Title
Monotherapy vs Combination Therapy for the Management of Alzheimer's Disease

Authors
Pandey, Shrina
Pandey, Ishna
He, Minyi
et al.

Publication Date
2021-04-01

Monotherapy vs Combination Therapy for the Management of Alzheimer's Disease

Shrina Pandey, Ishna Pandey, Minyi He, Ei Chit Paing

Mentor: Selina Pacheco

Public Health and Health Sciences Undergraduate Laboratory at Berkeley

University of California, Berkeley

Contents

Abstract

Alzheimer's disease is a neurological condition resulting from the death of neural cells that causes a deprivation in cognitive functionality, communication, and motor skills in patients. The condition has an inordinate impact on older populations, with 90% of all cases affecting those 65 years or older[1]. As the most common type of dementia, comprising approximately 70% of all dementia cases, Alzheimer's disease affects about 5 million people in the United States. The rate of the condition's extent among the population is increasing exponentially, with the number of people enduring Alzheimer's estimated to triple by 2060 in the United States.[1]

Due to the widespread impact of Alzheimer's, developing treatments that govern the symptoms of the condition is an immense priority for researchers all over the globe. In the spirit of the venture to aid patients suffering from Alzheimer's disease, also known as AD, researchers have in recent years been testing several monotherapies, as well as the combination of these drugs to examine the most viable treatment plan for Alzheimer's for several stages, ranging from mild to moderate to severe AD. Monotherapies are selective agents with a single therapeutic action that may or may not have other less-significant side effects. Combination therapies encompass multimodal agents including drug cocktails and multifunctional molecules that combine multiple mechanisms of therapeutic action which may have a wider range of side effects. This paper will explore the effects of combining monotherapies and the efficacy of several drugs on Alzheimer's patients by examining donepezil, memantine, the combination of donepezil and memantine, rivastigmine, rasagiline, and ladostigil.

Introduction

Furthering research by conducting comparative studies will be of critical value to synthesize the relative efficacy of AD treatments. AD research often focuses highly on the effects of a particular drug but often fails to underscore comparative potencies. In this manner, the effectiveness of a monotherapy in contrast to its augmented versions (via combination therapies) could receive more attention. While past research has concluded that there is a "pronounced advantage over individual-target drug or cocktail of drug"[3], this paper aims to qualify the generalizability of this statement. Ultimately, a better understanding of such comparative information could be a breakthrough in AD treatment, especially in circumstances where a combination of treatments is more effective than a particular pathway. This paper attempts to overcome these issues with a focus on acetylcholinesterase (AChE) inhibitors and coupling mechanisms including monoamine oxidation (MAO) inhibition and N-methyl-D-aspartate receptor (NMDAR) antagonists. With the fundamental goal of understanding the differences in efficacies of mono- and combination therapies in AD treatment, we will start with an evaluation of an AChE and NMDAR silver bullet in conjunction with their dirty drug. We will then assess AChE and MAO individually as well as their conjugated drug. We will finally conclude with an analysis of experimental type, AD experimental treatment measurements, and future recommendations for AD research.

Analysis of Donepezil and Memantine as Monotherapies vs in Combination

Donepezil [Monotherapy]

Background

Donepezil is a monotherapy used to treat Alzheimer's disease and dementia, both of which are brain disorders that impair a patient's ability to think coherently, perform normal tasks and daily activities, and communicate with others.[4] The drug has been approved in over ninety countries in the world for the treatment of Alzheimer's disease of severities ranging from mild to moderate, as well as severe Alzheimer's in various nations, such as the United States, Canada, Japan, etc. Some common side effects of utilizing this drug include nausea, vomiting, diarrhea, and other gastrointestinal problems.[5] While its benefits are often considered small in scale and donepezil does not damage or revamp the pathophysiology of Alzheimer's disease, its ability to treat symptoms of the condition has made it a defining pillar of the treatment of Alzheimer's in North American countries.

Mechanism

Donepezil is in a class of inhibitors referred to as cholinesterase inhibitors[6], denoting their functionality of binding to an allosteric site which later binds to acetylcholinesterase enzymes (AChE), the neural transmitter that breaks down acetylcholine at cholinergic synapses[5], which in turn increases the obtainability of acetylcholine at neuron synapses, boosting cholinergic transmission.[6] Although there is no current evidence pointing to how donepezil "alters the progression" of Alzheimer's, it has the ability to remedy certain symptoms by boosting cognitive function and improving behavior.

As a reversible drug,[7] donepezil disengages from the AChE, after which the regular functionality of the AChE is resumed,[8] making the impact of donepezil temporary. In addition to

this, donepezil is noncompetitive and therefore, unlike a plethora of drugs consumed by the general public, binds separate from the active site of an enzyme, binding instead at an allosteric site of the substrate. Therefore, the AChE inhibitor has an affinity for both the enzyme-substrate complex and the enzyme and when the inhibitor binds to either, it "deactivates", thus prohibiting the generation of the end product.[9]

Study Design and Results

Donepezil has been approved to aid in the treatment of symptoms in mild to moderate Alzheimer's disease for patients in over ninety countries. The first "multicenter, randomized, placebo-controlled clinical trial" that assessed the efficacy of donepezil in China was issued in 2002[4]. Patients aged 55 years and older with mild to moderate Alzheimer's Disease were tested with 5mg/d donepezil in a 12-week treatment trial, resulting in an "improvement in cognitive function, ADLs" (the Activities of Daily Living), "and global function" over the specified time period. A potential downfall of this study is that the sample size was small and could have been larger to minimize discrepancies. The patients received higher scores in assessments of the MMSE (Mini-Mental State Examination), CDR (Clinical Dementia), and ADL in comparison to a control group who were provided placebo.[4] In another prospective clinical trial of patients with mild to moderate Alzheimer's disease that spanned 72 months of treatment, providing more longevity than the previous study, the group provided with donepezil performed exceedingly better on cognitive function, ADLs, and global functions extending all the way to the end of the 72 months. A potential complication of this study is that it had an exceptionally high dropout rate of 55.8% among those in the donepezil group and 74.4% for the control group by the end of the clinical trial.[10]

Donepezil has also been demonstrated to be effective in the treatment of moderate to severe Alzheimer's disease. When comparing groups, those maintaining donepezil treatment had cognitive and functional benefits compared to groups with withdrawn donepezil amounts in patients with moderate to severe Alzheimer's disease.[7] In a 12-week study held in China spanning 40 cities and 60 hospitals, 808 dementia patients, about 67% of whom were at a stage of moderate to severe AD, were given 5 mg/d donepezil treatment. The results of this investigative study indicate that donepezil "significantly enhanced the patients' function in ADLs, cognitive abilities, and psychological symptoms." However, only the MMSE, or Mini-Mental State Exam, was utilized to examine subgroups that pertain to specific diseases within the study.[11] A multicenter, randomized, double-blind study with a placebo control group conducted over 24-week treatment with donepezil in patients with severe AD in China displayed substantial improvements in the cognitive abilities and global function of patients.[12]

Discussion

Donepezil is an effective treatment for the symptoms of Alzheimer's among patients with mild to moderate as well as moderate to severe AD. While some of the studies included limitations and downsides, their results were consistent with one another and concluded that donepezil increases cognitive function and global function, utilizing various techniques, such as MMSE (Mini-Mental State Examination), CDR (Clinical Dementia), and ADL. In comparison to the control group in all of the investigative studies and clinical trials conducted that were provided with placebo, donepezil provided significant improvement in the cognitive abilities of patients. It is important to note, however, that while global function and cognitive function are significantly improved among mild to moderate and moderate to severe AD cases, only the symptoms of AD are treated with donepezil and not the root mechanism of Alzheimer's,

meaning that donepezil cannot entirely treat patients, but has the ability to prolong the time period in which the symptoms of AD affect patients' everyday lives.

