UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS NATURAIS E EXATAS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS: BIOQUÍMICA TOXICOLÓGICA

Franciele Martini

AÇÃO NEUROPROTETORA DO COMPOSTO EBSELEN E DO EXERCÍCIO FÍSICO EM MODELOS DE DOENÇA DE ALZHEIMER ESPORÁDICA EM CAMUNDONGOS

Santa Maria, RS
2019

Franciele Martini

AÇÃO NEUROPROTETORA DO COMPOSTO EBSELEN E DO EXERCÍCIO FÍSICO EM MODELOS DE DOENÇA DE ALZHEIMER ESPORÁDICA EM CAMUNDONGOS

Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, da Universidade Federal de Santa Maria (UFSM, RS), como requisito para obtenção do título de **Doutor em Bioquímica Toxicológica**.

Orientadora: Prof.ª Dr.ª Cristina Wayne Nogueira

Santa Maria, RS
2019

MARTINI, FRANCIELE
AÇÃO NEUROPROTETORA DO COMPOSTO EBSELEN E DO EXERCÍCIO FÍSICO EM MODELOS DE DOENÇA DE ALZHEIMER ESPORÁDICA EM CAMUNDONGOS / FRANCIELE MARTINI.- 2019.
132 p.; 30 cm

Orientador: CRISTINA WAYNE
Coorientador: NOGUEIRA
Tese (doutorado) - Universidade Federal de Santa Maria, Centro de Ciências Naturais e Exatas, Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, RS, 2019

1. EBSELEN 2. EXERCÍCIO DE FORÇA 3. ALZHEIMER 4. NEUROPROTEÇÃO 5. ESTRESSE OXIDATIVO I. WAYNE, CRISTINA II. , NOGUEIRA III. Título.

Sistema de geração automática de ficha catalográfica da UFSM. Dados fornecidos pelo autor(a). Sob supervisão da Direção da Divisão de Processos Técnicos da Biblioteca Central. Bibliotecária responsável Paula Schoenfeldt Patta CRB 10/1728.

Franciele Martini

AÇÃO NEUROPROTETORA DO COMPOSTO EBSELEN E DO EXERCÍCIO FÍSICO EM MODELOS DE DOENÇA DE ALZHEIMER ESPORÁDICA EM CAMUNDONGOS

Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, da Universidade Federal de Santa Maria (UFSM, RS), como requisito para obtenção do título de **Doutor em Bioquímica Toxicológica**.

Aprovado em 22 de Fevereiro de 2019:

Cristina Wayne Nogueira, Dr.ª (UFSM)
(Presidente/Orientadora)

Roselia Maria Spanevello, Dr.ª (UFPEL)

Michele Sagrillo, Dr.ª (UFN)

Camila Simonetti, Dr.ª (UNIPAMPA)

Francieli Stefanello, Dr.ª (UFPEL)

Santa Maria, RS
2019

À minha família, em especial aos meus pais, que me deram o principal para a minha vida, o exemplo de conduta e perseverança!

À Dr. Cristina Wayne Nogueira, a quem considero minha mãe na bioquímica e me espelho na jornada de ser uma eterna aprendiz!

AGRADECIMENTO ESPECIAL

Cris,

É difícil acreditar que "acabou". Talvez porque, como você mesmo diz, "nunca acaba". Foram oito anos que se passaram desde que entrei na sua sala pela primeira vez, das vezes que vi sua porta aberta e estiquei o pescoço só para dizer um "Bom dia!", das vezes que você me perguntou "Está tudo bem?" e, depois da resposta afirmativa, uma nova pergunta "E os experimentos (o manuscrito, a tese), tudo bem?". A orientadora, a amiga, àquela que muitas vezes dividíamos as nossas angústias... Eles/Elas sempre eram sua maior preocupação. E continuará sendo. Porque nunca acaba. Os experimentos, as dúvidas, a vontade de fazer e discutir ciência, sua maneira sempre humana de se relacionar com seus orientandos e amigos, e a profunda admiração que tenho por você. Nada disso acabará... Pesquisadora brilhante, Professora por vocação e Inovadora como poucos.

Muitíssimo obrigada por tudo, Cris!

AGRADECIMENTOS

Agradeço a Deus pela vida, por iluminar meu caminho e me proteger sempre.

À minha família, meu pai Cláudio, minha mãe Ires e minhas irmãs Andréia e Rosieli, por estarem sempre ao meu lado, acreditarem em mim e nunca medirem esforços para me ajudar. Obrigada por todo apoio, cuidado e amor. Vocês são meus maiores incentivadores. Amo vocês!

Agradeço imensamente à minha orientadora Cristina, por toda dedicação, compreensão, paciência, ensinamentos que foram além do seu papel de orientadora.

Ao GZ, pela dedicação, exemplo profissional, carinho e amizade.

Ao meu amor, Bruno, obrigada por acreditar em mim, pela paciência, amor, cuidado e companheirismo em todos os momentos. Amo você!

Agradeço aos meus amigos, Hecson, Melise e Louise por compreenderem minhas faltas e torcerem por mim independentemente do tempo ou distância.

Ao meu companheiro de faculdade, mestrado e doutorado, Hecson obrigada pela amizade e parceria conquistada ao longo desta caminhada.

Aos meus coautores e amigos Ana, Suzan, Isabella, Bruna, César e Marlon pelo auxílio nos experimentos e pela amizade, vocês são parte desta conquista!

Aos colegas de Lab Cris: Caroline, Juliano, Lina, Marcel, Natália, Paulo César, Pietro, Sabrina, Suelen, Vanessa, Vinicius, pela ajuda, paciência, compreensão e amizade.

A todos antigos colegas do Lab Cris, especialmente ao César pelos incentivos, conselhos, amizade e ensinamentos profissionais.

Aos colegas do Lab GZ pela amizade.

Ao Rinaldo pelo cuidado com os animais e amizade.

À banca examinadora por participarem da avaliação dessa tese.

À Universidade Federal de Santa Maria e ao Programa de Pós-Graduação em Bioquímica Toxicológica pela possibilidade de realização desse curso.

À CAPES, pelo auxílio financeiro.

Enfim, agradeço a todos que de alguma forma contribuíram para a realização deste trabalho.

“Quando nada parece dar certo, eu penso no talhador martelando cem vezes a rocha, sem que apareça uma única rachadura. Porém, na centésima primeira pancada, a pedra se abre perfeitamente em duas. E eu sei que não foi só a última batida a que conseguiu isso, mas também todas as anteriores”.

(Jacob Riss)

RESUMO

AÇÃO NEUROPROTETORA DO COMPOSTO EBSELEN E DO EXERCÍCIO FÍSICO EM MODELOS DE DOENÇA DE ALZHEIMER ESPORÁDICA EM CAMUNDONGOS

