The Glutamatergic Hypothesis for Down Syndrome: The Potential Use of N-Methyl-D-Aspartate Receptor Antagonists to Enhance Cognition and Decelerate Neurodegeneration

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Abstract: Down syndrome (DS) is the most common genetically defined cause of intellectual disability and accounts for over 50% of the cases of Alzheimer-type dementia in persons younger than 50 years of age. At present, no pharmacotherapy aimed at counteracting either the neurodevelopmental or the neurodegenerative component of this genetic disorder has been approved. Recent preclinical and clinical work on the N-methyl-D-aspartate (NMDA) receptor antagonist memantine give us some reason for optimism, at least in relation to the potential for a partial pharmacological improvement of hippocampus dependent memory deficits associated with DS. Here, we will review briefly the roles of NMDA receptors in health and disease, including the glutamatergic hypothesis for Alzheimer disease. Then, we will describe the basis for a glutamatergic hypothesis for DS, by reviewing the available preclinical evidence and assessing potential molecular mechanisms for NMDA receptor dysfunction in DS. A short description of the first two clinical trials of memantine in young and older adults with DS will follow. We will conclude by reviewing three caregiver reports from our recent clinical study and some lessons we have learned designing and conducting the first translational study in the field of DS to arise directly from experimental results in animal models.

Keywords: Down syndrome, Alzheimer disease, Trisomy 21, Ts65Dn mouse, Memantine, N-methyl-D-aspartate receptor, Long-term potentiation, Long-term depression.

INTRODUCTION

Down syndrome (DS) is typically the phenotypic consequence of trisomy 21 [1]. At an approximate rate of live births of 1 in 691 [2], and a prevalence of 1 in 1000, DS is the most common genetically defined cause of intellectual disability [3]; with a moderate degree of intellectual disability being the most frequent occurrence [4, 5]. Although the neurodevelopmental disability displayed by individuals with DS is generally global in nature, disproportionate deficits in hippocampus and prefrontal cortex dependent functions have been well documented [4, 6, 7]. As the person with DS ages, he/she will inevitably develop a neuropathology indistinguishable from Alzheimer Disease (AD), which originally manifests itself in the mid-thirties to early forties [8, 9]. This neurodegenerative process most likely leads to the high prevalence of early-onset dementia in this population, which commonly occurs in their fifth or sixth decade of life [10]. Given that the life expectancy of persons with DS is quickly approaching 60 years in the industrialized world [3, 10] (mostly due to recent advances in the surgical and clinical management of the various comorbidities associated with DS [11]), it is now reasonable to say that the developmental and neuro-degenerative components of the syndrome may presently constitute the two greatest unmet therapeutic needs of this population.

In recent years, there have been several successful pharmacological rescuing studies using mouse models of DS (see references [12] and [13] for recent reviews). Such studies have demonstrated that, in spite of its underlying complexity, the possibility for the clinical development of drug therapies to tackle the developmental and neurodegenerative components of this genetic disorder might be within reach. As part of this special issue on DS, we will discuss the potential pathogenic role of deregulated glutamate receptor function in the brains of persons with DS (the glutamatergic hypothesis). This also will include a brief review of the current evidence supporting the possibility of modulating the function of this class of neurotransmitter receptor as a means to improving cognitive abilities and potentially counteracting neurodegeneration in persons with DS. To illustrate recent translational work inspired by the preclinical data on the mouse model of DS known as Ts65Dn [14-17], we will discuss the design and results of two recently completed clinical trials with the uncompetitive antagonist of the N-methyl-D-aspartate (NMDA) receptor, memantine [18, 19]. We will conclude by sharing some of the lessons that we learned in designing and conducting one of these trials.

NMDA RECEPTORS, SYNAPTIC PLASTICITY, LEARNING AND MEMORY

Glutamate is the most abundant excitatory neurotransmitter in the brain, and its receptors are generally

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categorized into ionotropic and metabotropic receptors [20]. This classification is based on whether the receptor molecule is a ligand-gated ion channel or whether it is a Guanosine 5'-triphosphate (GTP) binding (G-protein coupled) receptor activated by the neurotransmitter glutamate, respectively. Ionotropic glutamate receptors can be subdivided further into α-amino-3-hydroxy-5-methyl-isoxazole-propionic acid (AMPA), NMDA, and kainate receptors. These receptors were identified originally by electrophysiological and radioligand binding studies before their coding genes were cloned. Therefore, they were named based on their affinity for pharmacological agonist agents that do not occur naturally in the brain.

