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Hippocampal Injury at Mitochondrial Level Provoked by Iminodipropionitrile, Neuroprotective Effect of Alpha Lipoic Acid

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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 (250mg/kg body weight) on hippocampal mitochondrial enzyme activities such as MnSOD (manganese Superoxide dismutase), GPx (glutathione peroxidase), GST (glutathione-s-transferase) and GSH (reduced glutathione) respectively. The damage on mitochondrial membrane was estimated by MDA (malondialdehyde) level measurement were found to be significantly reduced after Iminodipropionitrile administration. Subsequent treatment with ALA significantly alleviated the depletion in the level of these enzymes. 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 counteract 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 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[6]. Ample literatures suggest that natural compounds with scavenging properties prevent the tissue from the attack of oxidative free radicals[7] generated by Iminodipropionitrile.

Antioxidants play a vital role in affecting various neurodegenerative disorders by quenching reactive free radicals[8]. ALA is a potent antioxidant utilized in prevention and cure of various neuronal diseases as proved by previous reports[9]. 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 mitochondrial damage[10]. The goal of our study is to gain insight into the Iminodipropionitrile induced oxidative damage in mitochondria of hippocampal region that is counteracted 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 Albino Wistar rats (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±2 ^{0}C) 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., Banglore, 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. Experimental animals were used after obtaining prior permission and handled according to the University and Institutional legislation as regulated by the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

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[11]. 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 MITOCHONDRIAL ANTIOXIDANT ENZYMES

The mitochondrial superoxide dismutase activity was assayed by the method of Misra and Fridovich[12]. The mitochondrial glutathione peroxidase activity was assayed by the method of Rotruck[13]. The activity of glutathione-s-transferase was assayed by the method of Habig[14]. The reduced glutathione in brain mitochondria was determined according to the method of Moron[15].

DETERMINATION OF MITOCHONDRIAL LIPID PEROXIDES:

The brain mitochondrial lipid peroxide content was determined by the thiobarbituric acid (TBA) reaction described by Ohkawa[16].

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.

RESULT AND DISCUSSION

The activities of mitochondrial antioxidant enzymes MnSOD, GPx and GST were significantly ($p < 0.05$) lower in the mitochondria of hippocampus of Iminodipropionitrile induced rats (group 2) as compared to that of control rats (group 1). Co-treatment of rats with ALA + Iminodipropionitrile (group 4) significantly reversed Iminodipropionitrile induced alterations in the activities of mitochondrial antioxidants (Table 1). The rats receiving ALA alone (group 3) did not show any significant change when compared with control rats (group 1) indicating that it does not produce oxidative stress.

Table 1: Effect of Iminodipropionitrile and Alpha lipoic acid on the activities of mitochondrial enzymic antioxidants in hippocampus of control and experimental rats

Groups	Control (Group 1)	Iminodipropionitrile Induced (Group 2)	ALA Alone (Group 3)	Iminodipropionitrile + ALA (Group 4)
MnSOD	21.98 ± 0.72	11.53 ± 0.92 [a],*	22.18 ± 0.64 [NS]	17.85 ± 0.36 [b],*
GPx	59.25 ±1.08	29.12 ± 1.58 [a],*	60.46 ± 0.97 [NS]	43.95 ± 1.56 [b],*
GST	36.51 ± 0.45	14.17 ± 0.63 [a],*	37.51 ± 1.2 [NS]	26 ± 0.46 [b],*

MnSOD, manganese Superoxide dismutase; GPx, glutathione peroxidase; GST, glutathione-s-transferase; NS-non significant.

Results are expressed as mean ±S.D. for six rats. Units: MnSOD, min/100mg Protein; GPx, min/mg Protein; GST, n moles of CDNB conjugated/min/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 ($p < 0.05$).