Autora: Franciele Martini
Orientadora: Dr.ª Cristina Wayne Nogueira

Sabe-se que a Doença de Alzheimer (DA) é uma doença neurodegenerativa que atinge cerca de 47 milhões de pessoas no mundo. Este número está previsto para aumentar nas próximas duas décadas. Uma gama de evidências tem mostrado que a patogênese da DA ainda não está completamente clara, afirma-se que seja heterogênea, pois seu desencadeamento está relacionado com o envolvimento de múltiplos fatores. O ebselen é um composto orgânico de selênio, que tem merecido destaque na literatura devido suas propriedades farmacológicas. De fato, os efeitos neuroprotetores do ebselen foram reconhecidos e seus alvos multifatoriais parecem ser uma vantagem para estratégias terapêuticas prospectivas. Ainda, a prática de exercício físico regular proporciona diversos benefícios, e um deles está associado a aspectos cognitivos, em idosos. Com isso, o objetivo principal desse estudo foi investigar a ação neuroprotetora do composto ebselen e do exercício de força na fisiopatologia da doença de Alzheimer em modelos animais de DA esporádica (**CEUA: 7372110915 – 6145050717**). Inicialmente, os resultados do **artigo 1** demonstraram que o ebselen inibiu, IC_{50} 33.14 (28.93 – 37.97) µM, a atividade da isoforma G4 da enzima acetilcolinesterase (AChE) hipocampal, *in vitro.* Nos modelos animais da DA esporádica, o ebselen (50mg/kg via i.p.) mostrou um efeito neuroprotetor em um modelo de amnésia induzida pela escopolamina, protegendo desses efeitos deletérios nos testes comportamentais de reconhecimento do objeto e Y- Maze em camundongos Swiss. Somado a isso, este composto apresentou uma inibição na atividade da AChE, no hipocampo dos camundongos. Estes resultados sugerem que o ebselen modulou a disfunção na neurotransmissão colinérgica induzida pela escopolamina. No **artigo 2** o protocolo experimental utilizando estreptozotocina (ETZ - 3 mg/kg via i.c.v.) visou um tratamento terapêutico e repetido com doses mais baixas de ebselen (1 e 10 mg/kg via i.p.). O tratamento com ebselen, nos camundongos, em doses baixas, foi eficaz em reverter a perda de memória ocasionada pela no teste de reconhecimento e localização do objeto e no Y-Maze. Além disso, o composto foi efetivo em reverter todos os parâmetros de estresse oxidativo e os níveis de proteínas da via apoptótica nas razões de Bax/Bcl-2, PARP clivada/PARP e nos níveis de caspase-3 no hipocampo de camundongos tratados com ETZ. O **manuscrito I** visou um tratamento terapêutico não farmacológico com um programa de exercício de força utilizando uma escada, onde os animais foram submetidos a um treino de força progressiva com duração de 4 semanas. O exercício de força aumentou os níveis de marcadores neurogênicos via sinalização BDNF/ERK-CAMK-II/CREB no hipocampo de camundongos, além de suprimir a perda de memória no teste do Morris water maze (MWM), em um modelo de DA esporádica. Finalmente, esta tese contribui para o esclarecimento dos mecanismos neuroprotetores envolvidos na ação do ebselen e reforça a hipótese de que composto pode ser uma interessante alternativa terapêutica para o tratamento da DA. Ainda, com relação ao exercício físico de força, os resultados contribuem para o entendimento dos efeitos do exercício de força sobre a DA.

Palavras – chaves: ebselen, exercício de força, Alzheimer, neuroproteção, estresse oxidativo.

ABSTRACT

NEUROPROTECTIVE ACTION OF EBSELEN COMPOUND AND PHYSICAL EXERCISE IN SPORADIC ALZHEIMER DISEASE MODELS IN MICE

Author: Franciele Martini
Advisor: Cristina Wayne Nogueira, PhD

Alzheimer's Disease (AD) is a neurodegenerative disease that reaches about 47 million people in the world and this prevalence is expected to increase over the next two decades. A range of evidence has shown that the pathogenesis of AD is still not completely clear; it is heterogeneous and involves multiple factors. Ebselen is an organoselenium compound, which has pharmacological properties. In fact, the neuroprotective effects of ebselen have been recognized and their multifactorial targets appear to be an advantage for prospective therapeutic strategies. In addition, regular physical exercise has several benefits, among them improvement of cognitive aspects in the elderly. Therefore, the main objective of this study was to investigate the neuroprotective action of ebselen and the strength exercise in the pathophysiology of animal models of sporadic AD (**CEUA: 7372110915 - 6145050717**). Initially, the results of **article 1** demonstrated that ebselen inhibited, *in vitro*. In the animal models of sporadic AD, ebselen (50 mg / kg via i.p.) inhibited IC50 33.14 (28.93 - 37.97) μM, the G4 isoform activity of the hippocampal enzyme acetylcholinesterase (AChE) showed a neuroprotective effect on a scopolamine-induced amnesia model, protecting against these deleterious effects in object recognition and Y-Maze behavioral tests in Swiss mice. In addition, this compound inhibited AChE activity in the hippocampus of mice. These results suggest that ebselen modulated dysfunction in scopolamine-induced cholinergic neurotransmission. In **article 2** the experimental protocol, repeated treatment with lower doses of ebselen (1 and 10 mg/kg via i.p.) in a model of AD induced by streptozotocin (STZ - 3 mg / kg via i.c.v.) was carried out. Ebselen treatment was effective in reversing the memory loss caused by STZ in mice, which was demonstrated in the object recognition and location tests and Y-Maze. In addition, the compound was effective in reversing all parameters of oxidative stress and protein levels of the apoptotic pathway in the Bax/Bcl-2, cleaved PARP/PARP ratios and caspase-3 levels in the hippocampus of STZ-treated mice. In the **manuscript I** mice were subjected to a non-pharmacological therapeutic treatment, a strength exercise program, which was carried out bin a ladder, where the animals underwent progressive force training (4 weeks). Strength exercise increased levels of neurogenic markers via BDNF/ERK-CAMK-II/CREB signaling in the hippocampus of mice, in addition to suppressing memory loss in the Morris water maze (MWM) test in a sporadic AD model. Finally, this thesis contributes to better understand the neuroprotective mechanisms involved in the action of ebselen and reinforces the hypothesis that this compound may be an interesting therapeutic alternative for the treatment of AD. Regarding strength exercise, the results help to understand the effects of strength exercise on AD.

Key words: ebselen, strength exercise, Alzheimer 's, neuroprotection, oxidative stress.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS

ACh – Acetilcolina

AChE – Acetilcolinesterase

AChEI – Inibidores da Acetilcolinesterase

AICD - Domínio intracelular da APP

APP - Proteína precursora do amilóide

βA - Fragmento β-amilóide

βA $_{1-42}$ - Fragmento β-amilóide contendo 42 aminoácidos

βA $_{1-40}$ - Fragmento β-amilóide contendo 40 aminoácidos

CAT – Catalase

CCL – Comprometimento cognitivo leve

ChAT - Colina acetiltransferase

SNC - Sistema nervoso central

CoA - Coenzima A

DA - Doença de Alzheimer

DEDA – Demência esporádica decorrente da doença de Alzheimer

E1/E2 - Domínios extracelulares conservados

ENF - Emaranhados neurofibrilares

EO - Estresse oxidativo

ER - Espécies reativas

ERN - Espécies reativas de nitrogênio

ERO - Espécies reativas de oxigênio

ETZ - Estreptozotocina

GPx - Glutationa peroxidase

GR - Glutationa redutase

GSH - Glutationa reduzida

GSSG - Glutationa oxidada

GST - Glutationa S-transferase

IBGE – Instituto de Geografia e Estatística

i.c.v. – Intracerebroventricular

MAP's - Microtubule associated proteins

NADPH - Nicotinamida adenina dinucleotideo fosfato reduzida

NFTs – Emaranhados neurofibrilares intracelulares

$(PhSe)_2$ - Disseleneto de difenila

PS - Placas senis

SOD - Superóxido dismutase

LISTA DE ANEXOS

SUMÁRIO

1. INTRODUÇÃO

1.1. DEMÊNCIA

Para fins de definição, pode-se conceituar demência, como uma condição heterogênea caracterizada por declínio cognitivo que não é suficientemente intenso para causar prejuízo funcional, evidenciando, dessa maneira, o diagnóstico de demência, ou também conhecido como Comprometimento Cognitivo Leve (CCL) (PETERSEN et al., 2014; ROCCA et al., 2011). Representando assim, um estado de transição entre o envelhecimento normal e a demência leve, tanto em termos cognitivos quanto neuropatológicos (GUILLOZET et al., 2003; MUELLER et al., 2005).

Dados epidemiológicos indicam um crescimento mundial da incidência de demência, particularmente do tipo Alzheimer, especialmente nos chamados países em desenvolvimento, é a forma mais comum de demência (cerca de 65 a 80%) (QIU, C., 2012). O relatório, de 2015, da "*Alzheimer's Disease International*" (ADI), estima que, 46,8 milhões de pessoas em todo o mundo convivem com a doença e as projeções são de que a prevalência deve dobrar até 2030, chegando a 74,7 milhões de pessoas (FRATIGLIONI et al., 2000; WIMO et al., 2017). Sendo assim, será a principal causa de dependência funcional e de mortalidade entre a população idosa (QIU, C., 2012).

O termo demência abrange um conjunto de sintomas envolvendo principalmente perdas de memória e de raciocínio decorrentes do próprio envelhecimento do indivíduo ou associada à doenças neurodegenerativas (GALLUZZI et al., 2013; PARK e HAN, 2015; PETERSEN e MORRIS, 2005). Claudia Suemoto, médica e professora da disciplina de Geriatria, da Universidade de São Paulo, uma das ganhadoras do prêmio "*L'Oréal Unesco para mulheres cientistas - 2016*", juntamente com seu grupo de pesquisa, desenvolve um projeto que trata da compreensão dos fatores de risco do mal de Alzheimer e mostra, em uma das suas pesquisas, que o ensino superior está associado a menor prevalência de demência e um aumento da reserva cognitiva (FARFEL et al., 2013). Assim, entre os principais fatores de risco relacionados a doença, estão a baixa escolaridade, o tabagismo, a diabetes, a depressão, a inatividade física, a obesidade e a hipertensão (WEINER et al., 2013).