AMPA receptors typically have a low affinity for glutamate, fast activation and inactivation kinetics, high permeability to Na^+ and K^+, and low Ca^{2+} permeability [21]. Their simple mode of operation makes them ideally suited to be the main carriers of the characteristically large and rapid glutamatergic synaptic currents. In addition, changes on the balance of continuous insertion and removal of AMPA receptors to and from the postsynaptic density are one of the main molecular events underlying the expression of most forms of long-term changes in the efficacy of glutamatergic synapses, or synaptic plasticity [22]. In contrast, NMDA receptors have high affinity for glutamate, require the binding of a co-agonist (typically, glycine, but also D-serine or D-alanine), and have slow activation and inactivation kinetics [23-25]. In addition to being permeable to Na^+ and K^+, NMDA receptors have high Ca^{2+} permeability [26]. However, under typical resting potentials, the channel is blocked by Mg^{2+}, which prevents Ca^{2+} to permeate its pore [27]. This last characteristic endows the NMDA receptors with the necessary properties to act as a detector of the coincident depolarization of the postsynaptic neuron and the release of glutamate by the presynaptic neuron [28]. When these two conditions are met, i.e., when the postsynaptic membrane is sufficiently depolarized to release the Mg^{2+} blockade and glutamate is being presynaptically released, the NMDA receptor channels allow Ca^{2+} to permeate into the postsynaptic neuron and initiate the cascade of molecular events that will lead to the induction of synaptic plasticity [29]. The resulting type of synaptic plasticity will depend on the amount of activation of the NMDA receptors, and the dynamics of postsynaptic Ca^{2+} activity elevation [30-32]. In general, a modest activation will lead to long-term depression (LTD), whereas a strong activation will lead to long-term potentiation (LTP). There is ample scientific agreement that the molecular mechanisms underlying these two forms of synaptic plasticity play critical roles in the encoding of memories in various regions of the brain, including the hippocampus, where the induction, expression, and maintenance of LTD and LTP have been characterized most extensively.

NMDA receptors are heterotetramers, consisting of two essential GluN1 subunits and combinations of two GluN2A-D subunits (which are thought to be the main determinant of channel gating kinetics and permeation properties [33-35]). GluN1 subunits have the binding site for the co-agonist, whereas the agonist glutamate (or L-aspartate) binds to the GluN2 subunits [36]. A developmental subunit switch from GluN2B to GluN2A, as the main GluN2 subunit in synaptic NMDA receptors, is known to occur in the first weeks of postnatal life in rodents and has been hypothesized to represent a molecular switch that turns on associative learning in different structures in both rodents and in human beings [33-35, 37]. This shift in subunit composition may also influence which form of plasticity is favored at a given synapse. The activation of GluN2B containing receptors seems to favor induction of LTD whereas the activation of GluN2A containing receptors seems to favor the induction of LTP [38].

THE GLUTAMATERGIC HYPOTHESIS FOR AD

Various neurotransmitter systems have been shown to be affected in AD [39-42]. Historically, the so-called cholinergic hypothesis was the first neurotransmitter-based hypothesis for the pathogenesis of AD to arise. It initiated as the result of three reports published in the mid-seventies indicating substantial neocortical deficits in choline acetyltransferase, which is the enzyme responsible for the synthesis of acetylcholine, and subsequent discoveries of reduced choline uptake, acetylcholine release and loss of cholinergic perikarya from the basal forebrain [39]. Additional support to the cholinergic hypothesis was provided by the mild, but significant symptomatic relief provided by the acetylcholinesterase inhibitors donepezil, galantamine, and rivastigmine in persons with AD [43, 44]. However, it has been exactly the fact that these drugs have not been very effective that has decreased widespread support for cholinergic deficiency as a major pathogenic mechanism in AD [45].

When compared to the cholinergic hypothesis, the rationale behind the glutamatergic hypothesis for AD has been less straightforward, and has evolved considerably over the years. One common thread in the many versions of the glutamatergic hypothesis for AD is the very robust experimental observation that excessive amounts of glutamate or excessive calcium permeation through NMDA receptors can lead to excitotoxic neuronal dysfunction and cell death [46]. In addition to AD, excitotoxicity may play an important role in other neurological diseases, such as Parkinson disease, Huntington disease, stroke, amyotrophic lateral sclerosis, and multiple sclerosis [47-49].

Excess of glutamate and excessive glutamatergic activity indeed have been shown to be present in AD [50]. This observation has eventually led to the idea that, in AD, glutamate does not exert its physiological role appropriately because NMDA glutamate receptors are tonically overactive, rather than being highly active only during phasic bursts [51]. The initial consequence of this dysfunctional state would be the excessive Ca^{+2} influx through the postsynaptic membrane and increases in "synaptic noise" and impaired neuronal plasticity [48, 52]. The chronic persistence of this state of excessive Ca^{+2} influx through NMDA receptors would then be expected to lead to permanent neuronal damage and cell death. In this view, disturbances in glutamate homeostasis could be potentially triggered by factors such as energy deficits, increased free radical formation, deficits in glutamate uptake and clearance from the synaptic space, and/or the toxic effect of Aβ peptides, Aβ oligomers, and misfolded tau proteins [42, 48, 52, 53].

A unique perspective has been provided by Parsons *et al*. [48], who have suggested that alterations in the ability of

Mg^{2+} to regulate the NMDA receptor function may be an important factor in the pathogenesis of AD. Such alterations in the voltage-dependent blockade of NMDA receptors by Mg^{2+} could be due to partial membrane depolarization, which might be attributable to decreased activity of Na^+/K^+ ATPase, mitochondrial dysfunction, AD-related changes in blood flow, and decreases in glucose metabolism and brain glucose supply. These authors also cite other factors like impairment of Ca^{2+} homeostasis, increased glutamate levels, and increased NMDA receptor sensitivity to glutamate.

