

Pharmacological drug strategies in Alzheimer's Disease

Estratégias farmacológicas de drogas na Doença de Alzheimer

Estrategias farmacológicas de fármacos en la enfermedad de Alzheimer

Rodrigo Lantyer Marques Dantas¹, Nathalia Clemente Baracho²,
Antoni Camins³, Miren Ettcheto⁴

1.Engenheiro Biomédico, Universidade Federal de São Paulo, discente do Programa Pós-Graduação Neurologia e Neurociências da Universidade Federal de São Paulo, Escola Paulista de Medicina, São Paulo-SP, Brasil.

2.Fonoaudióloga, Universidade Estadual de Ciências da Saúde de Alagoas, discente do Programa Pós-Graduação Neurologia e Neurociências da Universidade Federal de São Paulo, Escola Paulista de Medicina, São Paulo-SP, Brasil.

3.Professor Titular, Departament de Farmacologia, Toxicologia i Química Terapèutica, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona; Biomedical Research Networking Centre in Neurodegenerative Diseases (CIBERNED), Madrid; Institut de Neurociències, Universitat de Barcelona, Spain.

4.PhD en Departament de Farmacologia, Toxicologia i Química Terapèutica, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona; Biomedical Research Networking Centre in Neurodegenerative Diseases (CIBERNED), Madrid; Institut de Neurociències, Universitat de Barcelona, Spain.

Resumo

Introdução. A doença de Alzheimer (DA) é uma doença neurodegenerativa de mau prognóstico e sem cura que afeta milhões de pessoas em todo o mundo. Os medicamentos atualmente em estudos clínicos tentam investigar possíveis efeitos terapêuticos que progridem ou interrompem a doença. **Objetivo.** Esta revisão é necessária para criar um panorama atual em 2021, descrevendo as principais vias em estudo para inibir importantes vias de progressão da doença, como vias do sistema colinérgico e inibidores de ROCK, bem como novas perspectivas de tratamento com possível combinação de drogas, para diminuir neuroinflamação e alterar o curso da doença. **Método.** Avaliado ClinicalTrials.gov em 19 de janeiro de 2021, identificou todos os ensaios de agentes farmacológicos em desenvolvimento para o tratamento da DA na fase 3 do estudo clínico, obtendo assim um panorama global para conter essa doença devastadora, criando melhores perspectivas sobre o tratamento da DA. **Resultados.** Diferentes medicamentos (n=25) foram divididos por tipos de alvos de vias neurofisiológicas (inibidor de amiloide; inibidor de tau; receptores de neurotransmissores; anti-inflamatórios não esteroides (AINEs), mitocôndrias e funções metabólicas; plasticidade sináptica). **Conclusões.** Os avanços nos ensaios clínicos trazem esperança e novos caminhos, pois os alvos para o tratamento da DA são encorajados e prometem novas linhas de tratamento. Novos estudos com mais combinações terapêuticas que alterem o curso da doença devem ser incentivados.

Unitermos. Doença de Alzheimer; desenvolvimento de drogas; ensaios clínicos; inibidores ROCK

Abstract

Introduction. Alzheimer's disease (AD) is a neurodegenerative disorder with a poor prognosis and no cure that affects millions of people worldwide. Medicines today in clinical studies try to investigate possible therapeutic effects that progress or stop the disease. **Objective.** This review is necessary to create a current panorama in 2021, describing the main pathways under study to inhibit important pathways of disease progression, such as pathways cholinergic system and ROCK inhibitors, as well as new perspectives of treatment with possible combination of drugs, to decrease neuroinflammation and change the course of the disease.

Method. Reviewed ClinicalTrials.gov as of January 19, 2021, identified all trials of pharmacologic agents currently being developed for treatment of AD in phase 3 of clinical study, thus obtaining a global panorama to curb this devastating disease, creating better perspectives on the treatment of AD. **Results.** Different drugs (n = 25) were divided by types of targets of neurophysiological pathways (amyloid inhibitor; tau inhibitor; neurotransmitter receptors; non-steroidal anti-inflammatory drugs (NSAIDs), mitochondria and metabolic functions; synaptic plasticity). **Conclusions.** Advances in clinical trials bring hope and new avenues as targets for AD treatment are encouraged and show promise for new lines of treatment. New studies with more therapeutic combinations that change the course of the disease should be encouraged.