Mesmo que a idade seja o principal fator de risco para o desenvolvimento de uma demência, o impacto da idade e do sexo é inconclusivo no prognóstico do tempo restante de vida dos pacientes (CHI et al., 2014; HELZNER et al., 2008; REITZ et al., 2011). Em

geral, os pacientes com alguma forma de demência evoluem para a morte em torno de três a dez anos após o diagnóstico (BRODATY et al., 2012). Infelizmente, ainda não há cura conhecida ou medidas preventivas para a maioria dos tipos de demência.

1.2. DOENÇA DE ALZHEIMER

1.2.1. CARACTERÍSTICAS EPIDEMIOLÓGICAS E CLÍNICAS DA DOENÇA DE ALZHEIMER

A mais de um século, o médico Alis Alzheimer proferiu uma palestra no 37º Congresso de Psiquiatria do Sudoeste da Alemanha, sobre sua pesquisa intitulada "*Eine eigenartige Ekrankung der Hirnrinde"* - "*Uma doença Peculiar dos Neurônios do Córtex Cerebral*". Tratava-se do caso clínico de uma paciente de 51 anos que foi internada no Hospital de Frankfurt, em 1901, apresentando perda de memória progressiva, alucinações e delírios. Após sua morte, Alzheimer fez uma análise cerebral identificando as principais características neuropatológicas da doença, ou seja, a presença de placas senis e dos emaranhados neurofibrilares e a perda neuronal. Anos mais tarde, Emil Kraepelin, na 8º edição do *"Handbook of Psychiatry"*, após estudar casos parecidos, propôs que se denominasse essa patologia de "*Doença de Alzheimer's"*, em homenagem ao seu descobridor (GOEDERT e SPILLANTINI, 2006; SOUCHAY, 2007).

Sabe-se que a DA é uma condição de vida tardia, atinge cerca de 47 milhões de pessoas no mundo (PRINCE et al., 2013). Este número está previsto para aumentar nas próximas duas décadas (PRINCE et al., 2016). O custo total da demência foi estimado em torno de US $ 818 bilhões em 2010 e foi projetado para atingir US $ 1 trilhão até 2018 em todo o mundo (PRINCE et al., 2016). Isso se torna ainda mais dramático pois cerca de 60% das pessoas afetadas pela demência vivem em países de baixa e média renda. Diante disso, entre os anos de 2010 e 2011, nos Estados Unidos, foi realizado um Projeto de Lei de Alzheimer's Nacional (National Alzheimer's Project ACT - NAPA) (http://napa.alz.org/national-alzheimersproject-act-backgroun), pelo *Research Triangle Institute*, em que exigiu-se a criação de uma estratégia nacional para tentar solucionar a "crise" na DA, com o objetivo principal de "prevenir ou tratar de forma eficaz a Doença de Alzheimer até 2025".

No Brasil, segundo a Pesquisa Nacional por Amostra de Domicílios Contínua, divulgada pelo Instituto Brasileiro de Geografia e Estatística (IBGE), a tendência ao envelhecimento da população se manteve nos últimos anos, cerca de 4,8 milhões de idosos

desde 2012, superando os dados dos 30,2 milhões em 2017. Estes dados correspondem a um crescimento de 18% desse grupo etário, que tem se tornado cada vez mais representivo no Brasil. Ainda, entre 2012 e 2017, a quantidade de idosos cresceu em todas as unidades da federação, sendo os estados com maior proporção de idosos o Rio de Janeiro e o Rio Grande do Sul, ambas com 18,6% de suas populações dentro do grupo de 60 anos ou mais, segundo dados do IBGE.

A medida que a população envelhece surgem, ao mesmo tempo, questões de fundamental importância, tais como: Qual o limite para a expectativa de vida? Será o envelhecimento acompanhado por longos períodos de boa saúde, bem-estar, engajamento social e produtividade, ou é predominantemente despendido em doenças, incapacidade e dependência? Como a velhice afeta os cuidados de saúde e os custos sociais? São estas expectativas inevitáveis, ou há possibilidade para estabelecer uma infra-estrutura física e social capaz de promover uma melhora de saúde e bem-estar? (ALBERT et al., 2011).

Em contraste à isso, além do sofrimento pessoal, pacientes com a DA requerem cuidados médicos e hospitalares por muito tempo, logo constitui um sério problema de saúde pública, gerando custos à economia, mais de 1% do PIB global, levando a um "pesado fardo" para os pacientes e para a sociedade (PLASSMAN et al., 2007).

A principal preocupação a cerca desta doença está relacionada à sua fisiopatologia, caracterizada como uma espécie de lesão degenerativa crônica, progressiva e irreversível no sistema nervoso central (SNC), apresenta um início insidioso, com deterioração lenta na cognição, episódios de perda da memória, capacidade funcional, alterações comportamentais e de humor (DEL BO et al., 2009; HARDY e SELKOE, 2002; STIX, 2010). Ainda, os pacientes podem apresentar afasia, (distúrbio de linguagem com dificuldades de identificação, fluência, compreensão e associação das palavras), aprazia (desorganização no controle motor) e agnosia (incapacidade de reconhecimento de objeto e/ou familiares) (HARDY e SELKOE, 2002; HELMES e OSTBYE, 2002; STIX, 2010).

Uma gama de evidências tem mostrado que a patogênese da DA ainda não está completamente clara, afirma-se que seja heterogênea, pois seu desencadeamento está relacionado com o envolvimento de múltiplos fatores, incluindo hereditariedade, alerações nos níveis de neurotransmissores, além de fatores imunológicos e ambientais (UBHI et al., 2012). Morfologicamente a DA é caracterizada por mudanças neuropatológicas específicas, tais como: o depósito de fragmentos β-amilóide (βA) difusos e/ou aglomerando-se, formando as placas senis (PS); emaranhados neurofibrilares (ENF) decorrentes da hiperfosforilação da proteína Tau; surgimento concomitante de marcadores

de inflamação crônica (GOTZ e ITTNER, 2008; HARDY e SELKOE, 2002; MOORE e O'BANION, 2002; PARIHAR e BREWER, 2010).

Diante disso, muitas hipóteses etiológicas vêm sendo estudadas para explicar a DA, entre elas incluem-se a hipótese de alterações genéticas, que causam "mutações" na clivagem de proteínas precursoras das βA; diminuição dos fatores neurotróficos; disfunção mitocondrial; neurotoxicidade ocasionada pelo estresse oxidativo; mecanismos neuroinflamatórios; disfunção no metabolismo energético e autofagia (BLENNOW et al., 2006; YANG et al., 2016).

Assim, não é de surpreender que ainda faltam biomarcadores precisos e confiáveis para o diagnóstico precoce da doença. A adição de novos biomarcadores aos critérios diagnósticos provocou uma mudança na forma como a DA é considerada uma entidade patológica, isto é, ela não pode ser vista como uma doença que apresenta fases clínicas distintas e bem definidas, mas como um processo que acontece de forma multifatorial, sequencial e ininterrupto (SPERLING, 2011; VAN MAURIK et al., 2017). Esta ideia é suportada por estudos *post-mortem* que demonstraram que as mudanças fisiopatológicas começam a se desenvolver muito tempo antes dos sintomas cognitivos iniciais, em um estágio pré –clínico ou pré-sintomático (SPERLING, 2011).

Com isso, a partir de 2003, o Departamento Científico de Neurologia Cognitiva e do Envelhecimento da Academia Brasileira de Neurologia, recomenda para o diagnóstico da DA, no Brasil, a presença de pelo menos dois sintomas cognitivos ou comportamentais, que afetem no mínimo dois domínios: memória, funções executivas, habilidades visuais e espaciais, linguagem e personalidade ou comportamento. Aproximadamente 5% dos casos de DA são familiares, devido ao gene autossômico dominante de transmissão (mutações nos genes codificadores da proteína precursora amilóide (APP), presenilina 1 e 2) (TILLEMENT et al., 2011). Em contraste, a maioria dos casos são diagnosticados como uma forma esporádica da doença (demência esporádica decorrente da doença de Alzheimer - DEDA), ou seja, sua origem é desconhecida (NICOLAS et al., 2018).

