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Administration of CoQ_{10} analogue ameliorates dysfunction of the mitochondrial respiratory chain in a mouse model of Angelman syndrome

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ABSTRACT

Genetic defects in the *UBE3A* gene, which encodes for the imprinted E6-AP ubiquitin E3 ligase (UBE3A), is responsible for the occurrence of Angelman syndrome (AS), a neurodegenerative disorder which arises in 1 out of every 12,000–20,000 births. Classical symptoms of AS include delayed development, impaired speech, and epileptic seizures with characteristic electroencephalography (EEG) readings. We have previously reported impaired mitochondrial structure and reduced complex III in the hippocampus and cerebellum in the *Ube3a*$^{m-/p+}$ mice. CoQ_{10} supplementation restores the electron flow to the mitochondrial respiratory chain (MRC) to ultimately increase mitochondrial antioxidant capacity. A number of recent studies with CoQ_{10} analogues seem promising in providing therapeutic benefit to patients with a variety of disorders. CoQ_{10} therapy has been reported to be safe and relatively well-tolerated at doses as high as 3000 mg/day in patients with disorders of CoQ_{10} biosynthesis and MRC disorders. Herein, we report administration of idebenone, a potent CoQ_{10} analogue, to the *Ube3a*$^{m-/p+}$ mouse model corrects motor coordination and anxiety levels, and also improves the expression of complexes III and IV in hippocampus CA1 and CA2 neurons and cerebellum in these *Ube3a*$^{m-/p+}$ mice. However, treatment with idebenone illustrated no beneficial effects in the reduction of oxidative stress. To our knowledge, this is the first study to suggest an improvement in mitochondrial respiratory chain dysfunction via bioenergetics modulation with a CoQ_{10} analogue. These findings may further elucidate possible cellular and molecular mechanism(s) and ultimately a clinical therapeutic approach/benefit for patients with Angelman syndrome.

Introduction

Genetic defects in the *UBE3A* gene which encodes for E6-AP ubiquitin-protein ligase E3A (UBE3A), also known as E6-AP ubiquitin protein ligase, are responsible for the occurrence of Angelman syndrome (AS), a neurodegenerative disorder that arises in 1 in every 12,000–20,000 births (Hasegawa et al., 2012; Kishino et al., 1997; Knoll et al., 1989). Symptoms of AS include delayed development, severely impaired speech, ataxia, microcephaly and epileptic seizures with characteristic EEG readings (Bailus & Segal, 2014; Bird, 2014). Maternal deletions or paternal uniparental disomy of chromosomal 15q11-13 region accounts for 70% and 7%, respectively of Angelman syndrome. An additional 11% is due to point mutations or deletions of the *UBE3A* gene and 3% is accounted for by imprinting center defects (Kishino et al., 1997; Knoll et al., 1989; Jiang et al., 1998a; Jiang et al., 1998b; Nicholls et al., 1998).

Ubiquitin E3 ligase is important in several cellular functions, including protein degradation, protein transport, endocytosis and protein–protein interactions. Jiang et al. (1998a) generated and characterized the *Ube3a*$^{m-/p+}$ as an Angelman mouse model, having a deletion of the maternal *UBE3A* copy (Jiang et al., 1998a; Jiang et al., 1998b). Due to paternal imprinting, the *UBE3A* gene is silenced in certain brain regions, including the hippocampus and cerebellum, resulting in a lack of the UBE3A protein expression (Kishino et al., 1997; Jiang et al., 1998a). These mice exhibit pathology characteristic of Angelman syndrome, including motor coordination issues (ataxia), microcephaly, and epileptic-like seizures. These mice also display defects in the hippocampal long-term potentiation and cerebellar motor function (Huang et al., 2013; Gabriel et al., 1999). Our previous studies have demonstrated that hippocampal mitochondria of *Ube3a*$^{m-/p+}$ mice are small and dense with disorganized cristae. These mice also depict a reduction of complex III activity in the hippocampal region of the brain (Su et al., 2011). Several diseases with similar symptoms to AS, such as Rett

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syndrome have mitochondrial abnormalities (Condie et al., 2010; Gold et al., 2014). Our initial results that were suggestive of mitochondrial dysfunction in human AS led to this current investigation.

Idebenone is a CoQ_{10} analogue, the predominant form of ubiquinone in humans. To date, the only agents which have shown some therapeutic potential have been CoQ_{10} and its synthetic analogues. Idebenone is currently being used for the treatment of mitochondrial respiratory chain (MRC) disorders, which have been difficult to treat. We report that administration of idebenone, a CoQ_{10} analogue, to the *Ube3a*$^{m-/p+}$ mouse system corrects motor coordination and anxiety levels, but does not affect brain size, sociability or memory by novel object recognition (NOR) assay. We report that CoQ_{10} treatment also improves the expression of complexes III and IV in the neurons of hippocampus CA1, CA2, and CA3 regions of the *Ube3a*$^{m-/p+}$ mice. In addition, we report that oxidative stress measured by levels of glutathione disulfide (GSSG:GSH) and 4-HNE were increased in the cerebellum and hippocampus of *Ube3a*$^{m-/p+}$ mice, when compared to WT controls. To our knowledge, this is the first study to suggest an improvement in the dysfunction of the mitochondrial respiratory chain with a CoQ_{10} analogue, further elucidating a possible cellular/molecular mechanism(s) and ultimately potential therapeutic benefits for patients with Angelman syndrome.