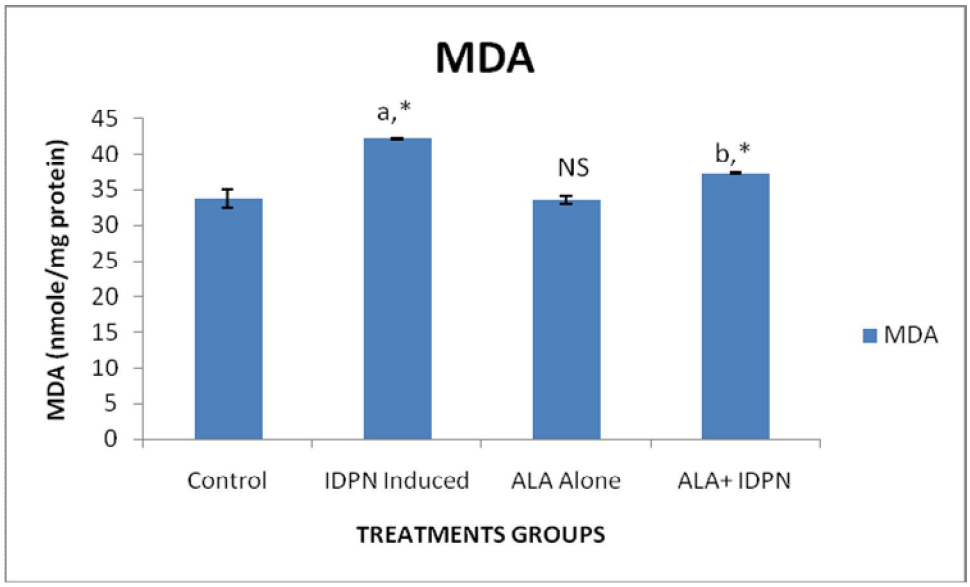

Figure 1: Levels of MDA (malondialdehyde) in the mitochondrial hippocampus of the experimental rats. Results are given as mean ±S.D. for 6 rats

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.

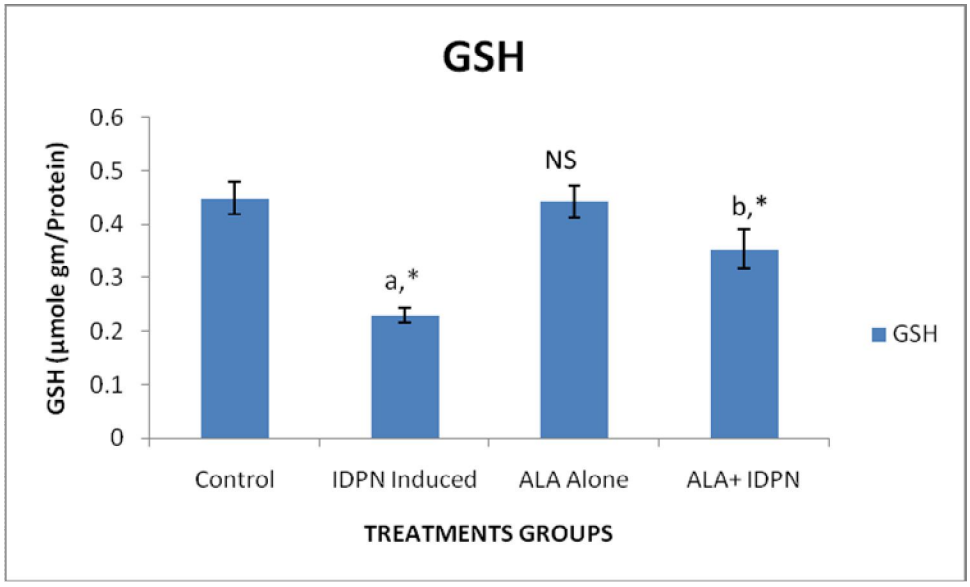

Figure 2: Levels of GSH (Glutathione) in the mitochondrial hippocampus of the experimental rats. Results are given as mean ±S.D. for 6 rats

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 1 shows the levels of mitochondrial MDA were significantly ($p < 0.05$) increased in Iminodipropionitrile induced rats (group 2) when compared to control rats (group 1). The MDA level reversed by the ALA + Iminodipropionitrile treated rats (group 4) which showed the alteration in the level of when compared to Iminodipropionitrile induced rats (group 2). The rats receiving ALA alone (group 3) did not show any significant change when compared with control rats (group 1) indicating that it does not have any adverse effect.