Keywords. Alzheimer's Disease; drug development; clinical trials; ROCK inhibitors

Resumen

Introducción. La enfermedad de Alzheimer (EA) es un trastorno neurodegenerativo de mal pronóstico y sin cura que afecta a millones de personas en todo el mundo. Los medicamentos de hoy en día en estudios clínicos intentan investigar los posibles efectos terapéuticos que progresan o detienen la enfermedad. **Objetivo.** Esta revisión es necesaria para crear un panorama actual en 2021, describiendo las principales vías en estudio para inhibir vías importantes de progresión de la enfermedad, como vías del sistema colinérgico e inhibidores de ROCK, así como nuevas perspectivas de tratamiento con posible combinación de fármacos, para disminuir neuroinflamación y cambiar el curso de la enfermedad. **Método.** ClinicalTrials.gov revisado al 19 de enero de 2021, identificó todos los ensayos de agentes farmacológicos que se están desarrollando actualmente para el tratamiento de la EA en la fase 3 del estudio clínico, obteniendo así un panorama global para frenar esta devastadora enfermedad, creando mejores perspectivas sobre el tratamiento de la EA. **Resultados.** Los diferentes fármacos (n = 25) se dividieron por tipos de dianas de vías neurofisiológicas (inhibidor de amiloide; inhibidor de tau; receptores de neurotransmisores; fármacos antiinflamatorios no esteroideos (AINE), mitocondrias y funciones metabólicas; plasticidad sináptica). **Conclusiones.** Los avances en los ensayos clínicos brindan esperanza y nuevas vías, ya que se fomentan los objetivos para el tratamiento de la EA y se muestran prometedores para nuevas líneas de tratamiento. Se deben impulsar nuevos estudios con más combinaciones terapéuticas que cambien el curso de la enfermedad.

Palabras clave. Enfermedad de Alzheimer; desarrollo de fármacos; ensayos clínicos; inhibidores de ROCK

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Endereço de correspondência: Rodrigo Lantyer Marques Dantas. E-mail: rodrigo.lantyer@unifesp.br

INTRODUCTION

In the XXist century, life expectancy has increased considerably, to a great extent thanks to advances in biomedicine which have decrease the mortality caused by multiple pathologies that some years ago were incurable. However, this increase has also been accompanied by the appearance of age-related diseases, such as Alzheimer's

disease (AD), which prevalence is rising due to the aging of the world population¹, entailing a great social and personal burden at the health level². AD is the most common form of dementia, progressive and, nowadays, incurable that begins approximately 15-20 years before symptoms onset. Histologically, AD is characterized by the accumulation of β -amyloid ($A\beta$) plaques and neurofibrillary tangles (NFT) in the brain. Specifically, $A\beta$ plaques are extraneuronal deposits composed by aggregates of various $A\beta$ peptides derived from amyloid precursor protein (APP). By contrast, NFT are intracellular filamentous inclusions of hyperphosphorylated Tau.

AD is classified into 2 groups: familial AD (FAD) and late onset or idiopathic AD (LOAD). FAD correspond to <5% of cases³ and normally appears between 30 and 50 years of age⁴. It has been associated to mutations mainly in three genes: APP, presenilin 1 (PSEN1) and presenilin 2 (PSEN2)⁵. By contrast, LOAD is the most common form which affects >95% of patients with this pathology. Its aetiology remains unclear; however, environmental and genetic factors seem to be involved in its development⁶, including APOE⁷.

Throughout the years, different hypotheses have been proposed to explain the origin of the disease, of which the amyloidogenic hypothesis has been one of the most accepted, proposed approximately thirty years ago. This hypothesis suggests that the accumulation of harmful $A\beta$ peptides in the nervous system would be the cause of AD. These plaques are produced through APP processing in which

the enzymes β -secretase and γ -secretase cleavage APP, forming A β deposits in the human brain⁸. Under physiological conditions, A β is quickly removed from the human brain by the withdrawal mechanism, ensuring a proper functioning of the system. However, in pathological situation, it is believed that there is a defect in the removal mechanism or excessive production which, culminates in an increase level of A β peptides, specifically those containing 42 amino acids^{7,9}. These peptides have a high tendency to bond generating oligomers, protofibrils, fibrils and A β plaques⁷⁻⁹. Likewise, it is now well known that the disease correlates better with increased levels of soluble A β than with plaque formation, causing alterations in dendritic spines and synapses that would be responsible for the cognitive alterations that are observed at the beginning of the disease in the stage of mild cognitive impairment (MCI). In fact, its excess together with Tau accumulation impairs synaptic communications between neurons leading to neuronal death¹⁰.

Despite the clinical symptoms observed and the biomarkers of cerebrospinal fluid (CSF) and positron emission tomography (PET) indicated strong evidence on AD in living patients, the definitive diagnosis of AD can only be achieved by evaluating post-mortem brain tissue^{7,11}. Abnormalities found in the CSF are low levels of A β peptides and increased levels of tau protein¹¹, not enough to conclude its diagnosis in its totality. So far, treatment for AD is restricted to the symptomatic level and there is no strategy

to combat the progressive neurodegeneration caused by AD and hence its fatal outcome¹².