Memantine [Monotherapy]

Background

Memantine(Namenda) has been approved by FDA for the treatment of moderate to severe Alzheimer's Disease (AD) symptoms since 2003.[13] The drug was first synthesized in the 1960s by Eli Lilly, who initially aimed to develop an antidiabetic agent.[14]Despite the limited evidence in managing blood sugar levels, it was not until 1984 when German scientists discovered that memantine affected the Central Nervous System and it could be effective in treating Parkinson's Disease.[15] In 1989, Bormann investigated the ability of memantine as an N-methyl-D-aspartate receptor (NMDAR) antagonist and discovered its action of temporarily blocking NMDAR,[16] which inspired the later experimentations and discussions for the development of memantine as a potential therapeutic option for treating AD.

Mechanism

Memantine targets the extrasynaptic NMDARs that are located on the dendrites.[17] NMDAR functions as a cationic channel to which glutamates bind,[18] and it has an important role in the progression of AD. Memantine serves as a "low-affinity voltage-dependent uncompetitive antagonist" of NMDAR that binds to NMDAR so that NMDAR will not be activated by glutamate.[16]

In patients with AD, there is an excess release of glutamate in their brains which increases the activity of NMDAR. This excitement stimulates an influx of Ca^{2+}+ ions into the postsynaptic cells and results in the formation of neuronal oxidative stress. The consequence is

the destruction and loss of synaptic connections, which is embodied in decreasing learning abilities and memory capacity.[18] Memantine works by reducing the overstimulation of NMDAR in the presence of a high concentration of glutamate by binding with NMDAR to prevent the series of action leading to synaptic destructions. There is also evidence of memantine being able to reduce the accumulation of Aβ from preclinical data.[19] However, whether memantine could slow down the disease progression remains controversial.[20]

Study Design and Results

One of the first few clinical trials that treated AD patients with memantine can be traced back to 2003 with 32 U.S. centers participating. It recruited 252 patients with moderate-to-severe AD who were randomly assigned to a daily treatment of either 20 mg of memantine or a placebo treatment for 28 weeks. The efficacy measures were evaluated from two levels. The primary level included Clinician's Interview-Based Impression of Change Plus Caregiver Input (CIBIC-Plus) and the Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory modified for severe dementia (ADCS-ADLsev), and the secondary level focused on the Severe Impairment Battery (SIB) and cognition, function and behavior disturbances. 181 patients completed the study and results demonstrated that memantine alleviated the symptoms of AD, evident by improvements in assessment scores.[21]

Despite many years of clinical research, many later clinical trials indicated that there is still insufficient evidence of therapeutic effectiveness from memantine monotherapy as a symptomatic treatment for AD. A meta-analysis synthesized nine randomized controlled trials (RCTs) that compared memantine monotherapy to placebo. These studies were conducted in different countries including Austria, China, Japan, the United Kingdom, the United States, and

multiple European countries (in one collaborative study) from 2003 to 2013 and lasted for 31 weeks on average. The meta-analysis analyzed the therapeutic outcomes of a total of 2433 patients with mild-to-severe AD (averaging 76 years old) who had not received cholinesterase inhibitors (ChEI) treatment to make the distinction of the effects from the pure memantine treatment. The primary efficacy measures emphasized the cognitive function (quantified by SIB, ADAS-Cog, SMMSE, or MMSE) and behavior disturbances (quantified by NPI and Behave-AD) while the secondary efficacy measures included activities of daily living (quantified by ADCS-ADLsev, ADCS-ADL19, ADCS-ADL23, BADLS, CIBIC-Plus, FAST, or GAF). Although the results showed that memantine monotherapy led to improvement in cognitive function, behavior disturbances and activities of daily living, the effect sizes were argued to be too trivial (with a Standardized Mean Difference from -0.09 to -0.27 across different tests) to prove clear clinical benefits.[22] The statistical results from the meta-analysis, therefore, suggested that memantine has limited clinical benefits in managing AD symptoms.

Discussion

It is important to note that one of the RCTs in this meta-analysis is Reignsberg's clinical trial in 2003. Reignsberg's result, in addition to the synthesized results from the meta-analysis uniformly yielded improving scores from multiple AD-associated tests comparing memantine monotherapy to placebo. However, the combined results stressed that the effect size of quantifiable outcomes was not big enough to translate into clinical meaningfulness of memantine monotherapy. This might be because all these RCTs reported the mean differences between placebo and memantine groups from multiple AD-associated scales. One significant drawback of this outcome-reporting strategy is that the mean scores may not be necessarily representative of every individual in the study. There might be a few participants who scored significantly higher

or lower than the majority and their scores exerted a larger effect in the mean. For example, the smaller single trial might include more uniqueness in treatment outcomes as compared to the meta-analysis that takes into account a larger volume of data. This possibly explains why the meta-analysis result seems to partially contradict the single trial. It is still important to know the mean point differences, but it will be helpful to include a responder analysis that reports the clinical response because it shows the percentage of patients achieving a pre-defined outcome or effect, indicating the probability of a therapy achieving a particular clinical effect. Combining statistical analysis from test scores with the responder analysis might produce a more holistic picture of the therapeutic effect.

Donepezil & Memantine [Combination Therapy]

Introduction

Memantine is an FDA-approved drug for the treatment of moderate to severe Alzheimer's Disease while donepezil is approved for the treatment of mild to moderate Alzheimer's Disease. Both memantine and donepezil as monotherapies have proven their efficacy through increased cognitive functions and improved daily activities. Memantine, an uncompetitive antagonist of NMDAR, and donepezil, an acetylcholinesterase inhibitor, both follow two different mechanisms. These two different inhibitors can also be used as a combination therapy to treat moderate to severe AD. Recently, the FDA has approved a drug called Namzaric that uses memantine and donepezil as a treatment of moderate to severe AD. This drug can be taken orally by capsules in two dosages: 4 mg memantine hydrochloride extended-release and 10 mg donepezil hydrochloride (14 mg/10 mg) or 28 mg memantine hydrochloride extended-release and 10 mg donepezil hydrochloride (28 mg/10 mg) which should

be taken once daily.[23] Although the FDA has approved the use of the two drugs combined, ambiguity on the efficacy of this dirty drug has remained.

Background

There are many reasons why Donepezil and Memantine work together efficiently. It is important to find out the root cause of AD to truly understand the effectiveness of the combination therapy. Deposition of beta-amyloid peptide (Aβ) plays an important role in the early developmental stages of AD. Insoluble Aβ plaques deposits in the brain parenchyma that disrupt neural connectivity and synaptic dysfunction, leading to lower cognitive function and results in AD.[24]Although deposition of Aβ does not directly contribute to the severity of AD, the accumulation of it triggers a downstream cascade of inflammatory response such as the production of proinflammatory cytokines by CD4+ T cells. Th1 cells and Th2 cells are part of the CD4+ T cells family which are responsible for adaptive immunity. Th1 cells produce IL-2, IFN-γ, and TNFα that act as a defence against intracellular viral and bacterial pathogens. IFN-γ activates macrophages, promotes antigen presentation and natural killer cell function. Th2 thereby counteracts the proinflammatory response by secreting IL-4, IL-5, and IL-13 to promote an anti-inflammatory response.[25] Th2 cells repress Th1 with the help of astrocytes that clears Aβ deposition and protects neuronal synapses in the central nervous system. Astrocytes are components of the Neurovascular Unit (NVU) that are responsible for neurodevelopment, brain maintenance, and repair and support cerebral homeostasis. Astrogliosis is a process when astrocytes undergo molecular and morphological changes that induce them to eliminate toxic substances.[26] One of the morphological changes includes an increase in expression of the glial fibrillary acidic protein (GFAP). A study using 3xTg-AD mice with GFAP−/− astrocytes found inability to form a barrier-like structure around amyloid β (Aβ) deposits, suggesting a role for

GFAP in the structural alterations of reactive astrocytes surrounding plaques in Alzheimer Disease (AD).[27] In the early stages of AD, astrocytes also present Aβ to antigens of Th2 cells thus, suppressing Th1 cells. However, in the late stages of AD, astrocytes produce IL-1β and IL-6 which recruits proinflammatory Th1 and Th17 cells. Donepezil is used to inhibit Th1 and promotes Th2, while Memantine decreases the pro-inflammatory response by blocking glutamate receptor NMDA.[3] Due to their ability to target different pathways, a combination of memantine and donepezil is a potentially effective drug to treat moderate to severe AD.