1.3. FISIOPATOLOGIA DA DOENÇA DE ALZHEIMER

A DA é diagnosticada através de avaliações neurológicas e imagens de ressonância magnética. A confirmação da doença, no entanto, é obtida "*post mortem*"(ALBERT et al., 2011). Imagens do cérebro de pacientes com a DA podem exibir atrofia cerebral e encolhimento do hipocampo, dependendo do estágio de progressão da doença. (SINHA et

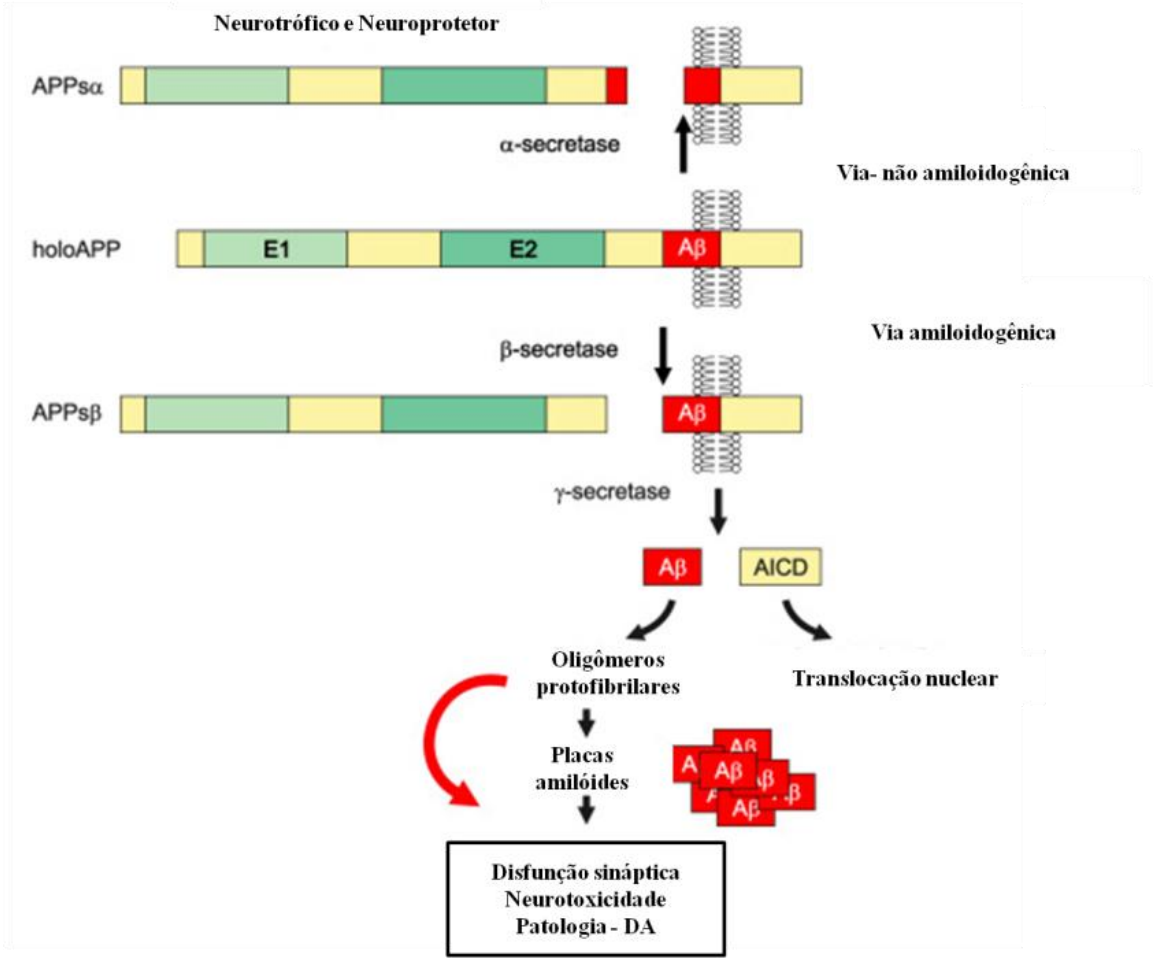
al., 2013). É importante salientar que o SNC do indivíduo com a DA não é uniformemente prejudicado; enquanto o cerebelo é altamente resistente, o hipocampo e o neocórtex são muitos sensíveis ao processo patológico (MU e GAGE, 2011). Por esta razão as habilidades cognitivas são mais afetadas do que, por exemplo, a movimentação ou homeostase corporal basal (MU e GAGE, 2011).

As características presentes nas regiões cerebrais associadas à memória dos pacientes com a DA são os emaranhados neurofibrilares intracelulares (NFTs) e as placas amiloides extracelulares (MEDEIROS et al., 2011). Os NFTs são formados pela fosforilação anormal de proteína tau (MEDEIROS et al., 2011; MORRIS et al., 2016), desestabilizando assim os microtúbulos e comprometendo a oxigenação e o transporte axonal de nutrientes aos neurônios (GOTZ e ITTNER, 2008; MORRIS et al., 2016). Recentemente, foi demonstrado que os emaranhados ocasionam comprometimento na memória espacial e morte neuronal (FU et al., 2017), fornecendo, supostamente, uma ligação entre a patologia da TAU e déficits cognitivos em estágios iniciais da DA. No entanto, acredita-se que as alterações patológicas da tau sejam eventos a jusante da deposição de βA (BENNETT et al., 2004). Desta forma, é plausível que, ambas, a tau e o βA atuem paralelamente devido aos seus próprios efeitos tóxicos e iniciem os eventos patogênicos que caracterizam a DA (BENNETT et al., 2004; SPIERS e BENDOR, 2014).

A hipótese da cascata amilóide sugere que o acúmulo cerebral do peptídeo βA é um evento central da DA, onde a proteólise da PPA acontece pela ação sequencial de um grupo de enzimas chamadas secretases, estas enzimas podem agir por duas vias distintas (KARRAN et al., 2011; SELKOE e HARDY, 2016). A primeira delas é a via não-amiloidogênica, na qual a α e γ- secretases clivam sequencialmente a PPA, formando fragmentos solúveis que não tem participação na patofisiologia da DA (KARRAN et al., 2011). No entanto, a PPA pode sofrer ação da β- e γ-secretases e gerar os fragmentos amiloidogênicos βA (mais comumente o βA_{1-40} e o βA_{1-42}), e estes, por sua vez, desencadeiam várias cascatas patológicas que levam a disfunções sinápticas e dendríticas, além de ativarem a microglia e os astrócitos e induzirem a morte celular (**Figura 1**) (MOORE e O'BANION, 2002; MULLER e ZHENG, 2012; SELKOE e HARDY, 2016).

Figura 1. Representação geral das funções fisiológicas e patológicas de proteínas da família APP. Representação do processamento de APP através da via não-amiloidogênica (topo) e amiloidogênica (inferior). Entre os vários produtos de clivagem da APP, o

ectodomínio APP secretado tem implicado na da sinalização neurotrófica e neuroprotectora, enquanto se acredita que a A e a AICD induzam predominantemente efeitos neurotóxicos. (AICD: domínio intracelular da APP; E1, E2: domínios extracelulares conservados).