Apart from Tau and A β , several studies have shown that there are several risk factors for triggering LOAD^{7,13,14}. A large study listed 20 risk factors for AD¹⁵. However, since its publication these data have been reviewed and, while some risk factors have been highlighted, others have fallen into disuse and others added¹⁶⁻¹⁸. Currently, it is known that the importance of a risk factor for AD is often assessed by the degree to which this factor influences APP metabolism, causing A β accumulation, neuronal death and, consequently, neural circuit damage. Although there is no consensus in the literature on all factors that are susceptible to AD development, the main risk factors currently considered and associated with AD have been: genetic, demographic issues (age, education, gender, race and social class), lifestyle (alcohol, lack of physical exercise and cognitive activity, malnutrition, poor diet, and smoking), medical conditions (cancer, cardiovascular disturbances, congestive heart failure, immune system dysfunction, micro heart attack, obesity, lack of control homeostatic cholesterol, lack of control of type 2 diabetes, stroke and head trauma), psychiatric disorders (depression, stress), environmental factors (air pollution, calcium deficiency, geographical location, metals: especially zinc, aluminium and copper, military service, organic solvents, type of work, vitamin deficiency) and infections (dental, fungicidal, viral and bacterial: *Chlamydomphila pneumonia*, *Treponema*)¹³.

Among these risk factors, the most strongly associated with the onset of LOAD is advancing age, cardiovascular changes and especially, the allelic variation of ApoE. However, in recent years diet-related factors such as obesity and associated diseases including type 2 diabetes mellitus (T2DM) have been gaining ground, which accelerate aging rate by triggering the pathophysiological cascade of LOAD¹³.

Another risk factor for LOAD widely discussed in the literature which interestingly also contribute to T2DM development, is the neuroinflammation caused by microglia. Microglia are macrophages that rest in the brain and spinal cord, and it has been classified as M2 or resting and M1 or activated. In physiological homeostatic equilibrium conditions, the resting M2 microglial cells are responsible for the removal of A β from the brain. In the case of LOAD, it has been suggested that A β could be the responsible of a process of over-activation of the microglia favouring the M1 state, causing a release of cytokines such as TNF α , interleukin 1 and other cytokines and chemokines that induces neuronal injury and death in AD¹⁹.

Based on the previously commented and considering the need to stop the progression of the disease, several protective factors have been described. A healthy lifestyle throughout life, with physical exercise, cognitive activities and a balanced diet minimize the predisposition to LOAD. This protection occurs by reducing the impact on pathophysiological processes that these behaviours provide. However, none of them has been able to delay or modify the

course of this devastating disease. For this reason, it is necessary to develop drugs that stop its progression, that is, the patient remains in the state of MCI and does not develop LOAD.

Therefore, the goal of this review is to analyse drug treatments to seek new solutions for the hopefully near future, minimizing the personal and social damage caused by AD.

Current drugs for the treatment of Alzheimer's Disease

Cholinergic system drugs have been shown to be an alternative treatment for AD, since studies have indicated that cholinergic neurons located in the basal forebrain are highly affected, contributing to memory and attention deficits^{20,21}. Acetylcholine (ACh) is the neurotransmitter used by cholinergic neurons which has a fundamental role in cognitive and motor processes, from memory acquisition to the recovery process^{22,23}. In AD brains, it has been observed that a clear reduction of ACh in the nervous system due to significant loss of neurons²⁴. Therefore, the most drugs approved by the food and drug administration (FDA) have focused on increasing ACh levels in the synaptic cleft by inhibiting acetylcholinesterase (AChE) enzymes, which also has been related to A β and A β fibrils formation and growth²⁵. The use of AChE inhibitors provides a significant improvement in the functional and cognitive aspects at an initial stage of LOAD impacting patients' quality of life.

However, there is no scientific evidence that this medication delays the disease's progression²⁶.

The main drugs currently used as AChE inhibitors are donepezil, galantamine and rivastigmine²⁷. Specifically, donepezil treatment in advanced stages of AD patients has indicated to be well tolerated at a daily dose of 5-10 mg and at a dose of 23 mg administrated on alternate days, being very efficient after 3 to 6 months of treatment ($p < 0.001$), with significant improvement not only in cognitive functions but also in language and visuospatial ability²⁸. In turn, galantamine has been shown to be a more effective medication than donepezil and rivastigmine, significantly improving the cognitive and functional processes in patients after 3 months of treatment, in view of significant improvements ($p < 0.001$) after 1 year of medication. The initial doses are 16 mg / day until reaching doses of 24 mg / day, which may be lower, depending on patient's tolerance to adverse effects such as nausea and vomiting^{29,30}. In the case of rivastigmine, daily treatment at a dose of between 6 and 12 mg/day has shown to improve the cognitive functions in patients with AD^{31,32}.

