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Mitochondrial modulators in experimental Huntington's disease: reversal of mitochondrial dysfunctions and cognitive deficits

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ABSTRACT

Huntington's disease (HD) is a chronic neurodegenerative condition involving impaired mitochondrial functions. The present study evaluates the therapeutic potential of combined administration of mitochondrial modulators: alpha-lipoic acid and acetyl-l-carnitine on mitochondrial dysfunctions in 3-NP–induced HD. Our results reveal 3-NP administration resulted in compromise of mitochondrial functions in terms of: (1) impaired activity of mitochondrial respiratory chain enzymes, altered cytochrome levels, reduced histochemical staining of complex-II and IV, reduced in-gel activity of complex-I to V, and reduced mRNA expression of respiratory chain complexes; (2) enhanced mitochondrial oxidative stress indicated by increased malondialdehyde, protein carbonyls, reactive oxygen species and nitrite levels, along with decreased Mn-superoxide dismutase and catalase activity; (3) mitochondrial structural changes measured by mitochondrial swelling, reduced mitochondrial membrane potential and ultrastructure changes; (4) increased cytosolic cytochrome c levels, caspase-3 and -9 activity along with altered expression of apoptotic proteins (AIF, Bim, Bad, and Bax); and (5) impaired cognitive functions assessed using Morris water maze and Y-maze. Combination of mitochondrial modulators (alpha-lipoic acid + acetyl-l-carnitine) on the other hand ameliorated 3-NP–induced mitochondrial dysfunctions, oxidative stress, histologic alterations, and behavioral deficits, suggesting their therapeutic efficacy in the management of HD.

1. Introduction

Huntington's disease (HD) is a hereditary autosomal-dominant disorder of the central nervous system (Zheng and Diamond, 2012). HD usually occurs in individuals in their mid 40s, with some exceptional cases of early onset (2 years of age) and late onset (in the mid 80s) (Finkbeiner, 2011; Vis et al., 1999). The underlying genetic defect involves abnormal expansion of the CAG triple repeats (>40) in exon 1 of huntingtin gene (*htt*) (HDCRG, 1993). The *htt* gene located near the telomere of the short arm of chromosome 4 (locus 4p16.3) encodes for huntingtin (*Htt*) protein (Lin and Beal, 2006). Although, mutation in *htt* gene was discovered more than 17 years ago, the role of *Htt* in pathophysiology of HD is still under investigation (Maroof et al., 2011). The most striking neuropathologic hallmark of this disorder is the atrophy of the striatal region, that controls movement, memory, and emotions, suggesting that striatal degeneration is an important aspect of the pathophysiology of HD (Rosas et al., 2002). In patients with HD, selective loss of medium spiny neurons has not only been observed in the caudate and putamen of the striatum of basal ganglia but also in pyramidal neurons of the cerebral cortex and to lesser extent in hippocampal and subthalamus neurons (Vonsattel et al., 1985). Neuronal loss to an extent of 80% has been observed in patients with HD (Ross, 2004).

Mitochondrial dysfunctions are suggested to be involved in the pathogenesis of HD (Lin and Beal, 2006). Evidence suggests that HD may be associated with impaired respiratory chain functions that can lead to an increase in reactive oxygen species (ROS) production (Colle et al., 2013). Activity of mitochondrial complexes II, III, and IV, including aconitase enzyme, are reported to be reduced in the caudate and putamen of HD brains (Beal et al., 1993). The hypothesis that mitochondrial dysfunctions contribute to the pathogenesis of HD was first tested pharmacologically with the use of 3-nitropropionic acid (3-NP), an irreversible inhibitor of succinate dehydrogenase (Brouillet et al., 1998). The 3-NP–induced HD model replicates most of the clinical and pathophysiologic hallmarks of HD, such as spontaneous choreiform and dystonic movements and cognitive deficits, including progressive degeneration of striatal

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tissue (Tasset et al., 2012). Specific motor abnormalities and cognitive deficits, including working memory deficits, also can be easily replicated in animals treated with 3-NP (Mehrotra and Sandhir, 2014). One of the mechanisms after the administration of 3-NP is the development of mitochondrial dysfunctions leading to bioenergetic failure. This bioenergetic failure involves three interacting processes: energy impairment, oxidative stress, and excitotoxicity (Damiano et al., 2010).

