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Protective Effect of Alpha Lipoic Acid on Nitrile Induced Oxidative Destruction of ETC And TCA Cycle Enzymes in Rats Brain

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ABSTRACT:

Iminodipropionitrile, one of the nitrile derivatives induces neurotoxicity. The proposed mechanism postulated generation of free radicals and mitochondrial role in this process. It has been reported that ALA (Alpha lipoic acid) by the virtue of its antioxidant nature may prevent free radical induced neurotoxicity. The present study aimed at investigating the effects of Iminodipropionitrile (250mg/kg body weight) and Alpha lipoic acid (100mg/kg body weight) on the activities of TCA cycle enzymes - ICDH (isocitrate dehydrogenase), SDH (succinate dehydrogenase), α-KDH (Alpha ketoglutarate dehydrogenase) and MDH (malate dehydrogenase) were found to be significantly reduced after Iminodipropionitrile administration. Subsequent treatment with ALA significantly alleviated the depletion in the level of these enzymes. The activities of ETC enzymes- NADH dehydrogenase and Cytochrome C oxidase were evaluated. Iminodipropionitrile administration significantly down regulated these activities whereas ALA restored the levels of these enzymes. It can be concluded that ALA effectively protects the hippocampal mitochondrial damage induced by Iminodipropionitrile.

KEY WORDS: Oxidative stress, Antioxidants, Iminodipropionitrile, Alpha lipoic acid, Neurotoxicity, Mitochondria.

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INTRODUCTION:

Apart from all tissues in the body, brain regions are more susceptible to oxidative stress. Neuronal cells meet huge oxygen demand due to their high-energy expenditure, which particularly constitutes a major site in oxidative damage[1]. Generation of free radicals disrupt the brain redox status that leads to oxidation of membrane lipids, nucleic acids and proteins which lead to degradation of neuronal cells[2].

The mitochondria are involved in diverse processes that modulate cell operation such as cell cycle regulation and apoptosis. Mitochondrial dysfunctions play crucial roles in many neurodegenerative disorders and neuronal damage. The main source of ROS in hippocampal mitochondria is the impairment of oxidative phosphorylation in ATP production. The transfer of electron between the enzyme complexes of electron transport chain[3] generates the superoxide radical. Mitochondrial antioxidant defense mechanisms counteracts these reactive species, but are exhausted when there is inordinate production of free radicals leading to disruption of mitochondrial membrane[4]. Free radicals culminating to membrane transition permeability pores (MTPP) result in insult of various mitochondrial enzymes.

Previous documentations finger towards nitriles causing cellular toxicity[5] and mitochondrial dysfunction[6] that pushes the cell towards apoptosis by the release of Cytochrome C and activation of several Apoptotic factors[3]. Iminodipropionitrile is one of those neurotoxicants that play a key role in the pathophysiology of neuronal damage. Previous reports stated that it caused severe profligation to neuronal cells by enormous generation of oxidative free radicals[7]. Ample literatures suggest that natural compounds with scavenging properties prevent the tissue from the attack of oxidative free radicals[8] generated by Iminodipropionitrile.

Antioxidants play a vital role in affecting various neurodegenerative disorders by quenching reactive free radicals[9]. ALA is a potent antioxidant utilized in prevention and cure of various neuronal diseases as proved by previous reports[10]. Mounting evidences show that ALA acts as a cofactor of enzymes involved in the oxidative phosphorylation in production of ATP in mitochondria. As documented by previous study ALA reprocesses endogenous antioxidants and hence quenches the free radicals due to its dual effect. Therefore, records of earlier investigations revealed the exceptional quality of alpha lipoic acid in mitigating healing effects on free radical induced cellular[5, 11] as well as mitochondrial damage[6, 12]. The goal of our study is to gain insight into the Iminodipropionitrile induced oxidative damage in mitochondria of hippocampal region that is counteracts by antioxidant effect of ALA.

MATERIALS AND METHODS

DRUGS AND CHEMICALS:

Iminodipropionitrile and ALA were purchased from Sigma Aldrich Chemical Company (Bangalore, India) and Hi-Media Lab (Nasik, India) respectively. The remaining chemicals were of highest purity and analytical grade.

ANIMALS:

The study was performed on male albino rats of Wistar strain (average weight 150-180 g), which were obtained from Experimental Animal Care Centre Vel's College of Pharmacy, Chennai, India. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) of Vel's College of Pharmacy, Chennai (Letter No. 290/CPCSEA/PHA-04-08) with CPCSEA Registration No. 290/CPCSEA/12-12-2000. The animals were housed under conditions of control temperature (25±20C) and were acclimatized 12 ± 1 hr day and night rhythm during the experimental period and they were given food and water supplied by Hindustan Lever Ltd., Mumbai, India under the trade name Gold Mohur rat feed and water *ad libitum.* Before experimentation, the animals were deprived of food for 24 hr but allowed free access to water throughout. The experiment was conducted according to strict guidelines of the committee.