On the other hand, the fourth drug approved by the FDA for the treatment of AD is Memantine, a non-competitive antagonist of the NMDA receptor (channel blocker) that protects neurons from glutamate excitotoxicity by preventing their apoptosis and has low toxicity. This non-competitive antagonism will never exceed the concentration of the agonist, which in this case is glutamate or glycine,

having a role in controlling the excitotoxicity of glutamate, which deal to the prevention of nerve cells death. Therefore, it provides a therapeutic power in delaying the progress of AD³³. The dose administered is 20 mg/day and it has been shown a significant improvement in patients with cognitive impairment, but the damage caused by the disease's progress is not repaired³⁴. In summary, the fourth drugs previously mentioned have demonstrated to promote an improvement in cognitive function of AD patients, however, none of them has been able to stop the progression of LOAD. Therefore, new strategies have been considered.

Recently, some studies have already linked intestinal microbiota disorders to AD. Personalized therapy and interventions in the intestinal microbiota are already evaluated as a possible treatment for AD, since the gut and brain axis play a fundamental role in the communication of brain function signalling³⁵. The remodelling of the intestinal microbiota using GV-971, which are linear acids of oligosaccharides that vary from dimers to decamers, have been demonstrated to be effective in reducing neuroinflammation through reconditioning the intestinal microbiota. Specifically, the process of neuroinflammation and cognitive loss occurs from the intestine when there is peripheral inflammation caused by an increase in the production of metabolites, which induce the migration of immune system cells to the brain and its penetration, in consequence, M1 microglia is activated. GV-971 drug causes a redirection of the microbiota and normalizes the disordered

metabolites. Hence, neuroinflammation is reduced improving cognitive performance^{36,37}.

Drug development for Alzheimer disease: phase 3 research studies

Currently, there are numerous drugs for the treatment of AD that are in phase III of clinical trials. This review summarizes the data posted from the database Alzforum.org and ClinicalTrials.gov as of January 19, 2021. Being returned on 25 agents in 39 tests that may be in the recruitment phase, or recruitment not started are summarized in Table 1.

Drugs targeting A β or inflammation processes represent 41.02% (n=16) of studies that have already started or are in the process of starting the recruitment of volunteers. Among them, aducanumab is a human monoclonal antibody under investigation. Its clinical development program included two phase 3 trials, EMERGE and ENGAGE, and the PRIME phase 1b study. In March 2019, Biogen discontinued the ENGAGE and EMERGE trials, as they were unlikely to meet the primary endpoints upon completion. However, later in October, the company announced a new analysis of a larger data set that showed that aducanumab reduced clinical decline in patients with earlier AD as measured by pre-specified primary and secondary endpoints. Preliminary results demonstrated that the drug decreases A β plaques with high doses of antibodies, being a promising drug and with the application made for commercialization with the FDA³⁸. Another monoclonal antibody which is in phase 3 is

gantenerumab. After a trial in futility-interrupted prodromal disease, obtained results suggested that higher doses may be effective. By contrast, Solanezumab in combination with gantenerumab has not been shown to be effective compared to the placebo group, but a new test is underway to verify the effectiveness in delaying the progression of AD-related brain damage³⁹⁻⁴¹.

Other drug with promising results is Albutein® (therapeutic albumin, Grifols) which was developed as a therapeutic strategy with the aim to reduce the load of A β in the brain by inducing changes in the dynamics of A β transport across the blood-brain barrier. The idea was based on the existence of soluble oligomers of A β , more toxic than fibers, in plasma, bound to albumin in a high percentage, indicating that this protein may play a relevant role in avoid the aggregation of A β and the existence of a dynamic equilibrium between the peripheral and central A β levels. For this reason, the therapeutic strategy is focused on plasma exchange in which the extracted plasma is replaced with an equivalent volume of plasma. In therapeutic apheresis, the extraction of plasma aims to eliminate the pathogenic elements present in it. Thus, the sequestration of A β in plasma could increase the transport of free A β from the CSF to the plasma, to restore the intrinsic balance between the brain and the blood of the levels of A β and reduce the load of A β in the brain. According to the amyloid hypothesis of AD, the alteration of this balance could be central in the pathogenesis and progression of AD.