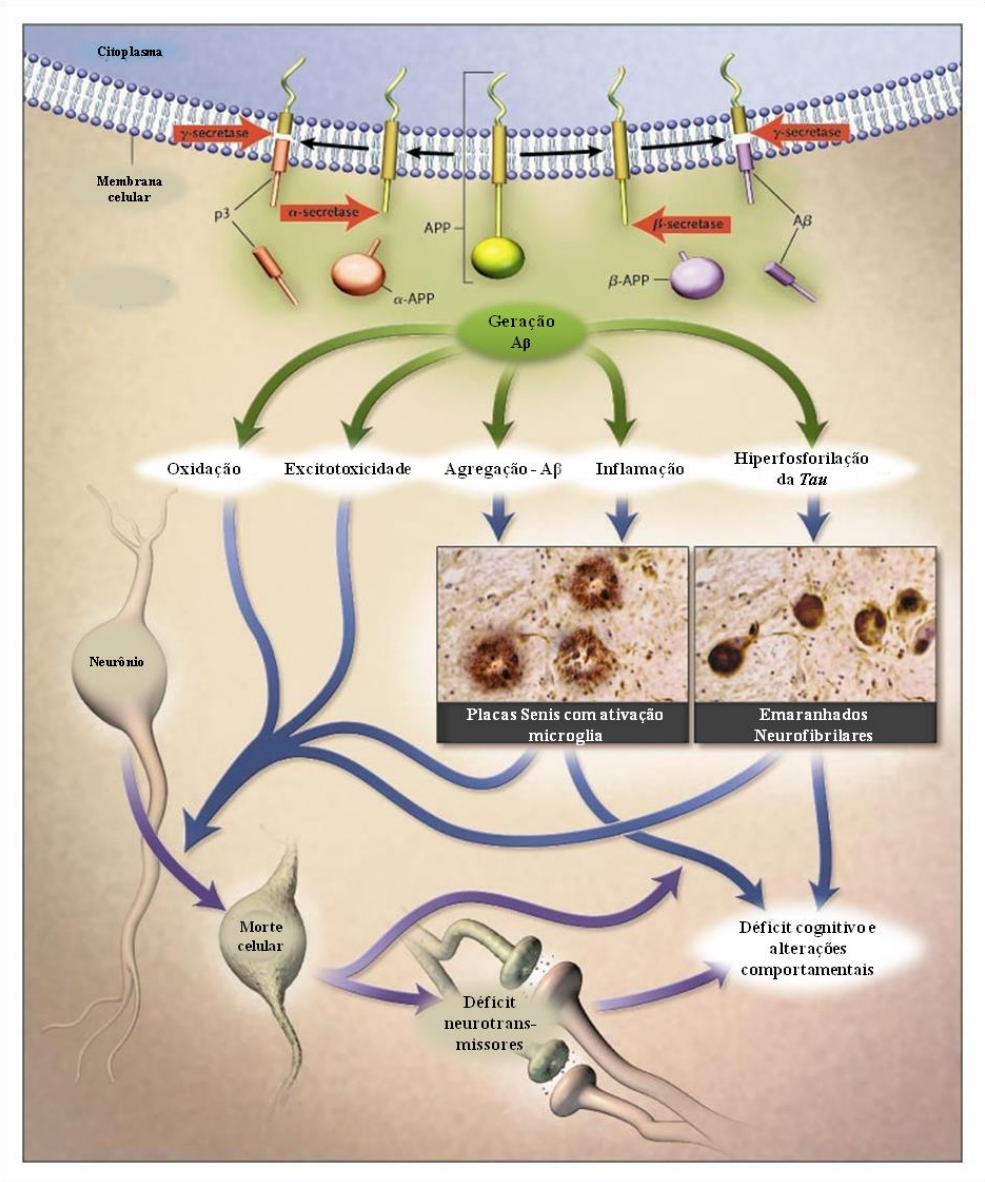


Fonte: Adaptado de Müller et al., 2012. *Experimental Brain Research.*

O βA é fisiologicamente degradado pelas enzimas peptídicas (IWATA et al., 2001; QIU, W. Q. et al., 1998), ou então pode ser eliminado para a circulação periférica, principalmente pela barreira hematoencefálica, através do fluxo volumétrico do líquido intersticial ou pelas vias linfáticas (TARASOFF-CONWAY et al., 2015). Além disso, os agregados βA podem ser fagocitados e degradados pela ação da microglia. Uma falha nestes "sistemas" de degradação poderia levar a um desequilíbrio entre a relação da produção e a depuração de βA no cérebro, resultando em uma disfunção neuronal e subsequente neurodegeneração (TARASOFF-CONWAY et al., 2015).

Assim, embora a patofisiologia da DA não esteja completamente elucidada, assume-se que ambas as alterações morfológicas induzidas pelo Aβ, PS e ENF, iniciam uma cascata patológica que resulta na disfunção neuronal e perda das conexões sinápticas, devido, principalmente, à inflamação e ao estresse oxidativo (**Figura 2**) (WANG, C. et al., 2014a).

Figura 2. Representação da fisiopatologia da Cascata Amilóide na DA.



Fonte: Adaptado de CUMMINGS (2004). *New England Journal of Medicine*.

1.4. HIPÓTESE COLINÉRGICA E MEDICAMENTOS DISPONÍVEIS À DA

O papel do sistema colinérgico no processo neurodegenerativo está bem estabelecido. A ACh, conhecida como um neurotransmissor-chave no sistema nervoso, interage com receptores associados a processos de aprendizagem e memória. No cérebro, está associada ao controle motor, memória e cognição (DREVER et al., 2011). Sua síntese é realizada pela colina-acetiltransferase (ChAT) através da catálise dos substratos colina e acetil CoA. Esta reação é limitada pela concentração de colina, a qual tem sua concentração dependente da ação da AChE, enzima que lisa a acetilcolina em colina e acetato (BLOKLAND, 1995; SCOTT et al., 2014).

A AChE é uma importante enzima responsável por regular a transmissão de impulsos nervosos através das sinapses colinérgicas, hidrolisando o neurotransmissor excitatório ACh (SCHETINGER et al., 2000). Além disso, está associada com o desenvolvimento, aprendizagem e memória, no cérebro (BALLARD et al., 2005a). Dessa forma, a "hipótese colinérgica" da DA (BALLARD et al., 2005b) começou com evidências na diminuição da expressão da enzima colina acetiltransferase em pacientes com DA, esse estudo foi realizado por laboratórios no Reino Unido em 1978 (DAVIS, K. L. e YAMAMURA, 1978). Esses achados levaram ao primeiro teste em pacientes com DA do tratamento com inibidores da AChE (AChEI), um estudo realizado em 1981 (GIACOBINI, 2004), além de uma demonstração de melhora na memória em um grupo de pacientes em 1982 (PARSONS et al., 2013; PERRY et al., 1982). Houve também uma demonstração de que os neurônios colinérgicos mais afetados na DA se originavam no núcleo basal de Meynert e que havia uma relação entre a inervação colinérgica e a deposição de amilóide em placas neuríticas (DREVER et al., 2011). Após estes estudos, os AChEI foram testados clinicamente em pacientes com a DA, levando à primeira aprovação pelo FDA de um medicamento para a DA, a tacrina, em 1993 (CRISMON, 1994). De muitas terapias colinérgicas, apenas a abordagem de AChEI mostrou um benefício consistente (CRISMON, 1994). No entanto, apesar do foco na hipótese colinérgica, é evidente que a DA abrange muito mais do que danos nos neurônios colinérgicos (TIRABOSCHI et al., 2004).

Como já mencionado, a DA é um importante problema de saúde que provavelmente aumentará progressivamente com o tempo (BROOKMEYER et al., 2007), e o principal objetivo dos pesquisadores tem sido descobrir drogas eficazes para tratar esta doença. Muitos já existem, mas ainda não foi encontrado tratamento para reverter à progressão da doença ou para controlar seu processo neurodegenerativo (SPUCH et al., 2012). A primeira classe terapêutica aprovada pelo FDA foram os AChEI, cujo mecanismo consiste

em impedir a degradação de ACh pela enzima AChE, o que aumentaria sua concentração no cérebro. No entanto, a significante hepatotoxicidade observada pelo uso da Tacrina, (WATKINS et al., 1994), fez com que esta droga fosse retirada do mercado. A **Tabela 1** demonstra os medicamentos aprovados e utilizados para o tratamento da DA; todos foram desenvolvidos para tratar os sintomas e desacelerar a progressão da doença (WATKINS et al., 1994). Como podemos observar, são divididos em dois grupos: os anticolinesterásicos, que pemitem a produção de ACh e a memantina, que é um antagonista do receptor NMDA (N-metil-D-aspartato) agindo na entrada de cálcio nos neurônios, reduzindo assim a morte neuronal (JOHNSON e KOTERMANSKI, 2006).

Os AChEI podem ser classificados com base na reversibilidade e duração da inibição das colinesterases. A tacrina e a donepezila são inibidores reversíveis da AChE de curta e de longa duração, respectivamente (DEARDORFF et al., 2015). A inibição da enzima tem duração intermediária para o inibidor pseudo-irreversível rivastigmina, e longa para o inibidor irreversível metrifonato (DEARDORFF et al., 2015). Este último teve seus estudos clínicos descontinuados, devido à sua toxicidade. Entretanto, todos apresentam limitações clínicas quanto ao seu uso, devido à meia – vida curta ou por apresentarem efeitos adversos, como distúrbios gastrointestinais e hepáticos (PORCEL e MONTALBAN, 2006).