EXPERIMENTAL PROTOCOL:

The experimental animals were randomized into following four groups with six rats in each group:

Group 1: Control rats received normal saline (2ml/kg body weight) for 7 days.

Group 2: Rats received Iminodipropionitrile (250mg/kg body weight) dissolved in saline and administered intraperitoneally for 7 days.

Group 3: Rats received Alpha lipoic acid (100mg/kg body weight) alone orally for 7 days.

Group 4: Rats received Alpha lipoic acid (100mg/kg body weight) dissolved in saline and administered by oral gavage once daily 30 minutes before Iminodipropionitrile (250mg/kg body weight) for 7 days.

After the 7 days of experimental period (i.e., on the 8th day), all the animals were anaesthetized and decapitated. Brain tissues were immediately excised and rinsed in ice-cold physiological saline. The hippocampus region was isolated and homogenized in 0.01 M Tris – HCL buffer (pH 7.4) and aliquots of this homogenate were used for the assays. Blood was collected and serum was separated for estimation of biochemical parameters.

BIOCHEMICAL ESTIMATIONS

MITOCHONDRIAL STUDIES

Isolation of Brain mitochondria

The mitochondria of brain were isolated by the method of Johnson and Lardy[13]. 10% (w/v) homogenate was prepared in 0.05 M Tris-HCl buffer containing 0.25 M sucrose and centrifuged at 600 × g for 10 minutes. The supernatant fraction was decanted and centrifuged at 15,000 × g for 5 minutes. The resultant mitochondrial pellet was then washed and resuspended in the same buffer.

Determination of TCA Cycle Enzymes:

The activity of Isocitrate dehydrogenase was assayed by the method of King[14]. The activity of α-ketoglutarate dehydrogenase was assayed by the method of Reed and Mukherjee[15]. The activity of succinate dehydrogenase was assayed according to the method of Slater and Bonner[16]. The activity of malate dehydrogenase was assayed by the method of Mehler[17]. The substrate used was oxaloacetate and determination of enzyme activity was carried out by measuring the rate of oxidation of NADH.

Determination of Enzyme Complexes of Electron Transport Chain:

The activity of NADH dehydrogenase was assayed according to the method of Minakami[18]. Cytochrome C oxidase activity was assayed by the method of Pearl[19].

Statistical Analysis:

All the grouped data were statistically evaluated with Statistical Package for Social Sciences (SPSS), Version 7.5. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. A '*P*' value of less than 0.05 was considered to indicate statistical significance. All the results were expressed as mean + S.D. for six animals in each group.

RESULTS & DISCUSSION

There was significant reduction in the level of mitochondrial TCA cycle enzymes ICDH, KDH, SDH and MDH of hippocampus in Iminodipropionitrile induced rats (group 2) when compared to control rats (Table 1). These enzyme levels were reversed in ALA+ Iminodipropionitrile co-treated rats (group 4) which showed alteration in levels when compared to the Iminodipropionitrile induced rats (group2). The rats receiving ALA alone (group 3) did not show any significant change when compared to control rats (group1) indicating that it does not have any adverse effects.

Table 1: Effect of Acrylonitrile and Alpha lipoic acid on the activities of mitochondrial TCA cycle enzymes in hippocampus of control and experimental rats.

Groups	Control	Acrylonitrile Induced	ALA Alone	ALA+ Acrylonitrile
SDH	43.57 ±2.35	12.65 ±4.55*,[a]	43.94 ±1.47 [NS]	26.25 ±1.35*,[b]
MDH	875.85 ±32.65	553.43 ±26.38*,[a]	870.05 ±31.41 [NS]	711.59 ±26.26*,[b]
KDH	54.27 ±3.97	23.43 ±1.83*,[a]	52.84 ±2.73 [NS]	38.46 ±1.52*,[b]
ICDH	155.57 ±3.88	99.41 ±6.25*,[a]	160.64 ±5.58 [NS]	134.02 ±3.57*,[b]

α-KDH, Alpha ketoglutarate dehydrogenase; SDH, Succinate dehydrogenase; ICDH, Isocitrate dehydrogenase; MDH, Malate dehydrogenase.

Results are expressed as mean ±S.D. for 6 rats. Units: SDH, nmole of succinate oxidized/min/mg protein; MDH, nmole of NADH oxidized/min/mg protein; KDH, nmole of ferricyanide formed/h/mg protein; ICDH, nmole of α-ketoglutarate formed/h/mg protein.