After years of apparently contradictory findings, some consensus seems to be emerging regarding the direct effects of Aβ peptides and Aβ oligomers on synaptic plasticity in AD and how the nature of these effects might change over the course of disease progression. Palop and Mucke [54] recently summarized the accumulated evidence in a hypothetical scheme in which intermediate levels of APP/Aβ would produce synaptic facilitation through actions on presynaptic terminals. Such synaptic facilitation would occur through enhanced endocytosis and processing of amyloid precursor protein (APP) at these terminals, followed by presynaptic release of Aβ and activation of presynaptic α7 nicotinic receptors, which would increase the presynaptic Ca^{2+} levels, and increase the probability of synaptic vesicle fusion and neurotransmitter release. In contrast, high levels of APP/Aβ would produce postsynaptic depression through increased internalization of NMDA and AMPA receptors, activation of perisynaptic NMDA receptors (believed to be enriched in GluN2B subunits), metabotropic glutamate receptors (with mGlu5 receptors playing a major role), and even α7 nicotinic receptors. These events would lead to less steep rises in postsynaptic Ca^{2+} levels, and activation of LTD-related pathways, such as those involving calcineurin, P38 mitogen-activated protein kinases (P38 MAPK), and glycogen synthase kinase 3 beta (GSK-3β), which would eventually lead to synaptic loss [53].

Similar to the cholinergic hypothesis, the Achilles' heel of the glutamatergic hypothesis is the modest efficacy of the uncompetitive NMDA receptor antagonist memantine in the treatment of AD. Memantine was approved for the treatment of patients with moderate to severe AD in 2002 in the European Union and in 2003 in the United States [48]. It has provided a small, but significant symptomatic improvement in 6-month, placebo-controlled randomized trials assessing cognitive, functional, and global outcomes of inpatients with moderate-to-severe AD (defined as a Mini Mental State Examination score below 20) [55]. Recent systematic reviews have concluded that although for moderate-to-severe AD "memantine appeared to be the most cost-effective treatment" [56], "evidence is lacking for a benefit of memantine in mild AD, and there is meager evidence for its efficacy in moderate" AD [57]. The combination of memantine and acetylcholinesterase inhibitors appears to be "of greater benefit in AD than [acetylcholinesterase inhibitors] alone, but the clinical relevance depends on exactly which studies are included so [any additional benefit of this combination] is not robustly demonstrated" [58].

There are several potential explanations for the observed modest therapeutic efficacy of memantine in the treatment of AD. The most obvious one is that the alterations in glutamatergic neurotransmission and glutamatergic-dependent synaptic plasticity observed in AD might be simply an epiphenomenon of the underlying AD pathology, and have little effect on the rate of disease progression or even on the manifestation of the most important signs and symptoms of AD. The second possibility is that memantine may not be selective enough to counteract effectively the glutamate receptors responsible for activation of LTD-related pathways due to high levels of APP/Aβ, such as GluN2B-containing NMDA receptors and mGluR5 receptors. A third possibility is that the clinical syndrome, with the underlying pathology of amyloid plaques and neurofibrillary tangles, known as AD, may in reality represent the end state of heterogeneous neuropathological states with equally heterogeneous molecular etiologies. Therefore, in the absence of specific biomarkers, it would be unreasonable to expect any single pharmacological or even immunological intervention to be successful in potentially heterogeneous cohorts of patients. Finally, one has to entertain the possibility that, by the time the cognitive deficits associated to AD become clinically detectable, it might be already too late for any pharmacological or immunological intervention to be strikingly successful in terms of symptomatic relief or disease modification.

THE GLUTAMATERGIC HYPOTHESIS FOR DS

Review of the Preclinical Evidence

As aforementioned, the association between DS and AD has been well documented for more than 60 years [3]. However, what inspired our work on building the case for a glutamatergic hypothesis for DS was the theoretical speculation that overexpression of chromosome 21 gene products (e.g., *RCAN1* and *DYRK1A*), coupled to potentially elevated amounts of reactive oxidative species (ROS), in the brains of persons with DS might inhibit the activity of the protein phosphatase calcineurin [59]. What truly made this work compelling to us was our knowledge of the previously published work by Lieberman and Mody [60] showing that the catalytic subunit of calcineurin can directly modulate NMDA receptor kinetics by decreasing channel mean open time and opening probability. This work was so intriguing to us that we started to look for a confirmatory experiment that would be simple enough for us to implement it in a couple of weeks in the lab. The clue for such experiment came from the reading of the work by Miyakawa *et al*. [61], which had shown that a conditional, forebrain-specific calcineurin null-mutant mouse displays increased sensitivity to the locomotor-stimulating effects of the high-affinity noncompetitive NMDA receptor antagonist MK-801.