Figure 2 exhibits the level of mitochondrial GSH that was significantly reduced in Iminodipropionitrile induced rats (group 2). ALA+ Iminodipropionitrile co-treated rats (group 4) reverse the mitochondrial GSH level. The rats receiving ALA alone did not show any significant change (group 3) when compared to control rats (group 1) indicating that it did not have any toxic effect up to this concentration (group 2).

Nitrile derivatives are extensively acquired 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[17]. Massive free radical generation evoked sub-cellular damage as culminated by previous literatures[3, 18]. 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[19]. 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.

To combat the oxidative free radicals cells have their own antioxidant defense system comprising of enzymic antioxidants, SOD (superoxide dismutase) which carries out the process of conversion of highly reactive superoxide to hydrogen peroxide, hydrogen peroxide in turn is converted to water and oxygen by the action of CAT (catalase). GPx (glutathione peroxidase) is an enzymic antioxidant, which quenches hydrogen peroxide in the presence of GSH (reduced glutathione). GSH is a cellular non-enzymic antioxidant, which apart from quenching free radicals, maintains the redox status of the cell[20, 21].

Earlier reports revealed that nitriles initiate lipid peroxidation (LPO) by generating free radicals, which cause deleterious effect on mitochondrial membrane (both inner and outer) thereby leading to distortion and loss of membrane integrity[22]. Mitochondrial membrane repercussions provoked by generation of lipid peroxyl radicals ($LOO^{\cdot}$) give rise to degradation product MDA (malondialdehyde)[5],

that can be applied to assess lipid peroxidation in hippocampal mitochondria. Iminodipropionitrile induced rats show increased levels of MDA due to disruption of hippocampal mitochondrial membrane. As assured by indigenous studies, ALA prevents mitochondrial lipid bilayer from oxidative free radical attack[10]. In this study, ALA treatment demonstrated decreased levels of MDA due to its free radical quenching effect and membrane stabilizing effect.

To combat the mitochondrial membrane alteration due to oxidative stress, cells possess inherent antioxidant defense system[4]. GSH is a important constituent of antioxidant defense system, ubiquitous in all brain cells hence it was intriguing to suss-out that intracellular reduced glutathione (GSH) pools in brain cells were exhausted by reactive oxygen species[23]. Much evidence has been conglomerated to state that mitochondrial GSH undergoes oxidation of its thiol (-SH) group due to nitrile intoxication which eventually causes down regulation of mitochondria[24]. Therefore Iminodipropionitrile being a nitrile had potential to deplete the level and activity of GSH. As scrutinized earlier ALA engrosses in the uptake of cysteine therefore involves alterations in levels of GSH[19]. Hence, this study revealed that ALA co-treatment regained the activity of mitochondrial GSH. Mitochondrial antioxidant, manganese superoxide dismutase (MnSOD) is a metalloenzyme, which quenches superoxide radicals by converting to molecular oxygen and hydrogen peroxide. Glutathione peroxidase (GPx) eliminates the hydrogen peroxide by converting to H_2O as reported by previous documents[25]. Nitriles show attenuation in the levels of mitochondrial antioxidants (MnSOD, GPx and GST) by generating enormous free radicals as reported by literatures[5]. Iminodipropionitrile induction depicted lower levels of hippocampal mitochondrial antioxidant enzymes such as MnSOD, GPx and GST. The activity of hippocampal mitochondrial antioxidant enzymes (MnSOD, GPx and GST) was found to be increased upon treatment with ALA.

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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