Comparisons are made between the following:

[a]Group I and Group II;

[b]Group II and Group IV;

[NS]Group I and Group III,

*Statistically significant ($p < 0.05$).

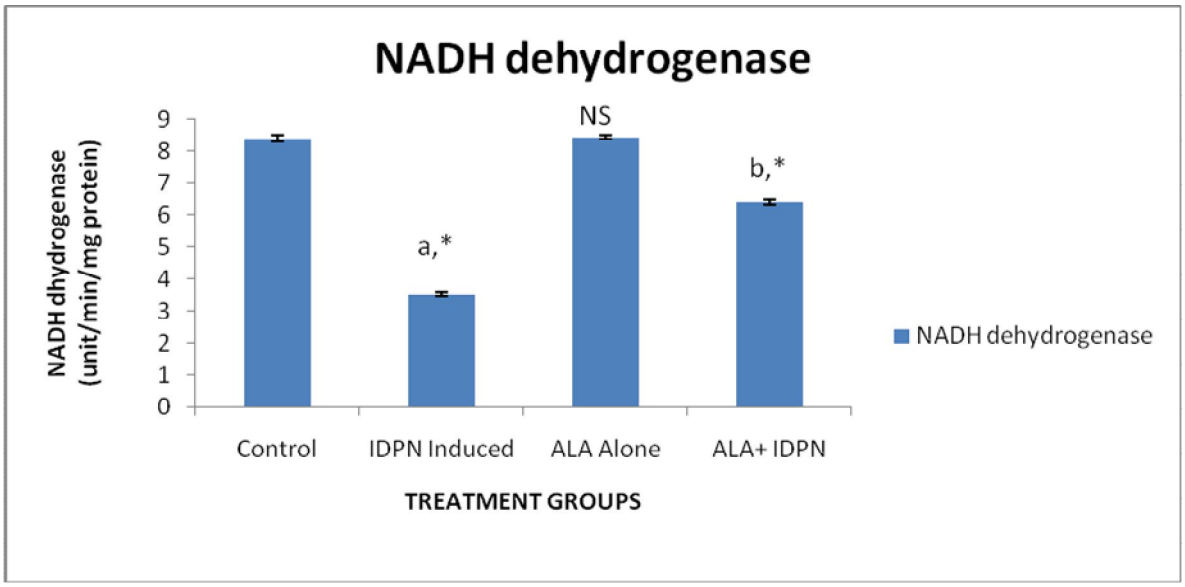

Figure 1: Levels of NADH dehydogenase in the mitochondrial hippocampus of the experimental rats. Results are given as mean ±S.D. for 6 rats.

Comparisons are made between the following: a. Group I and Group II; b. Group II and Group IV; NS. Group I and Group III. *statistically significant ($p < 0.05$); NS, non-significant.

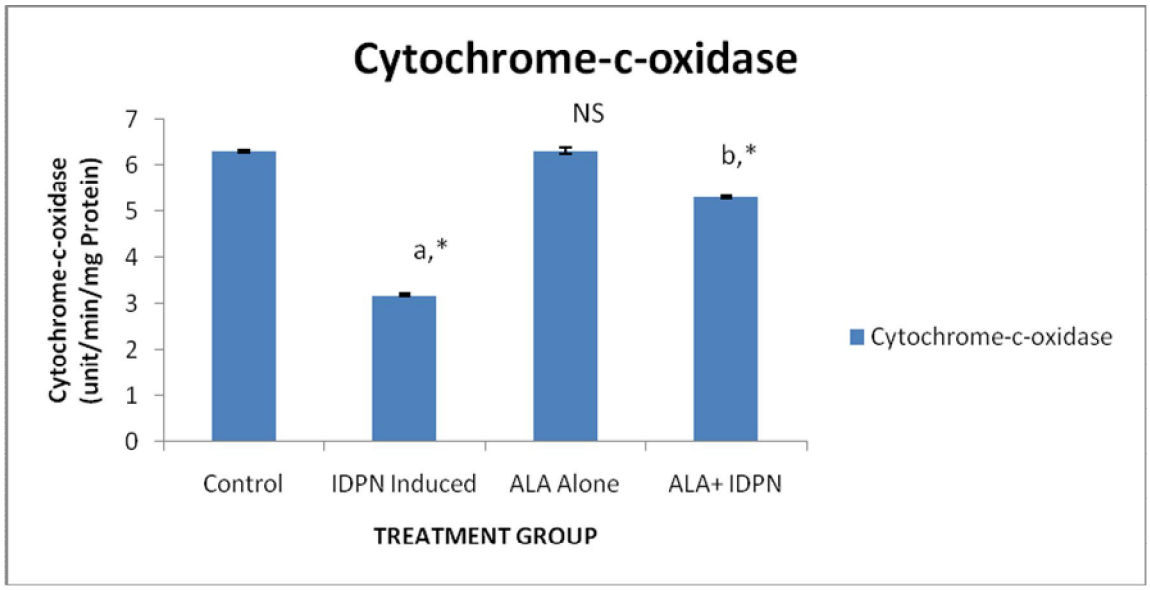

Figure 2: Levels of Cytochrome-C-oxidase in the mitochondrial hippocampus of the experimental rats. Results are given as mean ±S.D. for 6 rats.