Our simpleminded working hypothesis was that, if trisomy of chromosome 21 genes produces alterations on the functioning of NMDA receptors through the inhibition of calcineurin, we should see results similar to those described by Miyakawa and colleagues in Ts65Dn mice. (The Ts65Dn mouse is the best studied mouse model of DS and is trisomic for all chromosome 21 mouse homologous genes necessary to produce the theoretical effect on the activity of calcineurin.) Consequently, our first experiment involved quantifying the sensitivity of Ts65Dn mice to MK-801 [14]. To our surprise, we found that Ts65Dn mice display levels of hypersensitivity to the psychotomimetic effects of MK-801 comparable to what had been observed in calcineurin

null-mutant mice. Armed with the information that Ts65Dn mice display increased sensitivity of to MK-801 (an open channel blocker of NMDA receptors), the theorized decrease in calcineurin activity in these animals, and the results of Lieberman and Mody on calcineurin's direct modulation of NMDA receptor kinetics, our next step was to investigate whether an antagonist with lower affinity for the NMDA receptor than MK-801, memantine, could enhance the performance of Ts65Dn mice in a memory and learning task.

We chose to use a contextual fear conditioning test, because it is a behavioral measure that has been shown to be dependent on physiological levels of NMDA receptor activation [62]. In this Pavlovian conditioning experiment, mice are exposed to a novel context (conditioned stimulus) for a few minutes and then receive an electrical shock of moderate intensity (unconditioned stimulus). Twenty-four hours later, the animals are re-exposed to the same context without the unconditioned stimulus. In wild-type mice, the typical experimental finding is that these animals will spend a large percentage of their time on a crouching posture and mostly immobile, with the exception of movements associated with respiration and heartbeat, which is a species-specific defensive reaction known as 'freezing' [63].

The resulting findings from our experiments were informative in two ways: (1) they were the first demonstration that Ts65Dn mice display impaired performance in this specific behavioral task; and (2) we found out that this performance deficit could be pharmacologically rescued to levels statistically indistinguishable from those observed in control mice with a single injection of memantine 15 minutes before the first exposure to the conditioned stimulus and a second memantine injection 15 minutes before the re-exposure to the same environment. In subsequent studies, two independent research teams, using the Morris water maze [15] and a novel object recognition task [16], confirmed the pharmacological efficacy of memantine in Ts65Dn mice. These groups also demonstrated that, under chronic administration regimens, memantine maintains its memory-enhancing effects. Additionally, the first group detected reduced levels of brain APP and increased hippocampal vesicular glutamate transporter 1 levels in Ts65Dn mice chronically treated with memantine; and the second group found no histological signs of neuroprotection of basal forebrain cholinergic or locus coeruleus neurons in Ts65Dn mice following chronic treatment with memantine (i.e., no disease modifying effects).

More recently, we found that Ts65Dn mice display exaggerated NMDA-dependent LTD compared to euploid control mice, but control-levels of metabotropic glutamatergic receptor-dependent LTD. We also found that therapeutic levels of memantine pharmacologically rescues the observed exaggerated levels of NMDA receptor-dependent LTD in Ts65Dn mice [17], which has provided us with a potential mechanism by which memantine may be exerting its memory/learning enhancing actions on this mouse model of DS.

Alternative Molecular Mechanisms

It is important to notice once again that the short history of the glutamatergic hypothesis for DS so-far has been primarily the result of a somewhat naïve hypothesis, which arose initially in our lab. We proposed originally that the potential effects of the trisomy of chromosome 21 genes (or their mouse homologs in the Ts65Dn segment) on the function of NMDA receptors are exerted primarily *via* the inhibition of calcineurin activity. Although this simple hypothesis has withstood all the experimental tests it has been subjected up to now, these have not been many, and it is likely that the final picture will turn out to be much more complex than what we had proposed originally. For example, calcineurin activity has also been shown to be positively correlated to glycine-independent NMDA receptor desensitization [64, 65]. Also, several other chromosome 21 gene products are known to interact directly with NMDA receptors, such as the transient invasion and metastasis protein 1 (TIAM 1), intersectin 1 (ITSN1), and, as mentioned in the previous description of the glutamatergic hypothesis for AD, APP [66]. Additionally, the properties of NMDA receptors can be modulated by a large number of post-translational modifications, such as phosphorylation, palmitoylation, and S-nitrosylation [34], and alterations in the dynamics of subunit trafficking also can alter the final receptor composition and channel biophysical properties [32-34]. Interestingly, it has been suggested that NMDA receptor subunit trafficking may be abnormal in the Ts65Dn mouse [67]; and a recent study on a transgenic mouse for the chromosome 21 gene *DYRK1A* (which encodes the dual specificity tyrosine-phosphorylation-regulated kinase 1A, DIRK1A) has shown an increased GluN2A subunit expression in the brains of these animals [68].

In summary, the final diagram of the pathogenic effects of trisomic genes in DS on the function of NMDA receptors might involve a kinetic scheme in which multiple channel states are affected. These effects might be superimposed to multifarious post-translational modulations, shifts in subunit composition, and potential affinity alterations of the NMDA receptor channel to Mg^{2+}. One also has to consider the possibility that the primary pathogenic event altering glutamatergic neurotransmission in DS might not be located on the NMDA receptor level, but instead might involve downstream signaling processes.