Study Design and Results

A double-blind placebo clinical trial done by Howard et al. have shown that there were no notable benefits of adding memantine to a stable dose of donepezil.[28] This research was funded by the U.K. Medical Research Council and the U.K. Alzheimer's Society. The trial lasted 52 weeks with a total of 295 participants. Each person was assigned to one of the four subgroups: donepezil and placebo, only placebo memantine, and a combination of memantine and donepezil. All the selected patients (age 60 to 75) were required to have a stable dose of donepezil (10mg) for at least 3 months and have SMMSE scores between 5 and 13 that indicate moderate to severe AD. This study showed that patients that were assigned to stabilized donepezil doses had a higher SMMSE score by 1.9 points and a lower BADLS score by 3.0 points compared to patients that discontinued donepezil. Additionally, patients with continued memantine compared to discontinued memantine had higher SMMSE by 1.2 points and BADLS scores by 1.5 points. It is also noted that patients who were taking memantine and donepezil had higher SMMSE scores by 0.8 and lower BADLS scores by 0.5 compared to placebo and memantine. The researchers observed a slight increase (1.2 points higher) in SMMSE score ($P<0.001$) and a slight decrease in BADLS (1.5 points lower, $P=0.02$), suggesting that donepezil

and memantine efficacy "did not differ significantly in the presence or absence of each other".[28] Thus, the results of the trial were not significant enough to conclude that combination therapy is more effective relative to the monotherapy treatments. Therefore, patients that had either only donepezil or only memantine showed better efficacy than patients that were taking a combination of the two.

On the other hand, two 24 weeks long randomized placebo-controlled trials done by Atri et al. in several centers across the United States have proven that the addition of memantine to a stable dose of donepezil has a significant reduction in clinical worsening and an increase in safety and efficacy. The clinical worsening is a measure of deterioration from a baseline of three domains (cognitive, functional and global) and is determined when there is a decline of ≥ 4 points on ADAS-Cog or ≥ 5 points on SIB, and any decline on ADCS-ADL19/ADCS-ADL23 and CIBIC-Plus. All 510 patients were prescribed donepezil for at least 6 months with stable doses for 3 months and were separated into two groups: mild to moderate AD (171 patients) and moderate to severe AD (339 patients). The mild to moderate group was given 10mg of memantine once daily with a stable dose of donepezil while the moderate to severe group was given 20mg of memantine twice daily with a stable dose of donepezil as well. These two groups were then compared with a placebo group. This clinical trial has shown that the clinical worsening effect is lower by more than half for patients that were on combination therapy compared to patients stabilized on donepezil only for both moderate to severe group (8.7% of patients for combination and 20.4% of patients for donepezil only group) and mild to moderate group (5.9 % of patients for combination group and 15.0% for donepezil only group).[29]

A pooled area under the curve (AUC) analysis by Atri et al. performed a six-month randomized double-blind controlled trial of a total of 1,408 patients observed that all the

monotherapy groups had significantly higher cumulative worsening rates.[29] The combination therapy group had statistically significant cognitive function compared to monotherapy followed by the placebo group with almost no improvement in cognitive function. This study observed four different areas: cognition (SIB), function (ADCS-ADL19), behavior (NPI), and global status CIBIC-plus). Memantine and donepezil combination showed statistically significant compared to donepezil only (SIB ($P = 0.019$), NPI ($P=0.003$), and CIBIC-plus ($P<0.001$)). To compare with memantine only therapy, SIB ($P<0.001$), NPI ($P<0.001$), CIBIC-Plus ($P<0.001$). The results were not significant for ADCS-ADL19 measurement for both monotherapies (donepezil only, $P=0.407$) and memantine only, $P=0.310$) compared to combination therapy. Patients who were treated with donepezil and memantine had cumulative improvements in all four areas by 450%, while patients on placebo showed a decline in cognitive activity.[8] The observed statistically significant results suggest that further studies on the efficacy of the dirty drug could be of interest in future research.

Discussion

Donepezil and memantine combination therapy has proven to improve cognitive functions and daily activity. However, some studies have shown that the effect of combined drugs is not as significant compared to using donepezil or memantine alone. This inconsistency in the results shows that the combination therapy still needs to be further investigated and it is invalid to draw a conclusion of its effectiveness. Some of the variability in clinical trials that influence the confidence of each study include age, gender, underlying health condition, and the sample size drawn. The length of the trials also plays an important role in determining the effectiveness of the drug. Some studies have shown that cognitive function is only observed after 14 weeks of trial. Although donepezil and memantine combination therapy was FDA approved,

there is limited knowledge and information on how the drug works effectively. Therefore, further experimental research at different geographic locations, same age range, and same sample size might portray new information on donepezil and memantine combination therapy.

Donepezil and Memantine: Monotherapy vs Combination Therapy

A double-blind and randomized controlled clinical trial conducted between 8 medical centers in China provided a comparative analysis between donepezil and memantine monotherapy. 167 participants with a mean age of 69.95 years old and starting MMSE scores between 10 to 24 which categorized them into mild-to-moderate AD patients were selected, among which 80 received 10 mg memantine twice daily and 87 received 10 mg donepezil once daily throughout a 24-week period. Researchers set ADAS-cog, 20-item ADL and CIBIC-Plus as the primary outcome measure and NPI and MMSE as the secondary outcome measures. Results indicated a greater decrease in agitation in the memantine group and better naming ability in the donepezil group. Any of the four test items (memory, language, praxis, and attention) in the ADAS-cog did not change significantly in either group. In addition, NPI, 20-item ADL, CIBIC-Plus or MMSE scores did not exhibit any statistically significant differences in the changes from the start of the study in response to either treatment. Therefore, this study concluded that the differences in terms of treatment efficacy between donepezil and memantine monotherapy in mild-to-moderate AD patients is similar.[30]

