from previously reported results [7, 12] indicating significant increment of hepatic and renal rhodanese activities in cyanide-dosed rabbits.

Alkaline phosphatase is involved in the hydrolysis of a wide range of phosphomonoester substrates. Significant alterations of ALP activity associated with sublethal long term cyanide exposure have been documented in hepatic and renal tissues of rabbits [7, 9]. Indeed, Okolie and Osagie [24] showed significant decreases in ALP activity in the lungs of rabbits subsequent to chronic exposure to cyanide and suggested the existence of variabilities in tissue susceptibilities to the toxic effect of chronic cyanide exposure. However, the current study results found no significant difference in hepatic and renal ALP activity between two experimental groups.

As noticed above, some variations exist in the literature concerning the effects of cyanide poisoning on the tissue enzyme profile that might be associated to diversities in toxicokinetic parameters of cyanide compounds in various species, utilized dose, route and timing of exposure, tissue susceptibilities, experimental situations and procedures or other unknown factors.

CONCLUSION

Sub lethal cyanide exposure leads to disruption of some biochemical and physiological processes as showed by changes in some metabolic enzyme activities in liver and kidney of rats. However, further research should be focused on this issue for better understanding of the fine mechanism of cyanide effects upon enzyme activities.

ACKNOWLEDGMENTS

This work was supported by grant from Ferdowsi University of Mashhad, Mashhad, Iran. The authors declare that there is no conflict of interests.

REFERENCES

1. Isom GE, Borowitz JL, Mukhopadhyay S. Sulfurtransferase enzymes involved in cyanide metabolism. In: Charlene A.M. editor. Comprehensive Toxicology. Oxford: Elsevier. 2010. pp 485–500.

2. Shwetha A, Hosetti BB, Dube PN. Toxic Effects of Zinc Cyanide on Some Protein Metabolites in Fresh water fish, *Cirrhinus mrigala* (Hamilton). Int J Environ Res 2012; 6(3):769-78.
3. Wiemeyer SN, Hill AF, Carpenter JW, Krynitsky AJ. Acute oral toxicity of sodium cyanide in birds. J Wildlife Dis 1986; 22: 538–46.
4. Oruc HH, Yilmaz R, Bagdas D, Ozyigit MO. Cyanide poisoning deaths in dogs. J Vet Med A Physiol Pathol Clin Med 2006; 53(10): 509-10.
5. Cliff J, Lundquist P, Rosling H, Sorbo B, Wide L. Thyroid function in a cassava-eating population affected by epidemic spastic paraparesis. Acta Endocrinologica 1986; 113: 523-8.
6. Osuntokun BO. Cassava diet, chronic cyanide intoxication and neuropathy in Nigerian Africans. World Rev Nutr Diet 1981; 36: 141-73.
7. Okolie NP, Iroanya CU. Some histologic and biochemical evidence for mitigation of cyanide-induced tissue lesions by antioxidant vitamin administration in rabbits. Food Chem Toxicol 2003; 41: 463–9.
8. Okolie NP, Asonye CC. Mitigation of cataractogenic potential of cyanide by antioxidant vitamin administration. J Med Biomed Res 2004; 3(1): 48–52.
9. Okolie NP, Osagie AU. Liver and kidney lesions and associated enzyme changes induced in rabbits by chronic cyanide exposure. Food Chem Toxicol 1999; 37: 745-50.
10. Sousa AB, Soto-Blanco B, Guerra JL, Kimura ET, Gorniak SL. Does prolonged oral exposure to cyanide promote hepatotoxicity and nephrotoxicity? Toxicology 2002; 174: 87-95.
11. Soto-Blanco B, Gorniak SL. Milk transfer of cyanide and thiocyanate: cyanide exposure by lactation in goats. Vet Res 2003; 34: 213-20.
12. GholipourKanani H, Shahsavani D, Baghishani H. Effect of exposure to sublethal levels of potassium cyanide on serum and tissue enzymes in roach fish (*Rutilus rutilus*). Online J Vet Res 2013; 17(5): 245-55.
13. Kaneko JJ, Harvey JW, Bruss ML. Clinical Biochemistry of Domestic Animals, 5th Ed, Academic Press, London.1999. p. 829-44.
14. Baghshani H, Shahsavani D. Effects of lead acetate exposure on metabolic enzyme activities in selected tissues of common carp (*Cyprinus carpio*). Comp Clin Pathol 2013; 22:903–7.
15. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol 1957; 25: 56–62.
16. Burtis CA, Ashwood ER. Tietz textbook of clinical chemistry, 2nd Ed. Saunders, Philadelphia, 1994.p 625–888, 928–1081.
17. Sorbo B. Crystalline rhodanese: the enzyme catalyzed reaction. Acta Chemica Scandinavia 1953; 7: 1137–45.
18. Baghshani H, Aminlari M. Comparison of rhodanese distribution in different tissues of Japanese quail, partridge, and pigeon. Comp Clin Pathol 2009; 18: 217–20.
19. Jarrar BM, Mahmoud ZN. Histochemical demonstration of changes in the activity of hepatic phosphatases induced by experimental lead poisoning in male white rats (*Rattus norvegicus*). Toxicol Ind Health 1999; 15: 1–9.
20. Rahman MF, Siddiqui MK. Biochemical effects of vepacide (from Azadirachta indica) on Wistar rats during subchronic exposure. Ecotoxicol Environ Saf 2004; 59(3): 332-9.
21- Al-Ghanim KA. Effect of cypermethrin toxicity on enzyme activities in the freshwater fish *Cyprinus carpio*. Afr J Biotechnol 2014. 13(10):1169–73.
22. Elsaid FG, Elkomy MM. Aqueous garlic extract and sodium thiosulphate as antidotes for cyanide intoxication in Albino rats. Res J Med Med Sci 2006; 1(2): 50–6.
23. Manzano H, Sousa AB, Soto-Blanco B, Guerra JL, Maiorka PC, Gorniak SL. Effects of long-term cyanide ingestion by pigs. Vet Res Commun 2007; 31: 93–104.
24. Okolie NP, Osagie AU. Differential effects of chronic cyanide intoxication on heart, lung and pancreatic tissues. Food Chem Toxicol 2000; 38: 543-8.
25. Sylvester DM, Sander CC. Immunohistochemical localization of rhodanese. Histochem J 1990; 22:197–200.