Finally, one has also to take into account that the trisomy of some of the genes in chromosome 21 might have neuroprotective effects, and that some of the functional alterations observed in aneuploid mouse models of DS might be the result of developmental adaptations. For example, *GIRK2* is a gene located in chromosome 21, and its mouse homolog, *Girk2*, is located in the Ts65Dn trisomic segment. This gene encodes for K^+ channel subunits that form heterotetramers with other members of the GIRK subfamily. The resulting inward rectifying K^+ channels are regulated by a variety of Gi/Go-coupled inhibitory neurotransmitter receptors, such as muscarinic (M_2), serotonergic ($5\text{-}HT_{1A}$), GABAergic ($GABA_B$), somatostatin, opioid (μ, δ, κ), adenosine (A_1), and dopaminergic (D_3) receptors [69]. The potential role of trisomy of *GIRK2* in the pathogenesis of DS has been explored at some detail and was reviewed recently by Cramer *et al*. [70]. However, it is important to observe that one of the expected effects of the overexpression of GIRK2-containing K^+ channels would be to hyperpolarize the resting membrane potential of the neurons where they are typically expressed [73]. This effect would strengthen the

binding of Mg^{2+} to NMDA receptor channels, and, hence, counteract any potential excessive activation of NMDA receptors, which would be exactly the opposite of the aforementioned partial membrane depolarization hypothesized to occur in AD. Therefore, any pharmacological intervention in which the function of GIRK2-containing K^+ channels were to be antagonized (e.g., with the use of fluoxetine or $GABA_B$ receptor antagonists), in the absence of the normalization of NMDA receptor function, might also have the unwanted theoretical effect of accelerating the rate of neurodegeneration in persons with DS.

THE FIRST TWO CLINICAL TRIALS OF MEMANTINE IN DS

A side-by-side summary of the design and results of the two recently completed clinical trials on the effects of memantine on persons with DS can be seen in the Table **1**. One of these studies was the direct result of the success of pharmacological rescuing studies in the animal model of DS Ts65Dn described in the previous section, coupled with the generally favorable safety profile of memantine in patients with AD. This was a small-scale, randomized, placebo controlled clinical trial of memantine in young adults with DS [18]. The main aim of this study was to test the hypothesis that a short drug regimen of memantine could be efficacious in improving scores of hippocampus-dependent tasks by participants with DS. Hippocampus-dependent measures were our main focus due to the following reasons: (1) the previous demonstration of disproportionate deficit in tasks thought to be dependent on the hippocampus in persons with DS [6]; (2) previous studies by our group and others showing that memantine rescues behavioral performance deficits in Ts65Dn mice on tasks thought to have a significant hippocampal dependent component such as contextual fear conditioning, Morris water maze deficits, and novel object recognition [14-16]; and (3) our finding that memantine can also reverse at least one form of hippocampal synaptic plasticity alteration in Ts65Dn mice [17].

In our double-blind clinical study on memantine [18] (NCT01112683; www.clinicaltrials.gov), we compared the effects of 16-week treatment with either memantine or placebo on cognitive and adaptive functions of 40 young adults (aged 18-32 years) with DS using a broad, and carefully selected set of neuropsychological outcome measures. The primary measures of this study were the Paired Associate Learning (PAL) and Pattern Recognition Memory (PRM) tests, which are both part of the Cambridge Neuropsychological Test Automated Battery (CANTAB) and were shown previously to be disproportionally affected in adolescents and young adults with DS [6]. We also included two additional secondary measures associated with the primary hypothesis that memantine therapy would produce improvements in test scores on hippocampus-dependent measures. These additional measures were the short form of the California Verbal Learning Test-II (CVLT-II) and the Rivermead Behavioral Memory Test-Children's version (RBMT). Safety and tolerability were also monitored. This was the first clinical study in DS to benefit fully from the lessons learned from preclinical work in animal models and recent neuropsychological findings in persons with DS.

Although no significant differences were observed between the memantine and placebo groups on the two

Table 1. Summary Table of the Design and Results of the Two Recently Completed Clinical Trials on the Effects of Memantine on Persons with DS

Number of Participants Randomized	40	173 (61 of whom had a clinical diagnosis of dementia)
Number of Participants who completed the study	37	146
Mean (Standard Deviation) Participant Age	23.0 (3.8) years	51.4 (7.1) years
Duration of the Treatment	16 weeks	52 weeks
Medication Dose	20 mg/day	10 mg/day
Genotype	Trisomy 21 (92%) or complete translocation (8%) (% of the participants who completed the study); confirmed by karyotype	Unknown, "[DS] was confirmed by karyotype when possible, but a clinical diagnosis was accepted if the karyotype was not available"
Concomitant Medications Taken by the Participants During the Trial	Antidepressant (2.7%), stimulant (2.7%), and antiepileptic drug (2.7%)	Cholinesterase inhibitors (5.5%), antidepressants (18.1%), neuroleptics (7.9%), anxiolytics (2.4%), hypnotics (3.9%), and "Medication for physical problems" (74%)
Primary Measures	PAL and PRM	DAMES and ABS I and II
Primary Measure Outcomes	Not significant	Not significant
Other Efficacy Measures Associated with the Primary Hypothesis	CVLT-II and RBMT	None
Efficacy Measures Associated with the Primary Hypothesis Positive Outcome	CVLT-II significant (p= 0.046) RBMT not significant	N/A
Tolerability	Well tolerated	Well tolerated