The development of Atuzaginstat (COR388) is based on the bacterial hypothesis of AD, associated to the discovery of bacterium *Porphyromonas gingivalis*. This bacterium commonly related with periodontitis, contains toxic virulence factors (proteases) called gingipains that have been identified in the brains of AD patients. In addition, elevated brain levels of gingipain have been correlated with tau⁴². Likewise, preclinical studies indicated that there was a blockade of A β 1-42 production, reduced neuroinflammation and preserved neurons in the hippocampus of mice⁴³. The clinical study of this drug (NCT03823404) is a randomized, double-blind, placebo-controlled study that will assess the efficacy, safety, and tolerability of 2 dose levels of COR388 oral capsules in subjects with probable AD dementia according to the National Institute on Aging-Alzheimer's Association criteria during 48-week treatment.

Other research line in which therapeutical strategies have focused are drugs that target tau protein inhibitors which represent 2.5% (n=1) of clinical trials at this stage. TRx0237 has long been used in research and for the treatment of malaria and other conditions. In this study they are investigating whether this drug progresses to AD. The trial is recruiting participants with behavioral variant frontotemporal dementia⁴⁴.

Moreover, neurotransmitter receptors also have been designed as strategy for AD which correspond to 30.7% (n=12) of drug in this clinical stage. The main expected effect in this phase is that there is a significant reduction in

A β plaques in relation to the control group in each study, causing a reduction in neuroinflammation in patients with mild, moderate LOAD⁴⁴.

Early 2021, the pharmacist Novo Nordisk published a note that the medication Semaglutide, which is already being studied for its efficacy and safety for T2DM, will start phase 3 with early AD patients. This drug is a hormone, with a metabolic function (10.25%; n=4) that stimulates insulin signaling. The aim of this strategy is to increase insulin signaling, due to it is thought to will improve the transport of glucose in the brain, reducing neurodegeneration⁴⁵. In fact, the use of metformin (insulin synthesizer) in previous phases of the study has demonstrated a significant cognitive and memory improvements, even though it was a study with a small sample (n=20), indicating a tendency to use this drug as a treatment for AD⁴⁶.

Ginkgo biloba is a tree of prehistoric origin highly resistant to viruses, fungi, and bacteria. The main component of ginkgo extract are flavonoids, which have been scientifically demonstrated that are able to protect neurons from oxidative stress, among other discoveries^{47,48}.

The last group of drugs under study that targets synaptic plasticity / neuroprotection represents 15.38% (n = 6) and most have the therapeutic goal of improving patients' synaptic function⁴⁴. Dysfunction of pathways of SV2A, which is directly involved in the distribution of exocytosis vesicles that are very important in the synaptic clefts where neurotransmissions occur, are related to neurodegenerative

diseases such as epilepsy and AD⁴⁹. In phase II, SV2A modulating drugs, such as AGB101 (low-dose formulation of levetiracetam) have been shown to be effective in increasing cognition and significantly improving memory task performance in patients with AD⁵⁰.

The contributions of metabolic diseases, vascular diseases are correlated with the loss of the dendritic spine, which can trigger the development and advancement of LOAD, so other drugs such as losartan, amlodipine and atorvastatin are studied⁵¹.

ROCK inhibitors as a strategy to improve cognition in AD

The process of storing information in the brain occurs due to synaptic connections which happen when neurons transmit information to other neurons through their axons and dendrites⁵². Adequate synapse function is an essential prerequisite for all neuronal processing, especially for higher cognitive functions like learning and memory in which cytoskeleton plays a crucial role. The process of synapse formation and their maintenance —i.e. 'synaptogenesis'— is considered the final step of neuronal polarization, where axonal growth cones navigate through a specific pathway until contacting the appropriate targets, like dendrites, forming the boutons. The protrusions along the dendrites, highly concentrated in actin filaments, are called dendritic spines⁵³.

Table 1. AD drug development in phase 3 Drugs in a different target type
(ClinicalTrials.gov accessed January 19, 2021).