Figura 3. Representação das drogas desenvolvidas para o tratamento da DA.

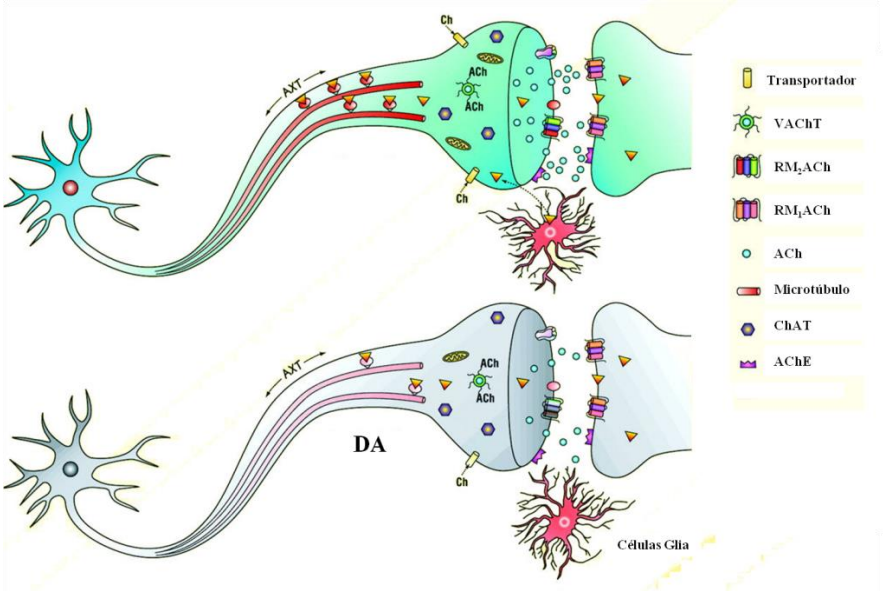
Droga	Classe	Mecanismo	Efeitos adversos
Donepezila (FDA – 1996)	AChEI Tratamento dos sintomas moderados à severos da DA	Mantém os níveis de Ach no cérebro	Náuseas, vômito e diarréia
Galantamina (FDA – 1996)	AChEI Tratamento dos sintomas moderados da DA	Mantém os níveis de Ach no cérebro e estimula os receptores nicotínicos à liberar mais Ach no cérebro	Náuseas, vômito, diarréia, diminuição do apetite e do peso corporal
Rivastigmina (FDA – 2000)	AChEI Tratamento dos sintomas moderados da DA	Mantém os níveis de Ach no cérebro	Náuseas, vômito, diarréia, diminuição do apetite e do peso corporal
Memantina (FDA – 2003)	Antagonista NMDA Tratamento dos sintomas moderados à severos da DA	Bloqueia o efeito tóxico associado ao excesso de glutamato e regula sua ativação	Tontura, dores de cabeça, constipação e confusão mental

Fonte: Adaptado CARVALHO et al., 2015. *Intelligent Information Management.*

Mais tarde, muitos estudos foram realizados e as evidências têm sugerido que a enzima AChE desempenha um papel chave no desenvolvimento das placas senis, acelerando a agregação e a deposição do peptídeo βA, uma das características mais relevantes na DA e esse efeito poderia ser atenuado por AChEI, como por exemplo a donepezila (ATTA UR et al., 2001; CUMMINGS, 2000; DAVIES, P. e MALONEY, 1976; ELLIS, 2005). Pode - se dizer que, essa classe farmacológica apresenta segurança e eficácia para o tratamento da DA (CUMMINGS et al., 2014), no entanto, são tratamentos a nível sintomático dos pacientes e melhoram temporariamente os problemas de memória, pois não tratam a causa subjacente da DA e não diminuem a taxa de declínio cognitivo (CHIAM et al., 2015; SHIMIZU et al., 2015). No entanto, o uso prolongado de donepezila pode ser limitado pelo aumento dos efeitos colaterais do tônus vagal associados à bradicardia, anorexia, dor abdominal, náusea e diarréia (TURON-ESTRADA et al., 2003). Drogas com menos efeitos colaterais, provavelmente, teriam uma maior taxa de adesão ao medicamento prescrito.

De fato, diferentes centros de pesquisa têm se dedicado a busca de novas drogas capazes de modificar e impedir a evolução progressiva da doença. No entanto, este desafio ainda não foi resolvido pela ciência, pois os mecanismos envolvidos na DA, apesar de muito estudado, ainda não são totalmente compreendidos (IQBAL et al., 2008).

Figure 4. Representação das alterações conhecidas e propostas nos neurônios colinérgicos que ocorrem no cérebro do idoso, no início da DA, em comparação com os neurônios jovens e saudáveis. Alterações na captação de colina, liberação de acetilcolina prejudicada, déficits na expressão de receptores nicotínicos e muscarínicos, e déficits no transporte axonal são representados no neurônio na DA.



Fonte: Adaptado de TERRY e BUCCAFUSCO (2003). *Journal of Pharmacology and Experimental Therapeutics.* (ChAT = colinacetiltransferase, AChE = acetilcolinesterase; RM_1Ach = receptor muscarínico M1; RM_2Ach = receptor muscarínico M_2; VAChT = transportador vesicular de acetilcolina; ACh = acetilcolina).

1.5. ESTRESSE OXIDATIVO

Como exposto anteriormente, uma das principais teorias da DA é um distúrbio na funcionalidade do sistema colinérgico. Entretanto, evidências indicam que a presença do estresse oxidativo tem um papel importante na DA. Essa hipótese tem um apelo teórico, visto que o cérebro é considerado vulnerável ao dano oxidativo por diferentes razões, entre elas, a alta utilização de oxigênio, alta concentração de lipídeos e sua modesta defesa antioxidante (GUTTERIDGE e HALLIWELL, 2018).

Em condições fisiológicas, várias defesas no organismo contribuem para a proteção das espécies reativas, incluindo a sua baixa produção, sua remoção por antioxidantes enzimáticos, não-enzimáticos e a reparação dos danos oxidativos (DAVIES, K. J., 2000). O estresse oxidativo é causado pelo aumento dos agentes pró-oxidantes e a diminuição das defesas antioxidantes. Os agentes pró-oxidantes responsáveis por causar o dano oxidativo são as espécies reativas de nitrogênio (ERN) e as espécies reativas de oxigênio (ERO), como os radicais livres (superóxido) e as moléculas como o peróxido de hidrogênio. As EROs são produzidas em todo o organismo em processos fisiológicos, porém em uma quantidade aumentada podem causar modificações no lipídeos, nas proteínas e no DNA (DURACKOVA, 2010; VAVAKOVA et al., 2015).

O interessante da hipótese do envolvimento das EROs nas doenças degenerativas é que o dano oxidativo é consequência de insultos patológicos que causam, em geral, um acentuado desequilíbrio entre as ER e os antioxidantes. No entanto, o dano oxidativo decorrente do metabolismo também ocorre, de forma muito lenta, mas cumulativa ao longo do tempo e pode ser responsável pelo aparecimento tardio, lento e progressivo destas doenças neurodegenerativas (COYLE e PUTTFARCKEN, 1993; MARKESBERY, 1997; PERSSON et al., 2014).

Um estudo realizado com cérebro de pacientes em diferentes estágios da DA demonstrou um aumento dos níveis de peroxidação lipídica, já nas fases iniciais da doença (BRADLEY et al., 2012). Embora o aumento das EROs seja uma característica patológica bem relacionada com a DA, a gênese deste processo não é ainda bem esclarecida. Uma das possibilidades seria a ação pró-oxidativa do peptídeo βA (CHAKRABARTI et al.,

2015). Por isso, acreditamos que a geração de espécies reativas desempenha papel fundamental na progressão da doença.

A proteção contra o dano oxidativo é providenciada pelas defesas antioxidantes, incluindo enzimas como a superóxido dismutase (SOD) que converte O_2^o em H_2O_2, a catalase (CAT) que é responsável pela detoxificação do H_2O_2 e a glutationa peroxidase (GSH-Px) que detoxifica os peróxidos orgânicos e inorgânicos derivados da oxidação de fosfolipídios de membranas (SCANDALIOS, 1993). As enzimas antioxidantes são consideradas as primeiras defesas para a proteção das macromoléculas biológicas contra o dano oxidativo (BUTTERFIELD et al., 2012). Além destas enzimas, a glutationa (GSH), os peptídeos de histidina, as proteínas ligadas ao ferro (ferritina, transferrina) e o ácido dihidrolipoico, são exemplos não enzimáticos de defesas produzidas pelo organismo. Defesas exógenas, provenientes da dieta, como o α-tocoferol (vitamina E), o ácido ascórbico (vitamina C), o β-caroteno (pró-vitamina A) e compostos fenólicos como os flavonóides, também possuem um papel importante na desativação das espécies oxidantes (HALLIWELL, 2006).

