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Aluminium Acetate: Role in Oxidative Metabolism of Albino Mice

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Abstract: Exposure to sublethal dose (3.5 mg kg^{-1}) of aluminium acetate has revealed significant changes in LDH, ICDH, SDH and GDH in brain, liver and kidney of albino mice. These changes are highly significant in multiple dose treated individuals compared to single and double dose aluminium acetate administered mice. In order to understand the energy related alterations in glycolysis and Kreb's cycle the activity levels of the selected dehydrogenases were estimated. The lactate dehydrogenase (LDH) activity showed increase in the tissues, where as succinic dehydrogenase (SDH) and isocitrate dehydrogenase (ICDH) were decreased in the tissues of aluminium acetate administered albino mice. The increased levels of LDH and decreased levels of SDH and ICDH confirms a shift in normal balance of glycolysis in favour of anaerobiasis. Glutamate dehydrogenase (GDH) activity was found to be elevated in all the tissues of aluminium acetate treated mice. The elevated GDH activity levels indicate its contribution to ammonia production and glutamate oxidation.

Key words: Aluminium acetate, LDH, ICDH, GDH, SDH, albino mice

INTRODUCTION

Living organisms maintain their complex order in a dynamic steady state by importing food and energy from their surroundings. They have the machinery needed to liberate and store chemical energy from foods and to create complex molecules from simpler one for the building of new structures. The metabolism involves the transformation by enzyme catalyzed reactions of both matter and energy (Davidson and Sittman, 1995). Depending upon the precise target role of metal, it's higher or lower concentration causes an established effect. Animals accumulate the metals in their tissues and the metals have high biological half- life leading to chronic damage. Aluminium is able to interfere with several biological functions, including enzymatic activities in key metabolic pathways (Zatta *et al.*, 2000). Aluminium acetate is harmful by inhalation, in contact with skin and if swallowed it causes possible risk of irreversible effects. Lactate dehydrogenase (LDH) is a key enzyme of anaerobic glycolysis and catalyzes the reversible oxidation of lactate to pyruvate in the terminal step of glycolysis. Any alteration in LDH activity indicates change in the production of pyruvate to lactate under anaerobic conditions favouring the reoxidation of NADH. Cell membrane damage in cells exposed to aluminium was confirmed by increased LDH release (Sargazi *et al.*, 2003). In view of its role in glucose oxidation, NAD dependent LDH activity levels were assayed to assess the metabolic significance of this enzyme in compensatory mechanism operating in the tissues of mice during aluminium toxicity. Isocitrate dehydrogenase (ICDH) is one of the key enzyme of TCA cycle. It catalyzes the isocitrate to alpha ketoglutarate through an unstable compound oxalosuccinic acid. Succinic dehydrogenase (SDH), a key enzyme of Kreb's cycle, catalyzes the reversible oxidation of succinate to fumerate. SDH is tightly bound to the inner mitochondrial membrane (Murray *et al.*,

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1995). Aluminium chloride caused a significant decrease in activity of succinate dehydrogenase in liver and gastrocnemius muscle, there by indicating altered oxidative metabolisms in these tissues (Chinoy and Memon, 2001). Glutamate dehydrogenase (GDH) is an important detoxification enzyme in nitrogen metabolism in mammals. GDH serves to link aminoacid and carbohydrate metabolism and regulates the level of ammonium ion. Therefore, the present study was designed to determine the aluminium acetate induced alterations in oxidative metabolism in different tissues like brain, liver and kidney of albino mice by selecting specific enzymes such as LDH, ICDH, SDH and GDH.

MATERIALS AND METHODS

Chemicals

Chemicals used in this study namely aluminium acetate was obtained from Sigma, USA. All the other chemicals were obtained from Qualigens and Loba Chemie, India.

Animal Exposure

Healthy adult albino mice (wistar) of same age group 60±2 days and weight 25±5 g were taken from veterinary college, Bangalore, India. The animals were housed at constant temperature (28±2°C) and relative humidity (60±10%)with a 12 h light : 12 h dark cycle. The toxicity of aluminium acetate was estimated as per Finney (1964) and was found to be 35 mg kg^{-1} body weight (Sushma and Jayantha Rao, 2005). Ten fold lower concentration of LD_{50} (3.5 mg kg^{-1} body weight) was selected as sublethal dose. The animals were divided into 4 groups. The first group of animals were considered as controls, received only distilled water without aluminium. To the animals of second group single dose i.e., 3.5 mg kg^{-1} body weight of aluminium actetate was given. Double doses (7 mg kg^{-1}) were given with 72 h interval to the third group of animals on 1st and 4th days. To the fourth group of animals multiple doses (14 mg kg^{-1}) were given with 72 h interval i.e., on 1st, 4th 7th and 10th day. After 72 h both control and experimental animals were sacrificed and the tissues like brain, liver and kidney were isolated in cold conditions. The tissues were stored in deep freezer at -20°C and used for biochemical analysis.

LDH activity in brain, liver and kidney of albino mice was assyed by the method of Srikanthan and Krishnamoorthy (1955). ICDH activity was assayed by Korenberg and Pricer (1951) method as modified by Mastanaiah *et al.* (1977). SDH activity was estimated by the method of Nachlas *et al.* (1960) as modified by Prameelamma and Swami (1975).

The GDH activity was assayed by the method of Lee and Lardy (1965).