Several metabolic modifiers have shown to ameliorate mitochondrial dysfunctions and oxidative stress in HD but have been found to be beneficial to a limited extent (Virmani et al., 2005; Hersch and Rosas, 2008). Therefore, improving mitochondrial functions has now become a prime focus to combat neurodegeneration in HD. Mitochondrial cofactors, particularly acetyl-l-carnitine (ALCAR) and alpha-lipoic acid (ALA) in combination, have been shown to effectively ameliorate mitochondrial dysfunctions, reduce oxidative damage to neurons, and improve behavioral functions in animals (Zaitone et al., 2012). 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 helping to maintain efficient mitochondrial functions (Rebouche, 1992). ALCAR also participates in cellular energy production and in repair of damaged neurons (Barnes et al., 1990). ALA also readily crosses the blood–brain barrier, where it is reduced to dihydrolipoic acid, a powerful mitochondrial antioxidant that recycles cellular antioxidants, including coenzyme Q, vitamin C, vitamin E, and glutathione (GSH), and also chelates transition metals like iron and copper (Moini et al., 2002). Combined supplementation with ALA and ALCAR has been reported to be more effective than the use of either alone in improving acquisition or memory performance in aged rats (Liu et al., 2002). In a recent study, supplementation with a combination of both ALA and ALCAR was found to be effective in improving cognitive and motor functions (Milgram et al., 2007). ALA and ALCAR combined supplementation have been shown to ameliorate age-associated mitochondrial functional changes (Shenk et al., 2009), mitochondria-associated structural damage (Aliev et al., 2009a) and oxidative stress in animals (Sun et al., 2012).

Therefore, the aim of the present study was to evaluate the propensity of combination of mitochondrial cofactors; ALA and ALCAR in ameliorating 3-NP–induced behavioral, biochemical, and histological changes.

2. Experimental procedures

2.1. Chemicals

All the chemicals used in the present study were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA), Merck Limited (Mumbai, India), Himedia Laboratories (Mumbai, India), Rankem (Bangalore, India), Qualigens Fine Chemicals (Mumbai, India), Loba Chemie (Mumbai, India), and Sisco Research Laboratories Pvt. Ltd. (Mumbai, India). ALA and ALCAR were gifts from Sami Labs Limited (Bangalore, India).

2.2. Animals and treatment schedule

Female Wistar rats weighing between 225 and 235 g (age 9–10 weeks) were procured from the Central Animal House of the University. The animals were allowed to acclimatize to the local vivarium for 7 days. 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. Animals were randomly segregated into following 8 groups with each group having 5 animals.

Control (Vehicle): Animals received vehicle alone.

ALA-treated: Animals were administered ALA at a dose of 50 mg/kg (intraperitoneally) for 21 days.

ALCAR-treated: Animals were administered ALCAR at a dose of 100 mg/kg (intraperitoneally) for 21 days.

ALA + ALCAR- treated: Animals were administered with combination of ALA + ALCAR at doses mentioned previously.

3-NP–treated: Animals were administered 3-NP at a subchronic dose twice a day intraperitoneally for 17 days; 7.5 mg/kg for 2 days, followed by 3.75 mg/kg for 7 days and at a dose of 2 mg/kg for 8 days. The dose of 3-NP used in the study is based on the doses reported in literature (Cirillo et al., 2010) and was optimized in the laboratory (Mehrotra and Sandhir, 2014).

3-NP+ALA–treated: 3-NP–treated animals were supplemented with ALA (50 mg/kg, intraperitoneally), once daily for 21 days.

3-NP+ALCAR–treated: 3-NP–treated animals were supplemented with ALCAR (100 mg/kg, intraperitoneally) once daily for 21 days.

3-NP+ALA+ALCAR treated: 3-NP–treated animals were supplemented with combination of ALA (50 mg/kg, intraperitoneally) + ALCAR (100 mg/kg, intraperitoneally) once daily for 21 days.

2.3. Isolation of rat brain mitochondria

On day 21, animals were sacrificed by decapitation under mild ether anesthesia. Mitochondria were isolated from the striatum by the method as described earlier with minor modifications (Cardoso et al., 2008). The purity of isolated mitochondria was evaluated by assessing the activity of succinate dehydrogenase (SDH).

2.4. Estimation of mitochondrial respiratory chain functions

The activities of mitochondrial respiratory chain enzymes were studied in the mitochondria isolated from the striatum of various groups. The activity of nicotinamide adenine dinucleotide (NADH) dehydrogenase (Complex I) was measured as described by King and Howard (1967). Activity of SDH (Complex II) was assayed according to the method of King et al. (1976). The activity of cytochrome oxidase (Complex IV) was assayed according to the method described by Sottocasa et al. (1967). The mitochondrial F_1F_0 synthase (Complex V) activity was measured as described by Griffiths and Houghton (1974). The 3-(4,5-dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed to assess the activity of the respiratory chain dehydrogenases present in the isolated mitochondria as described by Liu et al. (1997). The enzymatic activities of all the mitochondrial respiratory chain enzymes were normalized by the activity of citrate synthase as described by the method of Silva et al. (2013). Cytochrome levels in mitochondrial fractions were measured according to the method of Kumar et al. (2008).

2.5. Histochemical staining

The frozen brain sections were processed for SDH (Brouillet et al., 1998) and cytochrome oxidase histochemical staining as described by Gonzalez-Lima and Jones (1994). Animals were anesthetized and transcardially perfused with cold 0.1 M phosphate-buffered saline (PBS), pH 7.4, followed by cold 10% (v/v) glycerol in PBS. Twenty-micron-thick frozen sections of the brain were dried for 30 minutes and then activated in PBS at 37 °C for 10 minutes. The sections for SDH staining were incubated in a