While donepezil and memantine are similar in treatment efficacy, there are nuanced differences between the two drugs that indicate that the drugs are not guaranteed to be similar in efficacy, as proven by Zhang and Gordon's meta-analysis comparing various studies and summarizing their results[31]. In a multi-center study conducted in China comparing the efficacy of

donepezil and memantine, it was found that the effects of the two drugs were equivalent with some nuances in efficacy. Mild to severe Alzheimer's patients within the age group of 45-80 years old were divided into two groups, one of which was given 10 mg/day of donepezil, while the other was provided with 10 mg/day of memantine. On all basic outcome measures, such as CIBIC-plus (Clinician's Interview-Based Impression of Change plus Caregiver Input), 20-item ADL, ADAS-cog, the NPI, and the MMSE (with scores ranging from 3-24), the results of donepezil and memantine exhibited efficacy of equivalent levels.[12] However, in a secondary analysis, patients provided with donepezil displayed increased scores on the ADAS-cog (a behavior and mood test) in comparison to those in the mild to moderate subgroup who were given memantine, while the group provided with memantine exhibited more effective agitation scores on the NPI (The Neuropsychiatric Inventory).[30] In another study conducted by Hu HT, Zhang ZX, Yao JL, et al. to examine the different efficacies of donepezil and memantine in Alzheimer's disease patients, donepezil had a "trend toward better improvement in daily living" than those provided with memantine at the 8 week mid-point of the study, however, at the 16-week endpoint, there were no notable outcome differences between the two groups.[31]

Both Zhang et al.'s clinical trial[30] and Zhang and Gordon's meta-analysis[31] suggested that the efficacy outcomes maintained largely similar between donepezil and memantine groups in terms of the majority of AD-associated tests, and the changes in scores were not statistically significant to dictate either treatment's symptomatic benefits. However, both studies still manifested subtle yet statically significant differences in the subscales of the major tests; for example, different facets of cognitive function tests. In Zhang et al.'s study, the ability to name objects improved more in the donepezil group, while the agitation declined more in the memantine group. In Zhang and Gordon's meta-analysis that synthesized results regarding to

treatment outcomes between donepezil and memantine from multiple clinical trials, there was no significant difference in outcome between the two on all basic outcome measures, however, the secondary analysis pointed to variations, with donepezil increasing the ADAS-cog score, a behavior and mood test, and memantine exhibiting more effective agitation scores on the NPI.

A 24 weeks long double-blind, randomized placebo-controlled trial for moderate to severe AD was performed at 37 sites across the U.S in 2001 to study the effectiveness of the combination therapy. All 404 patients chosen were required to have a MMSE score between 5-14, at least 60 years old and have been prescribed 5-10mg donepezil for at least 3 months. Patients were assigned to one of two groups: memantine and donepezil group (203) or donepezil and placebo group (201). Statistical analysis was measured using SIB, ADCS-ADL19, CIBIC-Plus, and NPI. Patients were given 5mg of memantine with weekly increments of 5mg until it reached 20mg starting week 4 of trial and the placebo group were given placebo and a stable dose of donepezil. All analysis of data was taken from patients who had taken the baseline assessment for SIB and ADCS-ADL19 once. The addition of memantine to donepezil showed statistically significant results where the SIB p-value was less than 0.001 and the ADCS-ADL19 p-value was 0.03 after week 8. As a secondary measure, CIBIC-Plus for memantine and donepezil had a lower score and p-value of 0.03 for combination therapy, lastly, the NPI score was significantly lower for memantine-donepezil (P=0.002) when compared to donepezil-placebo at the end point.[32] The previously mentioned study by Howard et al. observed that patients who were taking memantine and donepezil had a higher MMSE score and a lower BADLS score, indicating that patients experienced better cognitive and ability to do daily activity.[28]

Discussion

According to the clinical trials and meta-analysis we have gathered in this paper, donepezil and memantine as monotherapies show firm proof of effectiveness and significantly improved AD symptoms. The question of whether combination therapy is better than monotherapy is still uncertain. From the clinical trials mentioned above, patients who had memantine and donepezil together had an increase in cognitive function, improvement in overall observation and behavior of patients, and significantly fewer behavioral symptoms. Some of the reasons why they obtained similar results may include having the same age range and requiring patients to be stabilized on donepezil for at least 3 months. However, it is still unclear to conclude whether monotherapy is better than combination therapy because each individual clinical trial may have different methods to test or measurements to determine what truly is a better treatment.

Analysis of Monotherapies Rivastigmine and Rasagiline vs Combination Therapy Ladostigil

Rivastigmine [Monotherapy]

Introduction

The semi-synthetic derivative of physostigmine- rivastigmine- developed by Martha Weinstock-Rosin played a key role in framing dementia treatment.[33] Rivastigmine first received global approval for mild to moderate dementia treatment for symptoms associated with Parkinson's disease (PD).[34] Having received approval from 60 major countries including that of the FDA in the US in 2000 for treatment of AD and other forms of dementia,[35] understanding the efficacy of rivastigmine in conjunction with its safety will be crucial.

Mechanism

Rivastigmine functions as a reversible inhibitor of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) that sustains its effects for at least 12 months of repeated administration.[36] As a fundamental building block of neural communication, AChE is characterized by a family of enzymes that catalyzes the hydrolysis of acetylcholine- a crucial neurotransmitter. Similarly, BuChE aids the hydrolysis of particular choline-based esters. A combination of the inhibitors actively works to block the AChE to limit the breakdown of acetylcholine in a reversible mechanism.[37] The resulting increase in acetylcholine levels in the brain provides relief to AD symptoms.

Study Design and Results

Rivastigmine couples BuChE and AChE inhibitors to inhibit AChE with the end goal of providing AD treatment. A retrospective statistical analysis of three double-blind databases (Investigation of transDermal Exelon in Alzheimer's disease [IDEAL; AD], EXelon in PaRkinson's disEaSe dementia Study [EXPRESS; PDD]; Alzheimer's Disease with ENA 713 [ADENA] database) conducted by Weintraub et al.[38] derived a twofold analysis of memory and language from ADAS-cog (Alzheimer's Disease Assessment Scale) to investigate this claim. Now considered the "gold standard" for mild to moderate AD analysis, the ADAS-cog utilized was categorically designed to "measure the change in cognitive deficits that characterize AD". Even though data were pooled and analyzed for 4,540 participants with AD and PD, this paper concerns itself with the 3,959 AD patients [IDEAL; EDNA] whose collective and subgroup data streams were aggregated and analyzed separately to the PD and placebo groups.[38]

In comparison with the placebo group ($Ps < 0.0001$) the rivastigmine-treated participants showed significant increases in ADAS-cog scores; however, with a $P > 0.05$ no statistically significant difference was observed in the adjusted scores in memory and language of AD population when accounting for placebo effects. Notably, the considerable sample size of 3,959 AP participants improves confidence in the final conclusions. Relatedly, the robust two-pronged approach (synthesized from 11 baseline ADAS-cog factors) lends credence to the study by providing statistical validation of a broader domain of factors that are affected by Alzheimer's. And by integrating data from multiple doses as well as delivery methods (1 to 4 mg/d, 6 to 12 mg/d by rivastigmine capsule; 293 to 9.5 mg/24 h, 303 to 17.4 mg/24 rivastigmine patch), and by clustering a larger placebo population (1350) a comparative analysis of titration quantity could be made. More specifically, it was observed that the greatest effect on memory and language

domains was for the 17.4 mg/24 h patch memory group. While the statistical significance was not designed for subgroup comparisons, studying such a comparison of titration quantity could be the focus of future research.[38]

Discussion

Despite progressive steps in the choice of outcome measurements, more holistic conclusions could be drawn using 'adverse event' data coupled with QoL (quality of life) indices that only certain studies in the database gauged. An underlying criticism is that the study accepted the assumption that the ADAS-cog system is a "gold standard" metric. Albeit, the original ADAS-cog consisted of 11 items including memory, language, praxis, and orientation,[39] but the component of patient well-being using QoL would fundamentally provide a more holistic approach to AD research. QoL data would ideally include physical, mental, social, and emotional health as well as adverse event analysis.[40] Future studies could incorporate activities in daily living and global functioning data to evaluate the efficacy and tolerability of rivastigmine in AD participants.

