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Research report

Mitochondrial cofactors in experimental Huntington's disease: Behavioral, biochemical and histological evaluation

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HIGHLIGHTS

- Therapeutic role of mitochondrial cofactors (ALA and ALCAR) in HD was investigated.
- Combined supplementation with ALA and ALCAR ameliorated motor and cognitive deficits.
- Oxidative stress was attenuated by combined supplementation with ALA and ALCAR.
- ALA and ALCAR supplementation improved histological changes observed in HD.

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ABSTRACT

The present study was carried out to evaluate the beneficial effect of mitochondrial cofactors; alpha-lipoic acid (ALA) and acetyl-L-carnitine (ALCAR) in 3-nitropropionic acid (3-NP) induced experimental model of Huntington's disease (HD). HD was developed by administering sub-chronic doses of 3-NP, intraperitoneally, twice daily for 17 days. The animals were assessed for their behavioral performance in terms of motor (spontaneous locomotor activity, narrow beam walk test, footprint analysis and rotarod test) and cognitive (elevated plus maze and T-maze tests) functions. 3-NP treated animals showed impairment in motor coordination such as decreased stride length, increased distance between inner toes, and increased gait angle. Increased transfer latency on elevated plus maze and T-maze tasks revealed cognition deficits in 3-NP treated animals. Increased lipid peroxidation and concomitant decrease in thiol levels were also observed. 3-NP administration also induced histopathological changes in terms of enhanced striatal lesion volume, presence of pyknotic nuclei and astrogliosis. However, combined supplementation with ALA + ALCAR to 3-NP administered animals for 21 days was able to efficiently improve behavioral deficits, attenuate oxidative stress and histological changes, suggesting a putative role of these two supplements if given together in ameliorating 3-NP induced impairments and thus could be engaged in managing HD.

1. Introduction

Huntington's disease (HD) is a neurodegenerative, hereditary disorder characterized by a variety of clinical symptoms. Among the most frequent symptoms are motor deficits involving chorea, emotional and behavioral disturbances manifested as depression and irritability, cognitive impairment and functional disability [1]. The neuropathological changes include progressive neuronal degeneration and atrophy affecting the striatum and other areas of the brain such as cortex, cerebellum, thalamus and sub-thalamic nucleus. This autosomal dominant disease is caused by CAG trinucleotide expansion within the first exon of *huntingtin* gene (*htt*), located near the telomere of the short arm of chromosome 4, locus 4p16.3 [2]. HD is found throughout the world with an estimated global prevalence of 4–5 per 100,000 in all ethnic groups, which makes it one of the most prevalent neurological diseases [3].

3-Nitropropionic acid (3-NP) induced experimental model of HD has been universally accepted and aides in phenotypic replication of chronic neurodegenerative processes involved in HD pathology [4]. This animal model have shown to mimic characteristic features such as neurobehavioral impairments involving alteration in impaired locomotor activity, gait disturbances and cognitive decline followed by striatal degeneration concomitant with astrogliosis as those observed in HD [5].

Mitochondria are considered as a major source of reactive oxygen species (ROS) generation and mitochondrial dysfunctions are believed to be involved in aging and a number of neurological disorders including HD [6]. Therefore, improving mitochondrial

Abbreviations: ALA, alpha-lipoic acid; ALCAR, acetyl-L-carnitine; DAB, 3,3′-diaminobenzidine; 3-NP, 3-nitropropionic acid; GFAP, glial fibrillary acidic protein; GSH, glutathione; HD, Huntington's disease; MDA, malondialdehyde; TTC, 2,3,5-triphenyltetrazolium chloride.

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function has now become a prime focus to combat neurodegeneration in HD. Mitochondrial cofactors, particularly acetyl-L-carnitine (ALCAR) and alpha-lipoic acid (ALA) have shown to be effective in reducing age-related mitochondrial dysfunction and their combination may decrease oxidative damage to neurons and improve locomotor and cognitive deficits [7]. ALCAR, an acetyl derivative of L-carnitine, is actively transported across the blood brain barrier and is required for the transport of long-chain fatty acids into the mitochondria for β-oxidation, ATP production and removal of excess short- and medium-chained fatty acids, thus helps to maintain efficient mitochondrial function [8]. Additionally, ALCAR participates in cellular energy production and in maintenance and repair of damaged neurons [9]. ALA also readily crosses the blood–brain barrier where it is reduced to dihydrolipoic acid, which is a powerful mitochondrial antioxidant that recycles cellular antioxidants, including coenzyme Q, vitamin C and vitamin E, glutathione (GSH) and also chelates transition metals like iron and copper [10]. Combined supplementation with ALA and ALCAR had been reported to be more effective than using either alone, in improving acquisition or memory performance in aged rats [11]. In a recent study, supplementation with a combination of both ALA and ALCAR were found to be effective in improving cognitive and motor performance [12]. The mechanism involved in protective effect offered by ALA and ALCAR appears to involve increased mitochondrial biogenesis [13].