Target Type	Name	Sponsor	Mechanism of Action	Therapeutic Effects Purpose	ID
I - Amyloid-Related and/or Inflammation					
	Aducanumab	Biogen	Monoclonal antibody directed at plaques and oligomers	Remove A β	NCT04241068
	Albutein	Instituto Grifols, S.A. Grifols Biologicals, LLC	Plasma blood	Reduced brain A β	NCT01561053
	COR388	Cortexyme	Bacterial protease inhibitor targeting gingipain produced by <i>P. gingivalis</i>	Reduce neuroinflammation and hippocampal degeneration	NCT03823404
	GV-971	Shanghai Green Valley Pharmaceuticals	Algae-derived Acidic oligosaccharides deaggregate A β	Reduced brain A β burden, tau hyperphosphorylation, and cognitive deficits	NCT04520412
	Gantenerumab	Chugai Pharmaceutical Co., Ltd., Hoffmann-La Roche	Monoclonal antibody; "brain-shuttle" gantenerumab	Remove A β	NCT04339413
					NCT04374253
					NCT03444870
					NCT03443973
					NCT01760005
	Lecanemab	Biogen, Eisai Co., Ltd.	anti-amyloid beta (A β) protofibril antibody	Reduce protofibrillar A β and A β plaques	NCT04468659 NCT03887455
	Levetiracetam	Beth Israel Deaconess Medical Center	SV2A modulator	Improve synaptic function; reduce A β - induced neuronal hyperactivity	NCT02002819 NCT03875638
	NE3107	Neurmedix Inc	Mitogen activated protein kinase 3 inhibitors; Mitogen-activated protein kinase 1 inhibitors; NF-kappa B inhibitors	Reduction neurodegeneration and neuroinflammation	NCT04669028
	Solanezumab	Eli Lilly & Co.	Monoclonal antibody directed at monomers	Remove A β and prevent aggregation	NCT01760005 NCT02008357

Table 1 (cont.). AD drug development in phase 3 Drugs in a different target type (ClinicalTrials.gov accessed January 19, 2021).

II -Tau					
	LMTM (TRx0237)	TauRx Therapeutics Ltd	Tau protein aggregation inhibitor	Reduce tau mediated neuronal damage	NCT03446001
III -Neurotransmitter Receptors					
					NCT04464564
	AVP-786	Avanir Pharmaceuticals, Concert Pharmaceuticals, Inc., Otsuka Pharmaceutical Co., Ltd.	Sigma 1 receptor agonist; NMDA receptor antagonist	Improve neuropsychiatric symptoms (agitation)	NCT04464564
					NCT03393520
					NCT02446132
					NCT03594123
	Brexiprazole	H. Lundbeck, Otsuka Pharmaceutical Co., Ltd.	D2 receptor partialagonist, serotonin-dopamine modulator	Improve neuropsychiatric symptoms (agitation)	NCT03548584
					NCT03724942
					NCT03620981
	Escitalopram	Johns Hopkins University, NIA	SSRI	Improve neuropsychiatric symptoms (agitation)	NCT03108846
	Guanfacine	Imperial College London, UK National Institute of Health Research	Alpha-2 adrenergic agonist	Modulation of noradrenergic deficit (cognitive enhancer)	NCT03116126
	Mirtazapine	University of Sussex	Alpha-1 antagonist	Improve neuropsychiatric symptoms (agitation)	NCT03031184
	Octohydroaminoacridine Succinate	Shanghai Mental Health Center, Changchun-Huayang High-tech, Jiangsu Sheneryang High-tech	AchE inhibitor	Improve acetylcholine signaling (cognitive enhancer)	NCT03283059
IV- Mitochondria & Metabolic Function					
	Ginkgo biloba dispersible tablets	Nanjing Medical University	Plant extract with antioxidant properties	Improve brain blood flow and mitochondrial function (cognitive enhancer)	NCT03090516
	Semaglutide	Novo Nordisk A/S	GLP-1 analogue Semaglutide	Improved memory function and reduced phospho-tau accumulation	NCT04707469*

Table 1 (cont.). AD drug development in phase 3 Drugs in a different target type (ClinicalTrials.gov accessed January 19, 2021).

Tricaprilin	Cerecin	Ketone body stimulant; caprylic triglyceride	Induce ketosis to improve mitochondrial function and neuronal metabolism	NCT04187547
Metformin	ColumbiaUniversity,NIA, EMDSerono	Insulin sensitizer	Improve CNS glucose metabolism	NCT04098666
V - Synaptic plasticity/ neuroprotection				
AGB101	AgeneBio, NIA	SV2A modulator	Improve synaptic function; reduce amyloid-induced neuronal hyperactivity	NCT03486938
ANAVEX2-73	Anavex lifesciences	Sigma-1 receptor agonist, M2 autoreceptor antagonist	Enhances cell signaling to ameliorate oxidative stress, protein misfolding, mitochondrial dysfunction and inflammation	NCT03790709 NCT04314934
Icosapent ethyl (IPE)	VA Office of Research and Development,University of Wisconsin,Madison	Purified form of the omega-3 fatty acid EPA	Improve synaptic function; reduce inflammation	NCT02719327
Losartan, amlodipine e atorvastatin + exercise	University of Texas Southwestern	Angiotensin II receptor blocker (losartan), calcium channel blocker (amlodipine), cholesterol agent (atorvastatin)	Vascular risk reduction; preservation of cognitive function	NCT02913664
Troriluzole	Biohaven pharma, ADCS	Glutamate modulator; prodrug of riluzole	Reduce synaptic levels of glutamate; improve synaptic functioning	NCT03605667

Specifically, the shape and number of them are continuously remodelled in adaptation to sensory stimuli or in learning and memory process, as occurs with synaptic activity.

