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Research report

Effects of long-term memantine on memory and neuropathology in Ts65Dn mice, a model for Down syndrome

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ABSTRACT

Memantine is a partial NMDA receptor antagonist that has been shown to improve learning and memory in several animal models, and is approved for the treatment of Alzheimer's disease (AD). Chronic treatments using memantine in animal models of Alzheimer's disease show disease-modifying effects and suggest a potential neuroprotective function. The present study assessed the effects of both short- and long-term memantine treatment in a mouse model of Down syndrome (DS), the Ts65Dn mouse. The Ts65Dn mouse contains a partial trisomy of murine chromosome 16, and exhibits hippocampal-dependent memory deficits, as well as progressive degeneration of basal forebrain cholinergic neurons (BCFNs). Ts65Dn mice were treated with memantine for a period of 6 months, beginning at 4 months of age. At the end of treatment the mice underwent memory testing using novel object recognition and water radial arm maze tasks, and then histologically analyzed for markers of neurodegeneration. Memantine treatment improved spatial and recognition memory performance in the Ts65Dn mice, though not to the level of normosomic littermate controls. Despite these memory improvements, histological analysis found no morphological signs of neuroprotection of basal forebrain cholinergic or locus coeruleus neurons in memantine-treated Ts65Dn mice. However, memantine treatment of Ts65Dn mice gave rise to elevated brain-derived neurotrophic factor expression in the hippocampus and frontal cortex, suggesting a mechanism of behavioral modification. Thus, our findings provide further evidence for memory facilitation of memantine, but suggest pharmacological rather than neuroprotective effects of memantine both after acute and chronic treatment in this mouse model.

1. Introduction

Down syndrome (DS) is the most common genetic cause of mental retardation, resulting from a trisomy of chromosome 21. In addition to developmental disabilities and learning impairments, DS individuals acquire the symptoms of dementia in the 4th and 5th decades of life at high rates and show neuropathology consistent with Alzheimer's disease (AD) with near uniformity [17]. While the early-onset dementia and neuropathology that develops in DS has much in common with that seen in AD, it is not clear whether cognitive decline in DS individuals arises through similar biological mechanisms.

Currently the most complete animal model for DS is the Ts65Dn mouse, which contains a partial trisomy of murine chromosome 16 confined to the region homologous to human chromosome 21. Ts65Dn mice exhibit characteristic age-related pathology associated with both AD and DS: degeneration of basal forebrain cholinergic neurons (BCFNs) [40], impaired hippocampal long-term potentiation [71], as well as increases in inflammation and oxidative stress [42,52]. In addition, these mice exhibit memory deficits on both spatial and non-spatial cognitive tasks [33,44,64]. Triplication of the amyloid precursor protein (APP) gene is required for a number of these pathologies [66], suggesting that elevated levels of APP or its toxic cleavage products, such as β-amyloid, may contribute to these deficits. In addition to its inflammatory potential, β-amyloid, has also been implicated in contributing to neuronal death by triggering excessive calcium signaling through NMDA receptors [22].

Other candidate genes for DS phenotype that may modulate NMDA receptor function, *Dyrk1A* and *DSCR1*, are also triplicated in Ts65Dn mice. Dyrk1A, a dual specificity kinase, prolongs calcium uptake after NMDA receptor stimulation when over-expressed [4], while *DSCR1*, which encodes calcipressin, can similarly facilitate Ca^{2+} influx by increasing NMDA receptor activity through the inhibition of calcineurin [49]. Ts65Dn mice exhibit an age-dependent reduction in Calbindin D-28k, a Ca^{2+} binding protein and important regulator of Ca^{2+} intracellularly, in the CA1 of the hippocampus [42]. The loss of Calbindin D-28k may further sensitize these neurons to

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excitotoxicity, since they would be less able to handle the elevated calcium load. Taken together, these studies provide strong evidence for altered NMDA receptor function, and consequent disrupted calcium signaling, increasing the potential for excitotoxic damage in DS, but chronic studies using neuroprotective therapies targeting calcium regulation or NMDA receptors have not been performed in Ts65Dn mice to date.

Conventional therapies for AD for many years have focused on cholinesterase inhibitors, which function by blocking acetylcholinesterase enzymatic activity resulting in increased levels of acetylcholine at the synapse, and have been used as the conventional therapy for AD for many years (for review: [37]). More recently NMDA receptor antagonists, such as memantine, have shown promise in their ability to not only facilitate cognitive function, but also to potentially halt or reduce the progression of the disease (for review: [50]). Glutamatergic NMDA receptors are required for several forms of synaptic plasticity and memory formation [6,20], but over-activation of these channels can disrupt these processes and lead to intracellular calcium toxicity. NMDA receptors, due to their high permeability to Ca^{2+} and their slow-gating kinetics, are also implicated in excitotoxic neuronal damage that has been implicated in several neurodegenerative disorders, including AD [50]. Cerebrospinal fluid levels of glutamate are elevated in AD patients [63], and impaired glutamate transporter function in these individuals may result in excessive glutamate at the synaptic cleft [54]. The potential efficacy of memantine lies in its low affinity, non-competitive antagonism for the NMDA receptor, which paradoxically acts as a stronger inhibitor under increasing glutamate levels [60], thus allowing memantine to preferentially reduce pathological NMDA receptor activity without impairing physiologic functioning necessary for learning and memory [59,67]. Importantly, several preclinical animal studies have found that memantine improves hippocampal-dependent memory function [57,84] and human clinical trials have found that memantine improves cognition and global mental status significantly in individuals with mild or moderate dementia after 28 weeks of treatment [29]. In addition, preclinical studies show that memantine attenuates neuropathological hallmarks, such as reducing amyloid plaque burden and neuroinflammation in AD mouse models [56,68], but the neuroprotective effects of this compound in animal models for DS have not been investigated.