Therefore, the purpose of the present study was to provide a more comprehensive behavioral assessment in 3-NP induced rat model of HD and to evaluate if the combination of mitochondrial cofactors possesses the propensity to improve behavioral, biochemical and histological changes. To this end, various locomotor and cognitive tasks were performed on rats to assess gait deformities, hind-limb impairment and muscular coordination. 3-NP induced oxidative stress was evaluated in terms of lipid peroxidation products and GSH levels. Lesion volume was measured using TTC staining (a dye, which is used as a marker for the presence of active dehydrogenases) and histopathological changes using hematoxylin–eosin and nissl staining were also conducted to evaluate the extent of neuro-anatomical damage produced by 3-NP administration. Here we report for the first time that, even though individual supplementation with ALA and ALCAR produced beneficial effect on various motor and cognitive performance tasks along with biochemical and histopathological changes, nevertheless combined supplementation (ALA + ALCAR) was more effective in reversing 3-NP induced changes.

2. Experimental procedures

2.1. Chemicals

All the chemicals used in the present study were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, MO, USA), Merck (Mumbai, India) and Sisco Research Laboratories Pvt. Ltd. (Mumbai, India). ALA and ALCAR were received as a gift for research purposes from Sami Labs limited (Bangalore, India), GFAP polyclonal antibody was procured from Abcam Plc (Cambridge, UK). Secondary anti-rabbit antibody was obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Animals and treatment schedule

Female wistar rats aged 9–10 weeks, weighing between 200 and 250 g were procured from the Central Animal House facility of Panjab University, Chandigarh, India. The animals were allowed to acclimatize to the local *vivarium* for 7 days. All the experiments were carried out between 09:00 and 15:00 h. The protocols followed were approved by the Institutional Animal Ethics Committee of the University and were in accordance with the guidelines for humane use and care of laboratory animals. The body weights of the animals were recorded daily and were randomly segregated into following eight groups with each group having five animals:

Control (vehicle): Animals received vehicle alone.
ALA treated: Animals were administered ALA at a dose of 50 mg/kg (i.p) for 21 days.
ALCAR treated: Animals were administered ALCAR at a dose of 100 mg/kg (i.p) for 21 days.
ALA + ALCAR treated: Animals were administered with combination of ALA + ALCAR at doses mentioned above.
3-NP treated: Animals were administered 3-NP at a sub-chronic dose twice a day intraperitoneally for 17 days. 7.5 mg/kg for the first 2 days, followed by 3.75 mg/kg for next 7 days, finally a dosage of 2 mg/kg for the last 8 days. The dose of 3-NP used in the study is based on the doses reported in literature and were standardized in our laboratory [14].
3-NP + ALA treated: 3-NP treated animals were supplemented with ALA (50 mg/kg, i.p), once daily for 21 days.
3-NP + ALCAR treated: 3-NP treated animals were supplemented with ALCAR (100 mg/kg, i.p) once daily for 21 days.
3-NP + ALA + ALCAR treated: 3-NP treated animals were supplemented with combination of ALA (50 mg/kg, i.p) + ALCAR (100 mg/kg, i.p) once daily for 21 days.

2.3. Behavioral studies

After the completion of respective dosages for each group, animals were assessed for locomotor and cognitive impairments using various neurobehavioral tasks.

2.3.1. Locomotor activity tests

2.3.1.1. Actophotometer test. The locomotor activity was measured using actophotometer [15]. The interruption of a beam of light falling on a photocell following the movement of the animal was recorded. Each rat was placed individually in the actophotometer for 3 min and the counts were recorded.

2.3.1.2. Narrow beam walk test. This behavioral test was used to evaluate motor performance in the animals, by progressively increasing the difficulty in the execution of the task as described by Masoud et al. [16]. The animals were trained in crossing a 150 cm long wooden beam, divided into three 50 cm segments, from a platform at one end to the animal's home cage at the other end, placed horizontally 60 cm above the floor. The number of paw slips onto an under-hanging ledge and the time taken to traverse the beam was recorded. The maximum time allowed for the task was 2 min. Occurrence of bradykinesia was quantified by calculating the average velocity of walking for treated and control animals.

2.3.1.3. Rotarod test. Rotarod treadmill test was performed to assess muscular strength of the animals in all groups [17]. The rotarod apparatus (IMCORP Instruments, Ambala, India) consisted of a rotating rod, 75 mm diameter, on which rats were allowed to hold. After twice daily training for 2 successive days (speed 8 rpm on the first day and 10 rpm on second day) the rotational speed of the rod was increased to 15 rpm on the third day in a test session. The time for each rat to remain on the rotating rod was recorded. The maximum time was 120 s per trial. The apparatus automatically records the time of fall. The animals were trained on the rod, so that they could stay on it at least for the cut-off time. Data were presented as retention time on the rotating rod.