Abbreviations used: PAL= Paired Associates Learning, which is part of the Cambridge Neuropsychological Test Automated Battery (CANTAB); PRM = Pattern Recognition Memory (also part of the CANTAB); CVLT-II = California Verbal Learning Test-II Short Form; RBMT = Rivermead Behavioral Memory Test - Children's version; DAMES = Down syndrome attention, memory and executive function scales; and ABS I and II = adaptive behavior scale parts I and II.

primary outcome measures, we found a significant effect of memantine therapy on the CVLT-II. The CVLT-II measures supraspan word learning ability (i.e., word lists with number of words larger than the typical 7±2 span of short-term memory) as an index of episodic verbal long-term memory. It is known to be sensitive to posterior hippocampal functioning (based on neuroimaging) and to be impaired in patients with various forms of degeneration or damage to the hippocampus. Additionally, the study showed a P-value <0.10 for one of the primary outcome measures: the number of stages completed in the PAL, which is a measure of non-verbal memory that requires the participant to learn associations between an abstract visual pattern and its location. A P-value <0.10 was also detected for one of the secondary outcome measures, the Recall of Digits test (which is part of the Differential Ability Scales; DAS-II). This is a measure of rote short-term verbal memory in which the participant is asked to repeat, in the same order, an increasingly longer string of single digit numbers verbally presented by the examiner. Memantine was well tolerated, with only infrequent and mild adverse events noted (two participants in the memantine arm showed increased anxiety and one displayed echolalia, as reported to the investigators by their caregivers). With only 37 participants (out of 40 recruited and randomized) completing the study, the small sample size was the obvious limitation of this study. Still, we considered the results promising enough to warrant a larger confirmatory trial, which we are currently in the process of designing.

The other clinical trial on memantine in persons with DS that has been published recently [19] was named "Memantine for Dementia in Adults Older than 40 years with Down's Syndrome" (abbreviated as 'MEADOWS'; NCT00240760; www.clinicaltrials.gov). It consisted of a randomized, double-blind, placebo-controlled trial to assess safety and efficacy of memantine on improving broad cognitive and adaptive function in older individuals with DS. In contrast to our study, the trial design rationale was not based on preclinical findings on mouse models or subsystem-specific neuropsychological assessments in persons with DS. Instead, it followed a more traditional approach, in the sense that it was based on the association between DS and AD and previous findings by members of the same research team demonstrating a progressive loss of function typically experienced by a subset of individuals with DS in their forties and beyond [74]. Accordingly, the primary endpoints were changes in cognition and function, as measured through the DS attention, memory and executive function scales (DAMES) and the adaptive behavior scale (ABS) parts I and II. These authors found that, although the 1-year-long treatment with memantine was well tolerated in their participant sample, the treatment produced no significant improvement on the scores of their primary or secondary efficacy measures.

It is important to notice that, because the two trials had very different designs and goals, it is not surprising that they also produced different results. In line with what has been said before about the modest effects of memantine on AD, there are several potential explanations for why memantine failed to produce a significant effect in the 'MEADOWS' trial. However, given the common underlying etiology in DS (i.e., trisomy 21), one of the explanations that can be excluded is the one relying on heterogeneous molecular etiologies for the age dependent loss of cognitive and adaptive skills that affects older adults with DS. Indeed, one can argue that the etiologic homogeneity of recruited cohorts with DS (i.e., trisomy 21) represents a great advantage for the study of AD-type pathogenesis in persons with DS compared to idiopathic AD in the general population.

It is tempting to blame the failure of the 'MEADOWS' trial to detect an efficacy signal on their use of only 10 mg per day of memantine (which is half of the dose used in our study and also half of the FDA-approved dose for the treatment of AD), or on the fact that the study was only powered to detect fairly large effects of memantine on dementia with the blunt neuropsychological assessment tools that were employed. For example, in retrospect, it is possible to speculate that a neuropsychological test battery rich in hippocampus and pre-frontal cortex depended measures, such as the cognitive test battery described by Edgin *et al*. [7], might have allowed the 'MEADOWS' team to detect a significant efficacy signal if it were available at the time of the study design. However, given their explicit goal of treating age-dependent cognitive decline in older adults with DS (51.4 ± 7.1 years, compared to 23.0 ± 3.8 years in our study; expressed as mean ± SD; see Table **1**), one can also argue that the 'MEADOWS' team made use of some of the best validated instruments currently available to assess the most clinically relevant signs and symptoms associated with this phenomenon. In the end, just as it is the case with most clinical trials on idiopathic AD in the general population, the most likely explanation for lack of efficacy probably lies on the fact that various irreversible neurodegenerative cascades were already well underway and the disease process might have reached a point of no return by the time pharmacological treatment was attempted. (For a more complete set of comments on the MEDOWS trial, see reference [72]).

THREE CAREGIVER REPORTS AND SOME LESSONS LEARNED

In this section, we will share three distinct caregiver reports that are only partially representative of the range of experiences of participants and their families during the course of our clinical trial of memantine on young adults with DS [18] to illustrate some of the 'human-level issues' involved on the design and conduct of this trial. The hope is that some of these experiences will help inform the design and conduct of future trials on potential cognitive enhancing therapies in DS, or even intellectual disability of other origins. Each report will start with a short edited account provided spontaneously by the participant's caregiver, followed by a brief commentary. All three cases represent events reported by caregivers of participants who were in the memantine arm of our study.