Rasagiline [Monotherapy]

Introduction

The inhibition of a particular group of mitochondrial-bound flavoprotein isoenzymes has surfaced as a viable outlet for clinical use in antidepressants and cognitive disorders. As the first type of antidepressant developed,[41]Monoamine Oxidase Inhibitors (MAOI) hold a long history of clinical experimentation and even received FDA approval for Parkinson's Disease Treatment in 2006. While the cross-application of a mechanism, such as MAO inhibition to cognitive disorders, would not be solely unique, its aforementioned clinical prominence could potentially

play a role in its usage for a patient facing a combination of depression and cognitive disorder symptoms. Moreover, the breakthroughs of MAOI in the treatment of neuropsychiatric disorders varying from Parkinson's disease to mood disorders has distinctly characterized MAOI as an untested yet versatile and unique candidate for the treatment of a range of mental disorders. In this paper, the known effects of MAO inhibition on Alzheimer's disease is of central concern.

Mechanism

The MAO isoenzymes catalyze the degradation of monoamines into its aldehyde counterpart, which can then be converted to acids or alcohols by aldehyde dehydrogenase and aldehyde reductase respectively. This process is regarded as one of the central mechanisms for the functioning of synaptic neurotransmission and consequently for the regulation of emotions, mood, and cognitive control.[42] In turn, particular isoenzymes of monoamine oxidase have been linked to apoptosis. The activity of MAO-A increased during apoptosis in PC-12 rat adrenal medulla cells,[43] and the presence of si-RNA (short interfering RNA) that downregulates MAO-A expression in human neuroblastoma SH-SY5Y cells was reported to reduce cell death and toxin binding.[44] Such in vitro findings implicate clinically testing the MAO pathway for cognitive disorder treatment where apoptosis is thought to play a role, such as Alzheimer's disease.[45] In particular, selegiline and rasagiline have been posed as tenable candidates.

Study Design and Results

Coupled with its previously reported promotion of anti-apoptotic activity, the irreversible MAO-B inhibitors such as rasagiline have been suggested as treatments for Alzheimer's (AD) and Parkinson's diseases (PD). While studies that link rasagiline to tau accumulation (or the buildup of a specific family of neurotoxic proteins[46]) such as that of the Cleveland Clinic surface

to attention, a deeper dive into the clinical and biochemical efficacy reveals that such generalized suggestions require further examination, as specificity and viability may not be unconditional.

In a Phase II "proof of concept" randomized clinical trial of the Cleveland Clinic, rasagiline potency in Alzheimer's treatment was investigated on patients with mild to moderate AD.[47] In addition to being a placebo-controlled, double-blind, and parallel-group study, demographic information pertaining to the age, sex, and education of the participants did not differ substantially between groups. Despite the limitation of a small sample size of 50, the longitudinal analysis of the experiment in conjunction with FDG-PET and Tau PET evaluations allowed for an interesting visual comparative analysis of tau aggregation. Crucially, tau- a protein linked to multiple brain diseases- had previously been found to agglomerate early in AD patients and this effect was found to increase with time.[48,49] By using Tau PET data over an interval of 24 weeks, the Cleveland Clinic Study expounded on prior findings while establishing new links to rasagiline and mild to moderate AD. Ultimately, the study met its primary objective of improving longitudinal metabolism- a key aspect of improvement in AD treatment- versus placebo in the frontal cortex (left $P < 0.012$ bilateral $P < 0.025$), anterior cingulate cortex ($P < 0.043$), and striatum ($P < 0.02$).[47] Utilizing the Digit Span, CGIC, COWAT, and NPI indexes for quality of life (QoL), a quantitative relationship was established between uniform improvements in QoL and receiving rasagiline treatment. Conversely, there was an observed decline in placebo participants. Hence, the study expands its evaluation of 'improvement' during treatment from mere quantitative or biochemical perspectives to considering the QoL, adverse events, and neuropsychiatric symptoms. Subsequently, this satisfies the need to understand the effect of treatments in daily life beyond the research laboratory, lending credence to the claims of improved wellbeing.

Discussion

These findings are promising for the future of rasagiline studies but the limitations on confidence must also be considered. Principally, a small sample size (noted by the authors themselves) renders the likelihood of the sample data (and consequent conclusions) in concert with generalizability to the larger Alzheimer patient population as questionable. As a further complication, 84% of the participants were taking AChE inhibiting medications such as memantine prior to and during the duration of the experiment. While the authors present that rasagiline effects were "incremental to the action of the medication", the true improvement in the dependent variable (baseline scores) that can be attributed to rasagiline is called into question. Additionally, the recognized imbalances in baseline MMSE, ADAS-cog, and QoL scores between treatment arms of participants that materialized by chance add another layer of complexity in analysis.[47] However, in contrast to several other MAO inhibitors, rasagiline could still be of viable interest for future studies. For instance, a Cochrane Database systematic review of selegiline (another MAO inhibitor) concluded that "[t]here would seem to be no justification (...) to use it in the treatment of people with Alzheimer's disease, nor for any further studies of its efficacy in Alzheimer's disease."[35] Thus, despite the identified drawbacks of lacking generalizable, controlled, and significantly conclusive results, the results seem promising and indicate that the repurposing of rasagiline in AD treatment holds a future in clinical trials.

Ladostigil [Combination Therapy]

Introduction

Ladostigil is a multimodal/multifunctional drug that was designed with the intention to treat dementia comorbid with extrapyramidal disorders and depression. It is a combination of the

neuroprotection properties of rasagiline and the cognitive enhancing properties of rivastigmine[50], both FDA approved drugs used to treat Parkinson's Disease and Alzheimer's Disease (AD) respectively. There have been two Phase 2 studies investigating ladostigil – one evaluating the course of escalating doses of up to 80mg twice daily in people with mild to moderate AD[51] and another evaluating a lower dose for its ability to delay progression from Mild Cognitive Impairment (MCI) to AD.[52] Although the developers intended to use ladostigil to treat AD it was later changed "to an indication of mild cognitive impairment (MCI)".[53]

Mechanism

Weinstock et al. developed Ladostigil with the intention of using it to treat AD alongside extrapyramidal disorders and depression.[54] One of the main reasons for the cognitive deficit in Alzheimer's Disease (AD) is the degeneration of cholinergic cortical neurons. Many subjects with AD experience extrapyramidal dysfunction and depression resulting from the degeneration of dopaminergic, noradrenergic and serotonergic neurons. For this reason, ladostigil was developed with both cholinesterase (ChE) and monoamine oxidase (MAO) inhibitory activity, as a potential treatment of AD.[54]

MAO is one of several contributing enzymes of oxidative stress, which "contribute to the observed synaptic dysfunction and neurodegeneration [in AD], which are most severe in the cholinergic system." The subsequent decreased levels of acetylcholine lead to cognitive and memory deficits — a key characteristic in AD patients.[55] Ultimately, ladostigil was created with the expectation that reduced cell damage and preservation of cholinergic neurotransmission would have a good chance at slowing down AD as well as maintaining cognitive function and the ability for self-care.[54]

Study Design and Results

Preclinical Research

Weinstock et al. conducted research on rats where they orally administered 35-100 mol/kg of ladostigil that was observed to inhibit ChE by 25%-40%. And after daily oral administration of 75 mol/kg for 2 weeks, it was found to inhibit MAO-A and B in the brain by about 80%. Ladostigil was also shown to have neuroprotective effects against a variety of insults in PC12 cells and in vivo. Additionally, it significantly reduced hippocampal cell damage caused by global ischemia in gerbils and the cerebral oedema induced by a closed head injury in mice and sped recovery of their motor and memory.[54] These findings displayed great potential in ladostigil being used to treat AD which led to further development and studies on the drug.