1.6. MODELOS ANIMAIS PARA O ESTUDO DA DOENÇA DE ALZHEIMER ESPORÁDICA

Os modelos animais são ferramentas importantes para que se compreenda melhor a patofisiologia da DA, possibilitando a descoberta de novos alvos terapêuticos para o seu tratamento. Além de reproduzir os sintomas clínicos da doença, que caracterizam a validade aparente dos modelos experimentais, outros dois critérios são úteis para a confiabilidade e reprodutividade destes modelos: I) a validade preditiva, que diz respeito à capacidade do modelo em detectar os tratamentos clinicamente úteis e II) a validade construtiva, referente à semelhança de fatores causais da doença no modelo experimental e em pacientes (MCKINNEY, 2001; WILLNER, 1997). Baseado nestes critérios, diversos modelos experimentais têm sido desenvolvidos para o estudo da DA em roedores.

1.6.1. MODELO DA ESCOPOLAMINA

A escopolamina é um fármaco antagonista competitivo não-seletivo de receptores muscarínicos. Portanto, ela não se liga fortemente a nenhum dos subtipos desses receptores metabotrópicos, tendo ação central e periférica (ANTOR et al., 2014). Quando administrada em altas concentrações, também pode antagonizar receptores nicotínicos.

Receptores muscarínicos estão distribuídos em todo nosso organismo e, no entanto os subtipos M1 e M5 estão mais presentes no cérebro. Portanto os efeitos centrais desse fármaco são mediados por esses dois subtipos (KLINKENBERG e BLOKLAND, 2010)

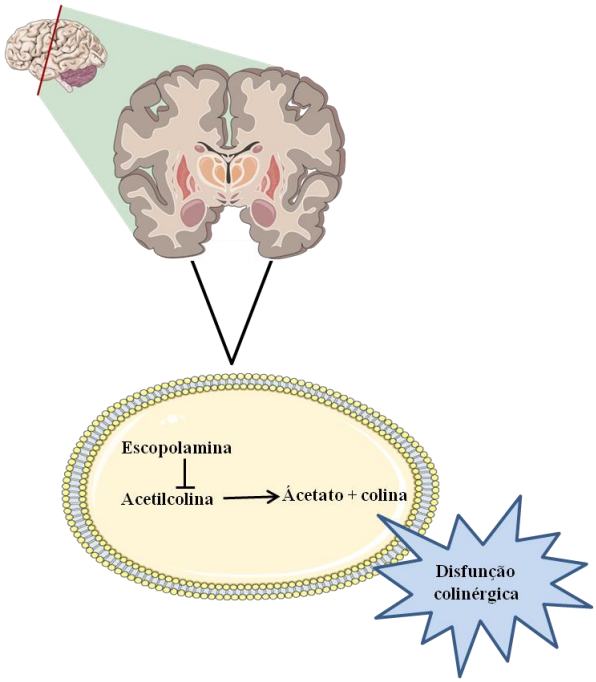
Sabemos que, quando há um acometimento da sinalização colinérgica, por lesão de áreas com grande quantidade de neurônios colinérgicos ou por uso de fármacos antagonistas dessa via, como a escopolamina, ocorre um déficit em processos mnemônicos (JACKLIN et al., 2015). Desta forma, o efeito amnésico da escopolamina é esperado, visto que ela tem sido usada como um modelo farmacológico de indução de amnésia em diversos estudos (DE BRUIN e POUZET, 2006; SCHABLE et al., 2012).

A administração de escopolamina está entre os modelos mais comumente utilizados pelos pesquisadores para investigar novas drogas (FLOOD e CHERKIN, 1986) para o tratamento da DA (YAMADA et al., 2004). O significado da ação colinérgica no cérebro para funções de aprendizagem e memória foi reconhecido há mais de três décadas, quando doses comparativamente baixas de alguns antagonistas muscarínicos do receptor de acetilcolina (escopolamina, atropina, beladona) foram estabelecidas para induzir déficits cognitivos temporários em voluntários jovens, humanos, que se assemelhavam àqueles visto em idosos (DRACHMAN e LEAVITT, 1974).

A redução na função do sistema colinérgico central pode estimular aspectos da demência como uma falha de memória e perplexidade, como visto na DA (CHEN et al., 2014). Tem sido observado que o dano dos neurônios colinérgicos geralmente ocorra em áreas do cérebro relacionadas com a memória e a aprendizagem, no hipocampo, córtex, e o núcleo basal de Meynert (GOVERDHAN et al., 2012).

No entanto, para estudos mecanicistas a escopolamina apresenta algumas limitações, pois não é capaz de replicar os aspectos patológicos e a progressão degenerativa observada na DA (VAN DAM e DE DEYN, 2006). Além disso, a principal crítica deste modelo é a falta de versatilidade, uma vez que as ações da escopolamina são limitadas ao bloqueio da função cerebral mediadas pelos receptores muscarínico (DORAISWAMY, 2002; TERRY e BUCCAFUSCO, 2003). Apesar dessa limitação, o comprometimento da memória induzido pela escopolamina fornece uma ferramenta fenotípica relativamente rápida para a descoberta e seleção de novas drogas para o tratamento de doenças neurodegenerativas (GRAHAM et al., 2005).

Figura 5. Representação do mecanismo de ação da escopolamina nos neurônios colinérgicos. Inibição do neurotransmissor acetilcolina são representados



Fonte: Própria do autor.

1.6.2. MODELO DA ESTREPTOZOTOCINA

O pioneiro do modelo de administração pela via intracerebroventricular (i.c.v.) de estreptozotocina (ETZ) em roedores é o professor Sigfried Hoyer (HOYER e LANNERT, 2007). A injeção i.c.v. de uma glicosamina derivada, em uma dose subdiabetogência em roedores, tem sido descrita como um modelo apropriado de DA esporádica, o qual é caracterizado por um progressivo déficit de memória acoplado a diversos efeitos citotóxicos como distúrbios na utilização da glicose e consequentemente do metabolismo energético, alterações no sistema colinérgico, estresse oxidativo e neurodegeneração (HOYER e LANNERT, 2008; LANNERT e HOYER, 1998; LESTER-COLL et al., 2006; MISHRA et al., 2018; PLASCHKE et al., 2010; SALKOVIC-PETRISIC et al., 2011; SHARMA et al., 2012).

Hoyer explica também que, o mecanismo pelo qual a ETZ induz citotoxicidade e déficit cognitivo pela injeção i.c.v. seja, possivelmente, pela dessensibilização dos receptores neurais de insulina e redução da atividade de enzimas glicolíticas (MISHRA et al., 2018; PLASCHKE e HOYER, 1993; PLASCHKE et al., 2010). Desta maneira, ocasionando uma queda no consumo cerebral de oxigênio e glicose, mas a queda do