In line with this process, it has been demonstrated that small changes in dendritic spines, such as density, number,

size, and shape cause cognitive deficits and neurodegenerative disorders, including AD⁵⁴. Likewise, numerous reports have shown that synaptic markers and/or dendritic spine loss precede the formation of A β plaques and NFTs, suggesting that these molecules and structures are strongly correlated with cognitive impairment in AD⁵⁴⁻⁵⁶. More specifically, the selective loss of thin spines is strongly linked with impaired ability to learn in aged rhesus monkeys⁵⁷. These alterations could be related with the MCI that is detectable in very early stages in AD patients⁵⁴, supporting that synaptic loss is central to the progression of the pathology⁵⁸ and providing cellular evidence that remodelling the structure of dendritic spines may be a mechanism of cognitive resilience. All these data sustain that synaptic structure and synaptic activity are clearly correlated with the cognition capacity. Therefore, the cellular and molecular events that control synapses can be an early target to treat cognitive impairment in AD.

The kinases proteins (ROCKs/Rho-kinase/Rho-kinase associated) belong to the serine/threonine family of small (~21 kDa) signalling G proteins which are part of the Ras superfamily. Several biological functions are mediated through the action of the ROCKs when they connect to GTPase⁵⁹. Two ROCK isoforms have been described in mammals, ROCK1 and ROCK2 that are powerful regulators of the actin cytoskeleton. Therefore, the synaptic alterations observed at early stages of AD should be correlated with modifications in the dynamic actin cytoskeleton, controlled

by ROCKs⁶⁰. Likewise, it has been described that A β induces aberrant stabilization of F-actin within dendritic spines, which impairs synaptic strength and plasticity, contributing to MCI.

Actin-depolymerization factor (ADF) is a group of small (15–22 kD) actin-binding proteins that include destrin, depactin, actophorin, collectively called ADF/cofilin family. Together with cofilin-1 (Cof1) regulate actin dynamics in dendritic spines, promoting actin depolymerization that contributes to the control of actin filament dynamics and reorganization⁶¹. Therefore, the close association between Cof-1 dysfunction and cognitive loss in AD and other neurological disorders is accepted⁶². This correlation would be explained by the fact that cofilin is crucial for the synaptic plasticity regulation⁶³. Thus, in long-term potentiation (LTP), a synaptic enhancement induced by high-frequency electrical stimulation, the actin cytoskeleton needs to be polymerized in an inactivated cofilin-dependent manner, while in the phase of long-term-depression (LTD), the actin cytoskeleton needs to be depolymerized by activated cofilin⁶¹. Indeed, activated cofilin inhibits tau-induced microtubule assembly promoting tauopathy⁶⁴. Finally, cofilin is also essential for the trafficking of AMPA receptors at the post-synaptic region during synaptic plasticity, which is associated with the memory acquisition^{65,66}.

Regarding to the regulation of cofilin activity, it has been shown that phosphorylation of the serine residue at position 3 (Ser-3) inactivates this protein⁶⁷. The serine phosphorylation is mainly mediated by LIM kinases (LIMK)

and testicular protein kinases (TESK), which are activated by Rho-GTPases. Rho GTPases influence both the maturation and the collapse of dendritic spines through cofilin activation⁶⁸. Specifically, activation of the RhoA/ROCK pathway results in phosphorylation of Cof1 and is sufficient to mediate A β -induced actin stabilization synaptic impairment and synaptic loss⁶³.

Importantly, brain tissue from AD patients and APP-expressing mouse models exhibits elevated ROCK levels and corresponding elevated levels of inactive p-Cof1⁶⁹. Furthermore, in other neurodegenerative diseases characterized by an early synaptic loss, such as Creutzfeldt-Jacob's disease, upregulation of p-Cof-1 has been described⁶⁹. These data highlighting that Cof-1 exerts a pivotal role in the synaptotoxic process of neurodegenerative diseases⁶⁷.