RESULTS

In the present study profound changes were observed in oxidative enzymes like LDH, ICDH, SDH and GDH (Table 1) in brain, liver and kidney of control and aluminium acetate treated mice. A significant increase in LDH and GDH activities (Table 1) and decrease in SDH (Table 1) activity were observed in multiple doses of all the three tissues of mice when compared to controls in response to aluminium acetate administration. In the case of aluminium treated mice a significant decrease was observed in ICDH (Table 1).

The percent changes in enzyme activities to aluminium acetate stress were as follows:

LDH-Liver > Kidney > Brain
ICDH-Liver > Brain > Kidney
SDH-Liver > Kidney > Brain
GDH-Liver > Brain > Kidney

Table 1: Changes in LDH, ICDH, SDH and GDH of Aluminium acetate Intoxicated Brain, Liver and Kidney of albino mice (µ moles of formazon formed/mg protein/h)

	Brain				Liver			
	Control	Single dose	Double dose	Multiple dose	Control	Single dose	Double dose	Multiple dose
LDH (µ moles of formazon/mg protein/h)	1.687±0.085	2.076±0.072 (+23.05)	2.610±0.095 (+54.71)	2.979±0.079 (+76.58)	2.086±0.053	2.360±0.057 (+13.13)	2.897±0.080 (+38.87)	3.280±0.071 (+57.23)
ICDH(µ moles of formazon/mg protein/h)	0.682±0.042	0.521±0.031 (-23.60)	0.396±0.061 (-41.93)	0.284±0.053 (-58.35)	0.712±0.032	0.672±0.048 (-5.61)	0.557±0.072 (-21.76)	0.411±0.082 (-42.27)
SDH(µ moles of formazon/mg protein/h)	1.342±0.073	1.248±0.041 (-7.00)	0.980±0.058 (-26.97)	0.642±0.061 (-52.16)	2.645±0.062	2.305±0.056 (-12.85)	1.831±0.078 (-30.77)	1.272±0.085 (-51.90)
GDH(µ moles of formazon/mg protein/h)	0.227±0.009	0.231±0.009 (1.762)	0.262±0.015 (+15.41)	0.306±0.015 (+34.80)	0.168±0.015	0.183±0.014 (+8.92)	0.201±0.017 (+19.64)	0.228±0.016 (+35.71)

	Kidney			
	Control	Single dose	Double dose	Multiple dose
LDH (µ moles of formazon/mg protein/h)	1.595±0.068	1.979±0.039 (+24.07)	2.474±0.073 (+55.10)	2.899±0.069 (+81.75)
ICDH(µ moles of formazon/mg protein/h)	0.595±0.028	0.509±0.037 (-14.45)	0.444±0.041 (-25.37)	0.317±0.078 (-46.72)
SDH(µ moles of formazon/mg protein/h)	2.694±0.073	2.261±0.083 (-16.07)	1.823±0.087 (-32.33)	1.168±0.094 (-56.64)
GDH(µ moles of formazon/mg protein/h)	0.137±0.013	0.144±0.015 (+5.11)	0.161±0.014 (+17.51)	0.188±0.017 (+32.11)

Values are mean±SD of six individual observations. Tissue was pooled from six to eight animals. (Values in parentheses indicate percent change)

DISCUSSION

Aluminium is a neurotoxic agent for animals and humans that has been implicated as an etiological factor in several neurodegenerative diseases and it alters the kreb's cycle enzymes and glutamate dehydrogenase in rat brain homogenate (Zatta *et al*., 2000). Lactate dehydrogenase catalyzes the reversible oxidation of lactate to pyruvate in the terminal step of glycolysis. It forms as a center of delicately balanced equilibrium between anbolism and catabolism of carbohydrates. It is also involved in gluconeogenesis in tissues which lactate is converted to glycogen through glycogenesis. Elevation in the specific activity of LDH as observed in the present investigation (Table 1) and this may be due to toxicity and related oxidative stress of aluminium. Increased LDH activity was reported by Vasantha Sena (2002) in brain and kidney treated with sodium selenite. The LDH activity increases during conditions favouring anaerobic respiration to meet energy demands, when aerobic respiration is lowered (Murray *et al*., 1995). Increased LDH activity in fresh water fish by arsenite treatment was reported by Shobha Rani *et al*. (2000). Aluminium inhibited the activities of oxidative enzymes like ICDH and SDH (Table 1). The drop in ICDH activity denotes the decrement in NAD+. Reduction through NAD-ICDH indicates lesser involvement of TCA cycle in the oxidative reactions. Significant reduction in the ICDH activity might also be due to the direct interaction of aluminium with mitochondria. Decreased ICDH activity was reported in Musbooduga tissues treated with benzene hexachloride (Harold *et al*., 1991). The drop in SDH activity denotes fluctuation of oxidative metabolism and also reflects the turnover of carbohydrates and energy output (Miroslaw, 1973). Decreased SDH activity in liver, kidney tissues of frog, Rana cynophlyctis by cythion treatment was reported by Tantarpalev and Kulkarni (2000). Increased GDH activity was observed in the experimental mice treated with sublethal dose of aluminium acetate. Elevation in GDH activity indicates increased oxidation of glutamate.

Increased GDH activity was reported in rat tissues treated with chromium (Backyavathy, 1986) and selenium (Samson Raju, 2000). Thus in order to understand the energy related alterations in glycolysis and Kreb's cycle, the activity levels of the selected dehydrogenases were estimated.

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