In the present study, we first examined whether long-term oral memantine treatment in Ts65Dn mice would improve performance in measures of hippocampal- as well as frontal cortex-dependent learning and memory tasks. An oral dosage of 20 mg/kg memantine was implemented, as this route is consistent with clinical administration. We hypothesized that since Ts65Dn mice share similar cognitive and neuropathological hallmarks to those observed in murine models of AD-like pathology, memantine treatment could have significant behavioral and morphological benefits in the Ts65Dn mouse such as facilitation of learning and memory function, neuroprotection of BFCNs and calbindin D-28k-positive cells in the hippocampus, and reduction of microglial activation observed in Ts65Dn mice [38,42,52]. Therefore in addition to evaluating Ts65Dn mice via water radial arm maze (WRAM) and novel object recognition (NOR) testing, we also assessed markers of cholinergic and noradrenergic degeneration, neuroinflammation, and hippocampal morphology after a 6-month chronic memantine administration. In a second study, we also evaluated the cognitive effects of acute administration of memantine in a separate set of Ts65Dn mice. We found that memantine facilitated performance in the WRAM and NOR tasks in Ts65Dn but not in normosomic mice in both chronic and acute settings, but did not alter markers of neurodegeneration in Ts65Dn mice.

2. Methods

2.1. Study 1: Long-term memantine treatment

2.1.1. Subjects

Mice partially trisomic for a segment of murine chromosome 16 just proximal to the gene for *App* and extending to the gene for myxovirus resistance (*Mx*) were developed by M. Davisson at Jackson Laboratories [21]. Controls for this experiment were normosomic littermates (NS) to the Ts65Dn mice with the same genetic background (B6C3HF1). As the C3H mouse strain carries the retinal degeneration allele (*rd*), the Ts65Dn and NS were screened and found free of retinal degeneration at Jackson Laboratories before shipment to MUSC. The trisomy is maintained by mating female carriers (the males are sterile) to C57Bl/6Jeicher × C3H/HeSnJ F1 males on a segregated genetic background [21].

Subjects consisted of male Ts65Dn mice and their NS littermates which were acquired from Jackson Laboratories (Bar Harbor, ME) and housed in our animal facility for 1–2 months before testing. All mice were singly housed, received food and water ad libitum, and were maintained on a 12-h light/dark cycle. Due to the subtle nature of the Ts65Dn phenotype, behavioral testing was conducted blind to the genotype of the animals. All experimental procedures were approved by the institutional animal care and use committee (IACUC) of the Medical University of South Carolina.

2.1.2. Treatment

Long-term memantine treatment in study 1 was initiated at 4 months of age (Fig. 1 schematically depicts the experimental timeline). Memantine hydrochloride (Tocris, Bristol, UK) was administered orally (via drinking water) at doses of 20 mg/kg/day on average for a period of 6 months, and was maintained during behavioral testing (see Fig. 1). This method of administration has proven successful previously in prolonged studies, producing steady state plasma levels within the therapeutic range [57]. Concentrations of 20 mg/kg have previously shown neuroprotective effects as well as memory facilitation in models of AD and stroke [12,19,23,82]. Water consumption was measured weekly throughout treatment to confirm average daily memantine intake over the course of each week. Controls received drinking water, and were also measured for water consumption. There were no differences in liquid consumption between groups (NS Control = 4.8 ± 0.7 ml/day; NS Mem = 5.1 ± 0.06 ml/day; Ts65Dn Control = 5.5 ± 0.3 ml/day; Ts65Dn Mem = 5.2 ± 0.5 ml/day; $p > 0.2$). During novel object

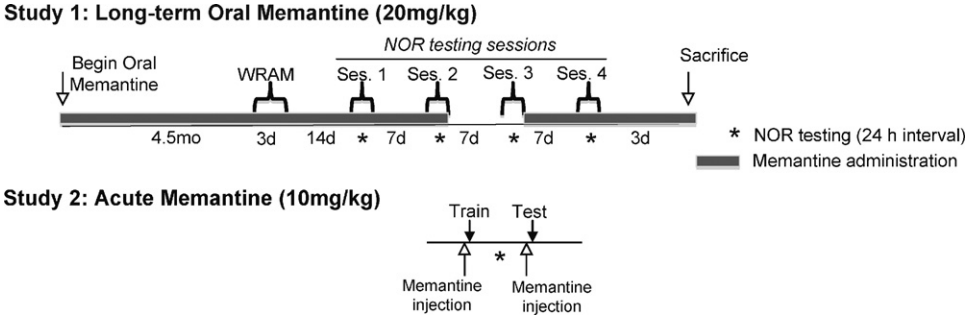

Fig. 1. Study timelines. Study 1 consisted of a long-term oral memantine treatment in which Ts65Dn mice and normosomic controls (NS) received memantine (20 mg/kg) for 6 months, beginning at 4 months of age. Mice from study 1 were first tested on a 3-day win-stay RA maze at 8.5 months of age. Following spatial memory testing, the mice underwent a series of four weekly NOR testing sessions. Prior to the 3rd NOR testing session, mice that had previously received memantine were switched to control fluids, such that the 3rd session occurred with mice "off" treatment. Following this session, mice were returned to their treatments for session 4. Study 2 evaluated the effects of acute memantine administration on recognition memory in Ts65Dn mice, as naïve Ts65Dn mice received acute memantine injections (10 mg/kg, i.p.) immediately prior to NOR training and testing periods.