Comparisons are made between the following: a. Group I and Group II; b. Group II and Group IV; NS. Group I and Group III. *statistically significant ($p < 0.05$); NS, non-significant.

Graph 1 and Graph 2 state the levels of respiratory marker enzymes NADH dehydrogenase and Cytochrome C oxidase respectively, the levels were significantly increased in mitochondria of hippocampus tissue in Iminodipropionitrile induced rats (group2) when compared to control rats (group1). ALA+ Iminodipropionitrile co treated rats (group 4) reverse the levels of these enzymes. The rats receiving ALA alone (group 3) did not show any significant change when compared to control rats (group 1).

Nitrile derivatives are extensively acquires in day-to-day life, among which Iminodipropionitrile exhibits behavioral syndrome in rodents, which is depicted by hyperactivity and repetitive head movements. Several preceding explorations revealed that Iminodipropionitrile exposure augmented synthesis of reactive species thereby culminating in oxidative stress[20]. Massive free radical generation evoked sub-cellular damage as culminated by previous literatures[3, 21]. ALA is a potent free radical scavenger and possesses the potential to cross blood brain barrier, due to this property, it recovers the neuronal cells from free radical damage[22]. The present investigation is rationalized on the effect of ALA on Iminodipropionitrile induced hippocampal oxidative damage on sub-cellular level, owing to its antioxidant effect and its profound ability to regenerate mitochondrial antioxidants.

Earlier documents have stated that excessive free radical load hampers various complexes of ETC (electron transport chain). NADH-dehydrogenase (flavin-linked) is complex I in ETC, responsible for removing electrons from NADH and transferring them to ubiquinone (Q) for ATP production[9]. Cytochrome-c-oxidase is complex IV in ETC; it carries out the transfer of electron to molecular oxygen thus producing water[3]. Free radicals deplete activity of GSH which decrease production of reducing equivalents NADH and NADPH that causes marked attenuation in activities of NADH-dehydrogenase and cytochrome-c-oxidase[23, 24]. Cytochrome c oxidase was previously documented to be oppresses by nitrile causing generation of more free radicals. Iminodipropionitrile being a nitrile had sufficient capacity to generate enormous free radicals[20] leading to depletion in activity of cytochrome c oxidase. The functional loss in the activity of NADH-dehydrogenase was observed in Iminodipropionitrile treatment due to enormous free radical generation. Previous documented evidence stated that ALA effectively increased the level of NADH dehydrogenase and cytochrome-c-oxidase[25]. However, in this study, administration of ALA ameliorated the levels of these complexes.

H_2O_2 as reported previously[26] directly inhibits alpha ketoglutarate dehydrogenase (α-KDH) and succinate dehydrogenase (SDH). Superoxide reacts with nitric oxide radical to form peroxynitrite[3] which inactivates α-KDH. SDH is also complex II in ETC of mitochondria and its activity is hampers

when its thiol groups are oxidize due to excessive free radicals. Free radicals damage the enzyme isocitrate dehydrogenase (ICDH) which is unable to produce NADPH, thereby leading to decreased production of GSH[23]. Malate dehydrogenase (MDH) gets inactivated by being susceptible to modification and degradation by oxidative free radicals. This study found that Iminodipropionitrile via free radical production inactivates and depletes TCA cycle enzymes like α-KDH, SDH, ICDH and MDH. Substantial amount of evidence proves that ALA preserves and protects the activity and levels of these enzymes. Culminating evidences reported earlier that ALA is involved in decarboxylation reactions by acting as coenzyme for various dehydrogenase complexes in mitochondria[25]. This study found that alpha lipoic acid on contrary increased the abated levels of alpha ketoglutarate dehydrogenase (α-KDH), succinate dehydrogenase (SDH), isocitrate dehydrogenase (ICDH) and malate dehydrogenase (MDH).

The study data concludes that ALA exerts a potent antioxidant action on Iminodipropionitrile induced mitochondrial hippocampal damage. It may have potential consumption in neurotoxic disorders caused by Iminodipropionitrile due to its casual access to all brain cells.

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