Phase 2: Safety and Efficacy of Ladostigil in Patients with Mild to Moderate Probable AD

This double-blind, randomized, placebo-controlled trial, sought to evaluate the safety and efficacy of ladostigil compared to placebo. The study consisted of two cohorts: one which received ladostigil from the start of the first 26-week period and another that received a placebo during that time. After the first 26-week period all participants received a 26-week treatment of ladostigil. The trial missed its primary endpoint of change on the Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog), and development for AD was terminated.[51]

Phase 2: Delaying Progression of MCI to AD

In this 3-year, randomized, double-blind, placebo-controlled phase 2 clinical trial 210 patients with mild cognitive impairment (MCI) and medial temporal lobe atrophy were allocated to placebo or ladostigil. The primary goal of this study was to explore its effect on ameliorating

progression from MCI to AD alongside the goals to assess the effect of ladostigil on cognition, daily functioning, and biomarkers.[52]

Over the three years, 20.4% of the placebo group progressed from MCI to dementia compared to the 14.1% of the ladostigil group. However, there were no statistically significant differences in secondary/exploratory outcomes when comparing the two cohorts. At the same time, there was a significantly reduced loss of whole-brain and hippocampal volume in the ladostigil-treated patients. Notably, the participants with MCI in the placebo groups were more cognitively impaired compared to those in the ladostigil trial which supports previous observations that decrease in brain volumes may precede cognitive impairment and dementia onset.[55]

Although this study ultimately showed that a low dose of ladostigil was well-tolerated and safe, and even had some potential benefits it is hard to say whether ladostigil is truly effective in delaying the neurodegeneration of MCI to AD. Conversely, even if that was clear, MCI diagnosis predicts over a 3-year period.[55]

Both Phase 2 studies failed to meet their primary research goals. Despite that, both clinical trials provided results that promote the continued development of ladostigil. Although ladostigil is no longer being considered for the treatment of AD it still seems to have the potential to treat MCI.

Monotherapies Rivastigmine and Rasagiline vs Combination Therapy Ladostigil

Given an overall significance value of $p<0.005$ and a significance threshold set to $p=0.045$ based on the O'Brien Method[56], there were no statistically significant differences between placebo and ladostigil treatment. And with an observed 20% progression in the placebo group, the Ladostigil group detected a non-significant risk difference of 7%.[57]

Similarly, while the rivastigmine-treated population showed statistically significant increases in ADAS-cog scores, adjusted memory and language scores had no significant differences ($P > 0.05$) when compared to the placebo group.

Meanwhile, Rasagiline silver bullet research detailed that the placebo trial observed a decline in longitudinal metabolism while improvements were observed in the rasagiline-treated participants (frontal cortex left $P < 0.012$ bilateral $P < 0.025$ among other statistically significant p-values). Importantly, improvements in QoL (quality of life) were observed in the rasagiline-treated population.[58]

Discussion

While each trial was conducted in appreciably distinct locations, had different sample sizes and administration methods, and measured efficacy through disparate experimental metrics, the pooled differences between the treatment and placebo groups for each independent study was made clear and is statistically sound.

Recognizing the flaws in direct commensurability of the three data streams, this paper chose to individually evaluate the efficacy of each drug in conjunction with questioning the experimental design and execution of each paper. Given this premise and the aforementioned data, we conclude that while rasagiline and rivastigmine silver bullet drugs showed signs of effectiveness, there are recognizable limitations such as a smaller sample size, exposure to confounding variables such as other AD drugs, and the use of suboptimal AD outcome measures that require further evaluation in future studies. However, the dirty drug ladostigil was largely ineffective. It can be reasonably inferred that this could have contributed to the repurposing of ladostigil for the delay of Mild Cognitive Impairment (MCI) to AD.

Conclusion

Overall, the relative effectiveness of "silver bullet" and "dirty" drugs of AChE inhibitors and their derivatives depends on a myriad of variables including the biochemical mechanisms, experimental administration, and limited data. This leaves no decisive conclusion, as re-evaluations would highly depend on the "type-specificity" of the inhibitor as well as circumventing issues of suboptimal experimental design. Even then, there may not be significant differences due to the lack of changes in biochemical efficacy of the augmented dirty drug relative to its silver bullet counterparts. However, confidence in experimental components could be improved by incorporating the aforementioned considerations: (1) avoiding significantly high dropout rates (2) combining point-based analysis accounting for mean point differences with the responder analysis (3) adjusting the time duration of observations based on cognitive function data (4) increasing the sample size of smaller studies and avoiding confounding variables of patients being exposed to other drugs (5) improving AD outcome measurement indices to account for the holistic quality of life in conjunction with biological components. Despite solely relying on comparative data between placebo trials and the administered treatment, we recognize the incommensurability due to experimental conditions and the statistical significance of such concerns that could affect conclusions. Future research that pools data in similarly controlled circumstances could address this factor.

Despite many chemical therapies targeting different disease etiologies that have been researched and developed, they only showed evidence in managing disease symptoms but lacked effects in preventing the onset of AD or intervening in the disease progression. This suggests the urgent need for novel therapeutic approaches to address those unrealized medical needs.

Among diverse options, immunotherapy, i.e. administering biopharmaceuticals to stimulate people's own immune response, has been attracting attention in AD therapeutic development. More specifically, immunotherapies that target Aβ protofibrils have been regarded as a hopeful approach to affect disease progression.[59] In addition, the popular, versatile CRISPR/Cas9 gene-editing technology sheds light on the strategy to target Aβ at the genetic level. The APPswe mutation plays a pivotal role in the heritability of AD, and researchers modified the mutant genes using CRISPR/Cas9 and gained evidence for both successful *ex vivo* and *in vivo* disruption of the APPswe allele which could potentially decrease the abnormally high level of Aβ that occur in the mutant-carrying population.[60] This would eventually prevent AD from taking place. While immunotherapies and gene therapies are still undergoing clinical trials up to phase III, both are highly promising therapeutic options in the future to fuel a revolution in the current AD treatment scheme.

References

1. MacGill, Markus. "Alzheimer's Disease: Symptoms, Stages, Causes, and Treatments." Edited by Seunggu Han, *Medical News Today*, MediLexicon International, 22 Sept. 2020, www.medicalnewstoday.com/articles/159442.
2. Cummings, J. L., Tong, G., & Ballard, C. (2019). Treatment combinations for Alzheimer's disease: current and future pharmacotherapy options. Journal of Alzheimer's Disease, 67(3), 779-794.
3. Benkler, C., Offen, D., Melamed, E., Kupershmidt, L., Amit, T., Mandel, S., Youdim, M. B., & Weinreb, O. (2010). Recent advances in amyotrophic lateral sclerosis research: perspectives for personalized clinical application. *The EPMA journal*, *1*(2), 343–361. https://doi.org/10.1007/s13167-010-0026-1.
4. U.S. National Library of Medicine. (n.d.). *Donepezil: MedlinePlus Drug Information*. MedlinePlus. https://medlineplus.gov/druginfo/meds/a697032.html#:~:text=Donepezil%20is%20used%20to%20treat,the%20ability%20to%20think%2C%20learn%2C.
5. *Therapeutics: Donepezil*. Alzforum: Networking for A Cure. https://www.alzforum.org/therapeutics/donepezil.
6. Kumar A, Sharma S. Donepezil. [Updated 2020 Aug 22]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK513257/.
7. Dooley, M., Lamb, H.M. Donepezil. *Drugs & Aging* 16, 199–226 (2000). https://doi.org/10.2165/00002512-200016030-00005.
8. Drug Action By Abimbola Farinde, et al. "Drug Action - Drugs." *Merck Manuals Consumer Version*, Merck Manuals, www.merckmanuals.com/home/drugs/drug-dynamics/drug-action#:~:text=from%20the%20body.-,Reversibility,the%20body%20manufactures%20more%20enzyme.
9. Delaune KP, Alsayouri K. Physiology, Noncompetitive Inhibitor. [Updated 2020 Sep 13]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK545242/.
10. Huang H, Li M, Jiang G, Mu X, Chen Q. Long-term efficacy observation of donepezil in the treatment of Alzheimer's disease. Zhonghua Lao Nian Yi Xue Za Zhi. 2012;31(2):98–101.
11. Peng D, Yu P. A survey of the consultative status of patients with Alzheimer disease and the efficacy and safety of donepezil treatment. Zhonghua Lao Nian Yi Xue Za Zhi. 2010;29(8):691–693.