The reactivation of cofilin occurs through dephosphorylation of p-Ser-3 by Slingshot family protein phosphatases (SSHs). In addition, a haloacid dehalogenase termed chronophin (CIN), and the more general proteins serine/threonine phosphatases, protein phosphatase 1 (PP1) and protein phosphatase 2A (PP2A) are also reported to be involved in cofilin dephosphorylation.

Globally, Rho GTPases function as key intracellular switches that regulate axonal and dendritic growth together with synapsis structure and activity through actin binding proteins such as Cof-1.

Hydroxyfasudil, which is a pan-ROCK inhibitor, is being used in studies of older rats and indicates improvement in the learning and working memory of these animals. The commercial drug (Fasudil), in several different dosage protocols, is considered safe and well tolerated in humans. Moreover, the results of the study associated with the positive clinical use of the drug corroborate that this ROCK inhibitor improves cognition and memory dysfunction in humans⁷⁰.

Interesting research carried out in the hippocampus of an in vivo mouse model suggested that ROCK2 is the most critical isoform for dendritic spine formation and synaptic function when compared to ROCK1. Interestingly, it was found that the ROCK2 isoform is involved in both pre-synaptic and postsynaptic transmission, while ROCK1 is involved only in postsynaptic transmission⁷¹.

In addition, it is well known that NSAIDs have been studied as a potential treatment of AD to inhibit the neuroinflammatory process as selective and non-selective cyclooxygenase (COX) inhibitors. NSAIDs such as ibuprofen, indomethacin, and sulindac have also been proposed to reduce the formation of A β 42⁷², by inactivating RhoA⁷³. Therefore, the inhibition of ROCK by some NSAIDs is not related to the inhibitory action of COX and constitutes a therapeutic target in the prevention of AD.

A β is also produced from ROCK2 phosphorylation in APP at some sites (T654 and S498), which highlights the importance of ROCK2 inhibition as a protective factor for AD

development, as ROCK2 acts as one of the mediators in axonal degeneration, leading to apoptosis⁷⁴.

SR3677 (ROCK2 inhibitor) reduced the action of the APP cleavage enzyme from β (BACE1) and the production of A β in mice. Alteration of the endocytic distribution of BACE1 and promotion of traffic from APP to lysosomes was also identified in this study. In addition, SR3677 blocked ROCK2 phosphorylation in threonine 654 (T654). These observations suggest that ROCK2 inhibition reduces levels A β through independent mechanisms in the rat brain⁷⁵.

As a therapeutic strategy for age-related memory loss and AD, pharmacological inhibition of ROCK1 and ROCK2 can be a promising treatment as it acts on increasing the density of the dendritic spines favouring synaptic transmission which improves the transmission of brain information and neural plasticity. However, more research should be done in order to clearly elucidate the specific role of each isoform and its specific targets.

Challenges and future of research in drug development for AD

The biggest challenge for the scientific class is to find an effective drug that reduces, slows down or regresses AD. Advances with monotherapies are evident in clinical tests. However, none of them has achieved its goal. In consequence, it has arisen the need to consider new strategies. One of them is the design of combinatorial therapies since it is well known that it has been effective for other diseases which share similar complexity, the fact that several pathogenic

pathways or multiple targets are involved in its development. In fact, it has been demonstrated that the combination of memantine + AchE inhibitor produced positive effects in patients with AD⁷⁷. By contrast, some studies with combined drugs such as: memantine + donepezil applied in patients with moderate to severe AD, did not show significant improvement ($p>0.01$) in relation to patients on monotherapy with one of the two drugs⁷⁶. This negative result, indicate possibly, the need to add more drugs in the combinatory treatment.

Actually, there are only six works in progress with combinatorial drugs consulted at ClinicalTrials.gov (accessed on January 22, 2021). A drug combination approach with amyloid pathway inhibitors and NSAIDs, such as the use of ALZT-OP1 (cromolyn and ibuprofen, NCT04570644). Drugs like Ginkgo combined with AchE inhibitors have also been the subject of studies (Ginkgo + Donepezil, NCT03090516). Another drug that is in the 1/2 phases is Dasatinib + Quercetin (Tyrosine kinase inhibitor and flavonoid, NCT04063124), which reduces senescent cells and tau aggregation⁷⁸.

We can conclude, AD is a complex disease where numerous pathways are involved in the neurodegenerative process. This complexity makes it necessary to address the disease by acting on different therapeutic targets such as the decrease in $A\beta$ levels, decrease the neuroinflammatory process, act by maintaining the stability of the dendritic spines, in addition to, acting on the mitochondria to maintain

adequate levels of ATP and decrease levels of oxidative stress. Finally, it must be considered that these drugs should be administered in a state of the MCI in the disease to be effective and to be able to modify the course of the disease and delay the process of cognitive loss.

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