Case 1: The 27-Year Old Female Participant 'RK'

> *"RK has not shown any negative changes or side effect since she started taking the pills you gave her".*
>
> *"RK has recently increased her productivity and has become one of [her worksite's] best workers".*

> *"[Her supervisor] also related how RK was happy at work and been more social as she chats with fellow workers".*
>
> *"I had not told anyone at [her] worksite […] that RK was in this double blind study".*
>
> *"I don't know if RK is working harder to earn more money or working better because she thinks the pills will help her work better or [if] the drug is actually helping her to think and act more clearly and quickly".*

This case exemplifies much of what most researchers would probably like to see in a trial like this. First, *RK* showed no adverse reactions to the medication. Second, independent observers, who were not aware of *RK* being part of a clinical trial, observed positive changes. Although *RK* (who, at baseline, already functioned at the top quartile of our sample) might have been positively influenced by the knowledge that she was participating in the study (placebo effect), at no time during the trial, the participant, her caregiver, or the members of our research team had any knowledge on whether she was taking memantine or placebo. This is reflected on the healthy level of skepticism by the mother, which might not have been the case had this study not been designed as a double blind, placebo controlled trial. Therefore, the very straightforward lesson that can be derived from this case is that, even in a pilot trial such as ours, the use of a placebo arm is critical to provide reliable efficacy data. This includes variance of individual effect sizes, which have already been essential for power analyses that are being performed to estimate ideal sample sizes for the next trial. In the case of *RK*, we observed a 29% improvement in her CVLT-II score, which was more consistent with the mean improvement in the memantine arm of the study (38%) than with what was observed in the placebo arm (18%).

Case 2: 21-Year Old Female Participant 'EK'

> *"EK displays what looks like a flushing reaction that starts approximately 15 minutes after she takes the pills. Her face turns red for several minutes and then goes back to normal in 5-15 minutes" [No hives or other allergic reaction signs or symptoms].*
>
> *"Something really strange happened last week, which is making me consider removing EK from the study: EK is normally a very placid girl who never questions authority. But last Saturday, when she came to her ballet class, and her instructor tried to point out that her posture was incorrect, she turned to her and expressed, in a way more forceful than I had ever seen, that she [the instructor] was embarrassing her in front of her friends".*
>
> *[After voicing her concerns to us, EK's mother decided to continue allowing her daughter to participate in the trial].*

This case has taught us a few valuable lessons. First, one should be on the lookout for unexpected physical reactions. The "flushing reaction" experienced by *EK* turned out to be self limited and inconsequential, but even for a drug with as long a track record of safety as memantine, one never knows exactly what to expect when using it on a different patient population. Second, assuming that memantine is producing cognitive enhancing effects, one has to remind constantly oneself that cognition does not exist in a vacuum. Therefore, alterations in the way the participant may be experiencing and reacting to stimuli in his/her social milieu are to be expected. In the case of this young lady, this may have manifested itself by an increased awareness and assertiveness, which in similar way to the physical reaction she experienced, were self limited and fairly inconsequential. In future studies, information regarding the potential for similar behavioral changes should be included in the informed consent form. Interestingly, *EK* also showed one of the largest relative improvements seen in the study on CVLT-II scores from baseline to the second neuropsychological evaluation performed at the 16th week of memantine treatment (59% improvement).

Case 3: 25-Year Old Female Participant 'EB'

> *Mother complained of that EB was displaying "increased anxiety".*
>
> *"EB is more talkative than I have ever seen her".*
>
> *"She has made some comments that have really amazed me".*
>
> *"But I also find her too needy and dependent. In the past, she used to stay in her bedroom most of the day and leave me alone. Lately, she is following me all the time asking me all kinds of questions, which is really draining me".*
>
> *[After gently and unsuccessfully trying to encourage the mother to maintain EB in the study, a discontinuation visit was scheduled in the fourth week of the study. Obviously, neuropsychological data were not collected].*

This case initially puzzled our team, but the more we thought about it the more the whole situation made sense. Although *EB*'s mother (who was her sole caretaker) seemed sincerely happy with some of the perceived progress displayed by her daughter during the study, she also realized she did not have the necessary family and social support structure in place to handle this new situation that was apparently created by *EB*'s participation in the trial. In our conversation with her during the discontinuation visit, we asked if what happened during the trial was not what she had in mind when she enrolled her daughter in the study. Her answer was that, "regardless of the long explanation we had given her and her daughter at the time the informed consent and assent forms were signed, she did not really know what to expect". She explained that, up to *EB*'s adolescent years, she struggled to provide the best education and training opportunities to her daughter. *EB* was now an adult living in her mother's home, and *EB* had been unemployed for a few years at the time of the trial. Given the limited training and occupational opportunities offered to *EB* by social agencies in the state, the situation in which she stayed "in her

bedroom most of the day" was far from ideal, but at least was a stable and manageable situation for the reality of their family unit. Therefore, in future trials, we hope to be able to provide even more written and verbal information during the consent and assent processes about potential consequences of the pharmacological intervention being tested. We will also aim at providing families with access to psychological and social counseling in case it is needed.