12. Cheng Y, Zhang N, Du H. Multicenter controlled randomized clinical trial of akatinol memantine for treatment of Alzheimer's disease. Zhonghua Shen Jing Ke Za Zhi. 2009;42(4):268–272.

13. U. S. Food and Drug Administration. Center for Drug Evaluation and Research. (2003). *Approval Package for: Application Number 21-487*. Retrieved from https://www.accessdata.fda.gov/drugsatfda_docs/nda/2003/21-487_Namenda_Medr_P1.pdf.

14. Gerzon, K., Krumkalns, E.V., Brindle, R.L., Marshall, F.J., Root, M.A., 1963. The Adamantyl Group in Medicinal Agents. I. Hypoglycemic N-Arylsulfonyl-N'-adamantylureas. J. Med. Chem. 6, 760–763. https://doi.org/10.1021/jm00342a029.

15. Schneider, E., Fischer, P.A., Clemens, R., 1984. Effects of oral memantine on symptoms of Parkinson's disease. Deutsche Medizinische Wochenschrift 109, 987–990. https://doi.org/10.1055/s-2008-1069311.

16. Bormann, J. (1989). Memantine is a potent blocker of N-methyl-D-aspartate (NMDA) receptor channels. *European Journal of Pharmacology*, *166*(3), 591–592. https://doi.org/10.1016/0014-2999(89)90385-3.

17. Léveillé, F., Gaamouch, F.E., Gouix, E., Lecocq, M., Lobner, D., Nicole, O., Buisson, A., 2008. Neuronal viability is controlled by a functional relation between synaptic and extrasynaptic NMDA receptors. The FASEB Journal 22, 4258–4271. https://doi.org/10.1096/fj.08-107268.

18. Lipton, S.A., 2005. The molecular basis of memantine action in Alzheimer's disease and other neurologic disorders: low-affinity, uncompetitive antagonism. Curr Alzheimer Res 2, 155–165. https://doi.org/10.2174/1567205053585846

19. Ito, K., Tatebe, T., Suzuki, K., Hirayama, T., Hayakawa, M., Kubo, H., Tomita, T., Makino, M., 2017. Memantine reduces the production of amyloid-β peptides through modulation of amyloid precursor protein trafficking. European Journal of Pharmacology 798, 16–25. https://doi.org/10.1016/j.ejphar.2017.02.001.

20. Post SG. Slowing the progression of Alzheimer disease: ethical issues. Alzheimer Disease and Associated Disorders 1997;11(suppl 5):S34‑9.

21. Reisberg, B., Doody, R., Stöffler, A., Schmitt, F., Ferris, S., Möbius, H.J., 2003. Memantine in Moderate-to-Severe Alzheimer's Disease. New England Journal of Medicine 348, 1333–1341. https://doi.org/10.1056/NEJMoa013128.

22. Matsunaga, S., Kishi, T., Iwata, N., 2015. Memantine monotherapy for Alzheimer's disease: a systematic review and meta-analysis. PLoS One 10, e0123289. https://doi.org/10.1371/journal.pone.0123289.

23. U.S. Food and Drug Administration. *NAMZARIC (memantine hydrochloride extended-release and donepezil)* (2014) Reference ID: 3678032

24. Murphy, M. P., & LeVine, H., 3rd (2010). Alzheimer's disease and the amyloid-beta peptide. Journal of Alzheimer's disease: JAD, 19(1), 311–323. https://doi.org/10.3233/JAD-2010-1221.

25. Kubick, N., Flournoy, P., Enciu, A. M., Manda, G., & Mickael, M. E. (2020). Drugs Modulating CD4+ T Cells Blood-Brain Barrier Interaction in Alzheimer's Disease. *Pharmaceutics*, *12*(9), 880. https://doi.org/10.3390/pharmaceutics12090880.

26. González-Molina, L. A., Villar-Vesga, J., Henao-Restrepo, J., Villegas, A., Lopera, F., Cardona-Gómez, G. P., & Posada-Duque, R. (2021). Extracellular Vesicles From 3xTg-AD Mouse and Alzheimer's Disease Patient Astrocytes Impair Neuroglial and Vascular Components. Frontiers in aging neuroscience, 13, 593927. https://doi.org/10.3389/fnagi.2021.593927

27. Xu K, Malouf AT, Messing A, Silver J (1999) Glial fibrillary acidic protein is necessary for mature astrocytes to react to beta-amyloid. Glia 25: 390–403. https://doi.org/10.1002/(SICI)1098-1136(19990215)25:4<390::AID-GLIA8>3.0.CO;2-7

28. Howard, R., McShane, R., Lindesay, J., Ritchie, C., Baldwin, A., Barber, R., Burns, A., Dening, T., Findlay, D., Holmes, C., Hughes, A., Jacoby, R., Jones, R., Jones, R., McKeith, I., Macharouthu, A., O'Brien, J., Passmore, P., Sheehan, B., Juszczak, E., … Phillips, P. (2012). Donepezil and memantine for moderate-to-severe Alzheimer's disease. The New England journal of medicine, 366(10), 893–903. https://doi.org/10.1056/NEJMoa1106668

29. Atri, A., Molinuevo, J.L., Lemming, O. *et al.* Memantine in patients with Alzheimer's disease receiving donepezil: new analyses of efficacy and safety for combination therapy. *Alz Res Therapy* 5, 6 (2013). https://doi.org/10.1186/alzrt160.

30. Zhang, N., Wei, C., Du, H., Shi, F.-D., & Cheng, Y. (2015). The Effect of Memantine on Cognitive Function and Behavioral and Psychological Symptoms in Mild-to-Moderate Alzheimer's Disease Patients. *Dementia and Geriatric Cognitive Disorders*, *40*(1–2), 85–93. https://doi.org/10.1159/000430808.

31. Zhang N, Gordon ML. Clinical efficacy and safety of donepezil in the treatment of Alzheimer's disease in Chinese patients. *Clin Interv Aging*. 2018;13:1963-1970. https://doi.org/10.2147/CIA.S159920.

32. Tariot PN, Farlow MR, Grossberg GT, et al. Memantine Treatment in Patients With Moderate to Severe Alzheimer Disease Already Receiving Donepezil: A Randomized Controlled Trial. JAMA. 2004;291(3):317–324. doi:10.1001/jama.291.3.317.

33. Kumar, V. (2006). "Potential medicinal plants for CNS disorders: An overview". *Phytotherapy Research*. **20** (12): 1023–1035.doi:10.1002/ptr.1970. PMID 16909441. S2CID 25213417.

34. Abreu, Vera. "Exelon (Rivastigmine)." *Parkinson's News Today*, 1 May 2018, parkinsonsnewstoday.com/exelon-rivastigmine/.