Materials and methods

Ethical statement

All experiments were done with the approval of the Institutional Animal Care and Use Committee (IACUC) of the University of California, Irvine (UCI) (IACUC Protocol #2007-2716-2), and in accordance with the guidelines established by the National Institutes of Health (NIH). Animals were housed in the vivarium and maintained under constant temperature (22 °C) and humidity with a controlled 12:12-hour light–dark cycle. Mice were provided standard rodent chow (Harlan Teklad Rodent Diet, Madison, WI) and water ad libitum.

Idebenone administration

Three-week old WT and *Ube3a*$^{m-/p+}$ mice on a C56BL/6 J background were randomly sorted into either treatment or control groups (n = 8–10 per group). Idebenone at 200 mg/kg body weight dissolved in corn oil was administered to the WT and *Ube3a*$^{m-/p+}$ groups by oral gavage, whereas the control groups received corn oil (vehicle), three times a week for three months. No adverse effects were noted with either treatment. Body and brain weights from the wild type and *Ube3a*$^{m-/p+}$ mice were recorded.

Behavioral studies

Rotarod performance test

To assess performance measurements, treated and untreated WT and *Ube3a*$^{m-/p+}$ mice were placed on the Rotarod apparatus, which was set to accelerate from 4 to 40 rpm in 5 min. Mice performed three trials with 45-minute to 60-minute inter-trial intervals on each of two consecutive days. Rotarod measurements were taken before and after idebenone administration.

Marble burying assay

Treated and untreated WT and *Ube3a*$^{m-/p+}$ mice were analyzed with the marble burying assay. Briefly, a cage was filled 5–10 cm deep with bedding spread evenly where twelve black glass marbles, evenly spaced, were placed on the surface of the bedding in four rows of three. Idebenone treated or untreated wild type or *Ube3a*$^{m-/p+}$ mice were placed separately in the cage and left undisturbed for 20 min. The number of marbles buried to 2/3 their depth was then counted and analyzed as previously described (Angoa-Perez et al., 2013).

Social three-chamber assay

The sociability assay was performed as previously described (Kaidanovich-Beilin et al., 2011; Silverman et al., 2010) to evaluate the sociability of treated and untreated WT and *Ube3a*$^{m-/p+}$ mice. Briefly, a rectangular three-chambered box apparatus was used (divided by clear plexiglass walls) with rectangular openings allowing access into each chamber. While one side contained an empty container, the other side of the container housed a mouse. The container had small bars to allow for social interaction. WT or *Ube3a*$^{m-/p+}$ mice treated/untreated with idebenone were first placed in the middle chamber and allowed to acclimatize for 10 min. Once the mouse was acclimatized, barriers separating the two side chambers were removed. The test mouse was left for 10 min to habituate to side chambers with no mouse present (empty apparatus). Finally, the mouse was given 10 min to interact and explore the three chambers when the mouse was present. The "social index" was measured as (chamber time in social chamber − chamber time in object chamber) / (total time in side chambers).

Novel object recognition (NOR) test

In order to assess memory recognition in the treated and untreated WT and *Ube3a*$^{m-/p+}$ mice, we performed novel object recognition (NOR) tests. Briefly, two novelty objects were placed into a sterile cage and fixed in place. Treated and untreated WT and *Ube3a*$^{m-/p+}$ mice were placed alone in the cage. The mouse was left for 15 min to explore and acclimate to the new environment before being moved back to its original cage. This was repeated for all mice twice on day 1 and day 2. On day 3, the acclimation step was repeated in the morning. In the afternoon (at least a 3 hour gap), one of the novel objects was replaced with a new object. Mice were then placed in the cage for 5 min. Mice interactions with the novel objects were recorded. Analysis of assay: Exploration was scored when the mouse touched an object with its forepaws, snout, licked, or sniffed the object from a distance of no more than 1.5 cm. The novelty index (NI) was calculated as NI = (Tn − Tr) / (Tn + Tr) (Silvers et al., 2007). 'Tn' represented the time exploring a novel object and 'Tr' the duration of a familiar object exploration.

Seizure activity and electroencephalogram (EEG) testing

Seizure activity in WT and *Ube3a*$^{m-/p+}$ mice (idebenone-treated and untreated) was monitored. Briefly, seizures were induced by scratching with a pair of long blunt forceps over the cage grid for 45 s and mice were visually monitored. All mice tested were 4–5 months of age. Inducible seizures (typically lasting 10–20 s) were noted when mice responded by increased activity including running and leaping followed by rigid extension of limbs and body (tonic and clonic seizures), as previously described (Jiang et al., 1998b).

In order to examine the characteristic brain activity in the treated and untreated WT and *Ube3a*$^{m-/p+}$ mice, we performed EEG analysis as previously described (Cattanach et al., 1992). Bipolar depth electrodes (PlasticsOne, Roanoke, VA) and optical fibers (0.37 NA, Low OH, 200 μm diameter, ThorLabs, Newton, NJ) terminated in 1.25 mm ceramic ferrules (Kientec Systems, Inc., Stuart, FL) were implanted ipsilaterally (posterior 2.5 mm, left 1.75 mm, ventral 1.25 mm with respect to bregma) and in some cases, also contralaterally at the same posteroventral position into the hippocampus, targeting the dorsal stratum oriens of the CA1 so that emitted light would illuminate the hippocampal formation. Optical fibers and electrodes were fixed to the skull using screws and dental cement (Teets Cold Curing, Sylmar, CA) and the animals were allowed to recover for several days before beginning the 24 hour video and EEG monitoring for seizures and subsequent closed-loop seizure detection and light delivery. EEG activity was monitored by data acquisition system via a lightweight, flexible, shielded, grounded multi-wire cable to ensure optimal recording conditions.

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