CONCLUSION

In this article, we have reviewed the basis for a glutamatergic hypothesis for the pathogenesis of DS and its relationship to the glutamatergic hypothesis for AD. We argued that in DS the theorized dysfunctional state of glutamatergic receptors (read, NMDA receptors) is expected to play significant pathogenic roles on both the neurodevelopmental and neurodegenerative components of DS. Results from behavioral and electrophysiological assessments in mouse models of DS have been instrumental in confirming the existence of NMDA receptor dysfunction in these animals, which is expected to mirror the human situation. Most importantly, pharmacological experiments with the uncompetitive NMDA receptor antagonist memantine demonstrated that this drug can rescue performance in behavioral tests of learning and memory and reset LTD in Ts65Dn mice to levels comparable to those observed in euploid control animals. In contrast, the results of our recently concluded pilot clinical study of a 16-week treatment with memantine on enhancing test scores of young adults with DS in hippocampus-dependent neuropsychological measures were certainly less impressive than the animal studies. However, this small-scale study on memantine has already shown that, by carefully choosing outcome measures informed by animal studies and newly available neuropsychological data in persons with DS, it may be possible to detect efficacy where it otherwise may have been missed.

Historically, the design of studies on potential pharmacotherapies for individuals with DS, has followed two different general models. The first, involves tackling the neurodegenerative component of DS in older adults (e.g., the 'MEADOWS' trial or a recently published trial on antioxidants by Lott *et al*. [73]). The second strategy is based on the recruitment of young adults with DS in an attempt to address the neurodevelopmental component (e.g., our study with memantine or the trial of the anticholinesterase agent donepezil by Kishnani *et al*. [74]). The first approach has typically involved recruiting individuals at ages in which cognitive decline is expected to occur, and hence an age when, theoretically, an up-to-one year drug therapy duration would have the greatest chance of producing measurable improvements in health outcomes. We argued here and elsewhere [72] that, similar to the case in AD, it might be too late to intervene by the time such therapeutic interventions are attempted. In a similar way, the second approach, which essentially involves recruiting young adults to investigate a quintessentially neurodevelopmental disorder, may also be flawed. Obviously, this originated not from the lack of knowledge of this fact by the investigators designing such trials, but instead it has been mostly the consequence of the greater regulatory simplicity of testing drugs already approved for adult use in adults with a different disorder. However, it still the case that, at the time of young adulthood, most developmental processes have already ended and, as illustrated previously in this article by the case of *EB*, the levels of social support (including constant stimulation, monitoring, and assessment of academic attainment) and expectation from caregivers have diminished considerably.

In the real world, one has to do research with practically available means. Even in the field of AD, projects involving long, disease modification trials have had a difficult track record in obtaining support. Therefore, in order to design relatively short trials with a chance of detecting an efficacy signal in research on the neurodegenerative component of DS, investigators will have to rely on the development and validation of more sensitive neuropsychological instruments and early biomarkers for disease progression. Additionally, in research on the neurodevelopmental aspects of DS, the inclusion of younger participants will also be necessary. This approach has been trail blazed by Dr. Prya Kishnani's group at Duke University, which has recently published on the use of anticholinesterase drugs in children and adolescents with DS [75, 76]. Future studies are also likely to benefit from new published baseline data generated by the application of cognitive test batteries rich in hippocampal and pre-frontal cortex dependent measures to individuals with DS aged 7-38 years [7].

Ultimately, even if the principle that DS may be amenable to pharmacological intervention is ever proven unequivocally, we still will have to undergo the necessary multiyear-to-decade long studies to evaluate the efficacy on adaptive and daily living skills of individuals with DS. As in other fields of study, the choice of which specific pharmacological agent, or combination of pharmacological agents, that will be taken to this next level should be selected from those deemed the safest and most efficacious.

The results from our pilot study on memantine give us reason to be optimistic that the translational approach in DS will bear fruit. With the presently available data, however, it would still be premature to make any recommendations on the clinical usefulness of memantine or any other drug to enhance the cognitive abilities of persons with DS. Still, there is good reason to anticipate that, within a decade or so, rational pharmacotherapies are likely to emerge as valuable tools in the armamentarium of clinical practitioners managing the neurodevelopmental and/or neurodegenerative components of DS. The translational approach, in which potential therapies are initially tested in animal and cellular (read, stem cells, particularly, induced pluripotent stem cells, or IPS cells) models of DS should become progressively more powerful in the coming years, as we resolve many pending issues, such as the development and validation of more accurate cross-species phenotypes and new cellular biomarkers. By pharmacologically addressing the neuropsychological and neurological consequences of trisomy 21, we would certainly be a step closer to the goal of improving the quality of life of individuals with DS and their families.

LIST OF ABBREVIATIONS

DS = Down syndrome

AD = Alzheimer disease

NMDA = N-methyl-D-aspartate

APP = amyloid precursor protein

LTP = long-term potentiation

LTD = long-term depression.

CONFLICT OF INTEREST

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