35. Birks, J. S., Chong, L. Y., & Grimley Evans, J. (2015). Rivastigmine for Alzheimer's disease. *The Cochrane database of systematic reviews*, *9*(9), CD001191. Advance online publication. https://doi.org/10.1002/14651858.CD001191.pub4.

36. Darreh-Shori, T., Almkvist, O., Guan, Z. Z., Garlind, A., Strandberg, B., Svensson, A. L., Soreq, H., Hellström-Lindahl, E., & Nordberg, A. (2002). Sustained cholinesterase inhibition in AD patients receiving rivastigmine for 12 months. Neurology, 59(4), 563–572. https://doi.org/10.1212/wnl.59.4.563.

37. Colović, M. B., Krstić, D. Z., Lazarević-Pašti, T. D., Bondžić, A. M., & Vasić, V. M. (2013). Acetylcholinesterase inhibitors: pharmacology and toxicology. *Current neuropharmacology*, *11*(3), 315–335. https://doi.org/10.2174/1570159X11311030006.

38. Weintraub, D., Somogyi, M., & Meng, X. (2011). Rivastigmine in Alzheimer's disease and Parkinson's disease dementia: an ADAS-cog factor analysis. *American journal of Alzheimer's disease and other dementias*, *26*(6), 443–449. https://doi.org/10.1177/1533317511424892.

39. Rosen, W. G., Mohs, R. C., & Davis, K. L. (1984). A new rating scale for Alzheimer's disease. *The American journal of psychiatry*, *141*(11), 1356–1364. https://doi.org/10.1176/ajp.141.11.1356.

40. "Intro to Neuro-QoL", *Health Measures*, Northwestern University, www.healthmeasures.net/explore-measurement-systems/neuro-qol/intro-to-neuro-qol.

41. "Monoamine Oxidase Inhibitors (MAOIs) Print." *Mayo Clinic*, Mayo Foundation for Medical Education and Research, 12 Sept. 2019, www.mayoclinic.org/diseases-conditions/depression/in-depth/maois/art-20043992.

42. Bortolato, M., Chen, K., & Shih, J. C. (2008). Monoamine oxidase inactivation: from pathophysiology to therapeutics. *Advanced drug delivery reviews*, *60*(13-14), 1527–1533. https://doi.org/10.1016/j.addr.2008.06.002

43. De Zutter GS, Davis RJ (2001) Pro-apoptotic gene expression mediated by the p38 mitogen-activated protein kinase signal transduction pathway. Proc Natl Acad Sci USA 98(11):6168–6173.

44. Yi H, Akao Y, Maruyama W, Chen K, Shih, Naoi M (2006) Type A monoamine oxidase is the target of an endogenous dopaminergic neurotoxin, N-methyl(R)salsolinol, leading to apoptosis in SHSY5Y cells. J Neurochem 96(2):541–549.

45. Naoi, M., Riederer, P., & Maruyama, W. (2016). Modulation of monoamine oxidase (MAO) expression in neuropsychiatric disorders: genetic and environmental factors involved in type A MAO expression. *Journal of neural transmission (Vienna, Austria : 1996)*, *123*(2), 91–106. https://doi.org/10.1007/s00702-014-1362-4.

46. Gomez-Ramos A., Diaz-Hernandez M., Cuadros R., Hernandez F., Avila J. Extracellular tau is toxic to neuronal cells. *FEBS Lett.* 2006;580:4842–4850. doi: 10.1016/j.febslet.2006.07.078.

47. Matthews, D. C., Ritter, A., Thomas, R. G., Andrews, R. D., Lukic, A. S., Revta, C., Kinney, J. W., Tousi, B., Leverenz, J. B., Fillit, H., Zhong, K., Feldman, H. H., & Cummings, J. (2021). Rasagiline effects on glucose metabolism, cognition, and tau in Alzheimer's dementia. *Alzheimer's & dementia (New York, N. Y.)*, *7*(1), e12106. https://doi.org/10.1002/trc2.12106.

48. Maass A, Landau S, Baker SL, et al. Comparison of multiple tau-PET measures as biomarkers in aging and Alzheimer's disease. Neuroimage. 2017;157:448-463.

49. Pontecorvo MJ, DeVous MD, Kennedy I. A multicentre longitudinal study of flortaucipir (18F) in normal ageing, mild cognitive impairment and Alzheimer's disease dementia. Brain. 2019;142(6).

50. Kantak, K., & Hofmann, S.G. (2011). Cognitive enhancers for the treatment of neuropsychiatric disorders: clinical and preclinical investigations. Pharmacology, biochemistry and behavior, 99(2).

51. Avraham Pharmaceuticals Ltd. (2011, February - 2013, March). Safety and Efficacy Study of Ladostigil in Mild to Moderate Probable Alzheimer's Disease. Identifier: NCT01354691. https://clinicaltrials.gov/ct2/show/study/NCT01354691.

52. Schneider, L. S., Geffen, Y., Rabinowitz, J., Thomas, R. G., Schmidt, R., Ropele, S., ... & Ladostigil Study Group. (2019). Low-dose ladostigil for mild cognitive impairment: A phase 2 placebo-controlled clinical trial. Neurology, 93(15), e1474-e1484.

53. AlzBiomaker Database, Version # (https://www.alzforum.org/therapeutics/ladostigil). Alzforum. 23 April 2021.

54. Weinstock, M., Goren, T., & Youdim, M. B. (2000). Development of a novel neuroprotective drug (TV3326) for the treatment of Alzheimer's disease, with

cholinesterase and monoamine oxidase inhibitory activities. Drug development research, 50(3‐4), 216-222.

55. Knez, D., Sova, M., Košak, U., & Gobec, S. (2017). Dual inhibitors of cholinesterases and monoamine oxidases for Alzheimer's disease. Future medicinal chemistry, 9(8), 811-832.

56. O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. Biometrics 1979;35:549–556.

57. Schneider, L. S., Geffen, Y., Rabinowitz, J., Thomas, R. G., Schmidt, R., Ropele, S., Weinstock, M., & Ladostigil Study Group (2019). Low-dose ladostigil for mild cognitive impairment: A phase 2 placebo-controlled clinical trial. *Neurology*, *93*(15), e1474–e1484. https://doi.org/10.1212/WNL.0000000000008239

58. Weintraub, D., Somogyi, M., & Meng, X. (2011). Rivastigmine in Alzheimer's disease and Parkinson's disease dementia: an ADAS-cog factor analysis. *American journal of Alzheimer's disease and other dementias*, *26*(6), 443–449. https://doi.org/10.1177/1533317511424892.

59. Lannfelt, L., Möller, C., Basun, H., Osswald, G., Sehlin, D., Satlin, A., Logovinsky, V., & Gellerfors, P. (2014). Perspectives on future Alzheimer therapies: Amyloid-β protofibrils - a new target for immunotherapy with BAN2401 in Alzheimer's disease. *Alzheimer's Research & Therapy*, *6*(2), 16. https://doi.org/10.1186/alzrt246.

60. György, B., Lööv, C., Zaborowski, M. P., Takeda, S., Kleinstiver, B. P., Commins, C., Kastanenka, K., Mu, D., Volak, A., Giedraitis, V., Lannfelt, L., Maguire, C. A., Joung, J. K., Hyman, B. T., Breakefield, X. O., & Ingelsson, M. (2018). CRISPR/Cas9 Mediated Disruption of the Swedish APP Allele as a Therapeutic Approach for Early-Onset Alzheimer's Disease. *Molecular Therapy - Nucleic Acids*, *11*, 429–440.https://doi.org/10.1016/j.omtn.2018.03.007