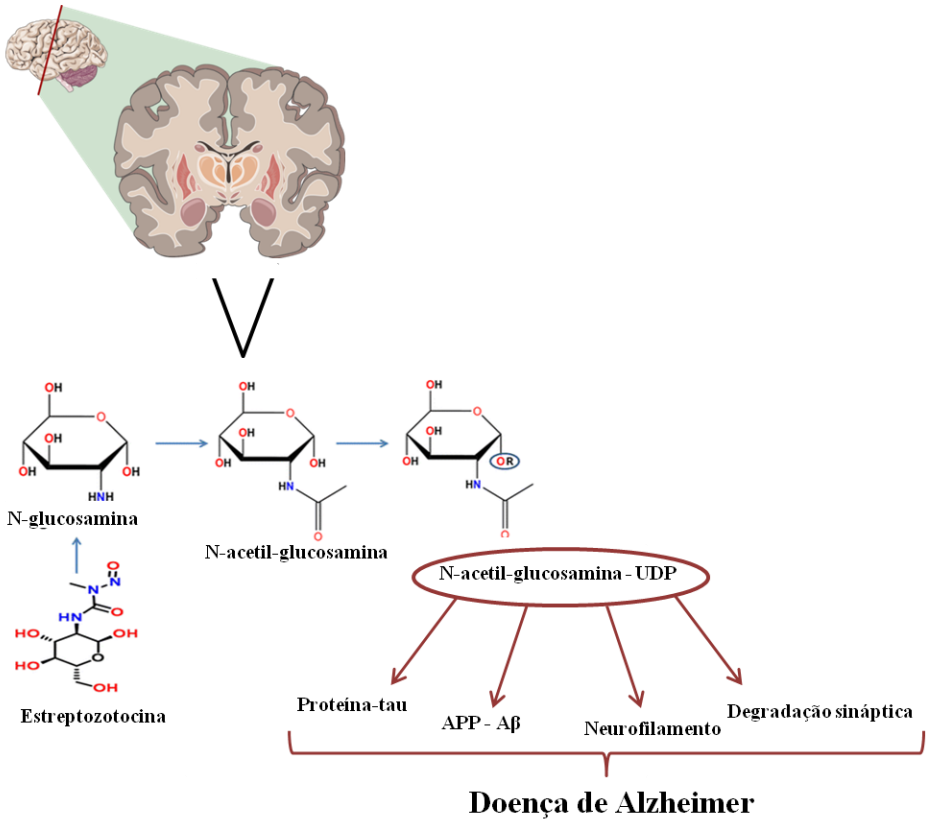
consumo de oxigênio é desproporcionalmente menor. Levando à hipótese de que a principal alteração bioquímica na DA se refere ao controle sobre o metabolismo da glicose cerebral, ou seja, uma subsequente falha de transdução de sinal do receptor de insulina cerebral.

O estresse oxidativo está envolvido na patogênese da DA induzida pela ETZ em roedores, desta forma, pode-se admitir que o surgimento de estresse oxidativo está relacionado aos efeitos deletérios induzidos pela ETZ. Pesquisas mostram que há uma maior geração de espécies reativas, peroxidação lipídica, carbonilação de proteínas, depleção de defesas antioxidantes não enzimáticas (AGRAWAL et al., 2010; DESHMUKH et al., 2009; ISHRAT et al., 2009; KUMAR et al., 2010; PRAKASH e KUMAR, 2009).

E por fim, recentemente, MISHRA et al. (2018) confirmou em um de seus trabalhos que a injeção de ETZ – i.c.v. induz eventos fisopatológicos como a diminuição do metabolismo enrgético cerebral, disfunção do receptor de insulina. Além disso, estresse oxidativo, neuroinflamação, disfunção mitocondrial, excitoxicidade, aumento da fosforilação da *tau*, deposição de βA, perda sináptica e de memória (PERSSON et al., 2014). Por fim, juntos esses eventos são observados na DA esporádica (CARUSO et al., 2018).

No entanto, não há dúvida que este modelo também apresenta limitações relevantes, uma vez que é um modelo experimental para estudar a DA esporádica metabólica e as possíveis terapias. E, ainda é uma questão desconhecida como é a dessensibilização do receptor de insulina no cérebro, se é um efeito secundário do hipometabolismo da glicose cerebral ou uma causa primária da DA (GRIEB, 2016).

Figura 6. Representação química da ETZ onde inicia o processo químico pela formação da N-glucosamina e N-acetil-glucosamina e finalmente os metabolitos tóxicos glucosamina-N-acetil - UDP. Estes metabolitos tóxicos promovem a formação da proteína tau, do neuropeptídeo Aβ, de neurofilamentos e um processo de degração de sinapse. Esses processos bioquímicos e a formação anormal de metabólitos tóxicos no cérebro, consequententemente, causa neurodegeneração que é uma característica da DA esporádica.



Fonte: Adaptado por KAMAT (2015). *Molecular Neurobiology*.

1.7. A BUSCA POR NOVOS TRATAMENTOS E OS COMPOSTOS ORGÂNICOS DE SELÊNIO

O Selênio (Se) é um micronutriente no organismo que representa um componente essencial de selenoproteínas, que desempenham um papel importante em muitos processos biológicos, incluindo as respostas imunes adaptativas e inatas e as defesas antioxidantes (BEHNE et al., 1990; KRYUKOV et al., 2003). Para fins nutricionais, o Se pode ser utilizado tanto na forma inorgânica (selenito e selenato) como também, na forma orgânica (selenocisteína, selenocistina e selenometionina) que possui menor toxicidade e maior biodisponibilidade quando comparadas às formas inorgânicas (PAPP et al., 2007). Suas funções biológicas são atribuídas às selenoproteínas, que contêm resíduos de selenocisteína (SeCys) responsáveis pela sua atividade específica. Selenoproteínas, tais como a glutationa peroxidase (GPx), ao participar na redução de peróxido de hidrogênio e lipoperóxidos, regulam o estresse oxidativo (PAPP et al., 2007).

Ao longo das últimas décadas, o interesse por compostos orgânicos de selênio sintéticos têm aumentado devido às diversas propriedades farmacológicas que estas moléculas apresentam numa vasta gama de modelos de patologias humanas (NOGUEIRA

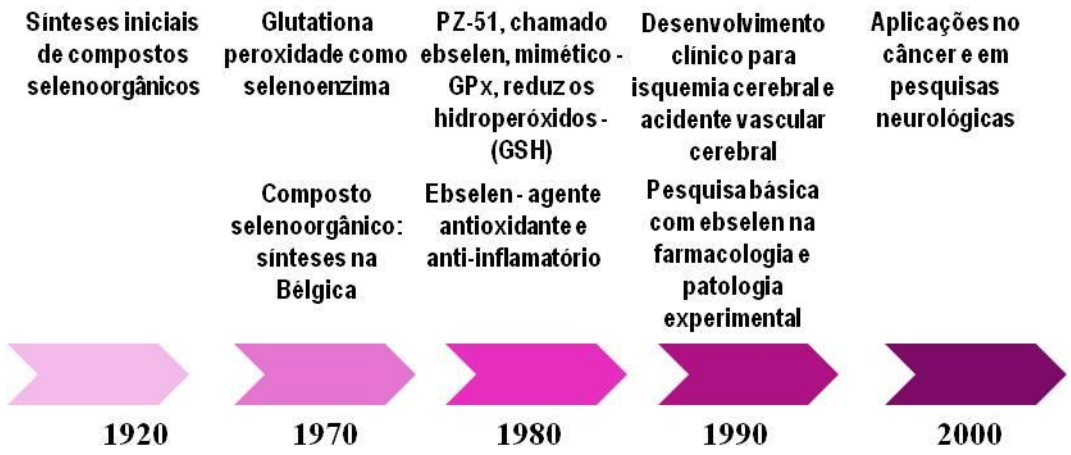
e ROCHA, 2011; NOGUEIRA et al., 2004). Dentre os compostos estudados por nosso grupo de pesquisa, o ebselen, um composto selenoorgânico multifuncional (NOGUEIRA et al., 2004), onde atualmente encontra-se em fase de ensaios clínicos para esquemia cerebral (GABRYEL and MALECKI, 2006) e transtorno bipolar (MASAKI ETA l., 2016; SINGH et al., 2013; WANG et al., 2014). Além disso, a eficácia deste composto já foi comprovada em um modelo transgênico de DA familiar (XIE et al., 2017).

1.7.1. EBSELEN

O Ebselen (2-fenil-1,2-benzisoselenazol 3(2H)-on) (Figura 4), também chamado PZ 51, é um composto orgânico de selênio, que tem merecido destaque na literatura pelas suas propriedades farmacológicas. O ebselen foi descrito e caracterizado como um mimético da GPx em 1984 (MULLER e ZHENG, 2012; SCHEWE, 1995; WENDEL et al., 1984), e desde então tem - se expandido a aplicabilidade de suas atividades farmacológicas. A partir dessa década cresceu enormemente o número de estudos onde nos quais o ebselen exerce proteção em diferentes tipos celulares para os mais diversos tipos de patologias. Foi um dos primeiros compostos sugeridos para a terapia de inativação de hidroperóxidos na presença de glutationa (PARNHAM, M. J. e KINDT, 1984).

A reação catalisada por estes compostos é similar à reação catalisada pela GPx, e é de particular significância para células vivas porque decompõe H_2O_2, em um intermediário que pode dar origem a ERO, prevenindo assim a formação de HOº e peroxidação de lipídeos (NOGUEIRA et al., 2004). É um composto não-tóxico, com baixo peso molecular e dotado de potente atividade mimética da GPx (IMAI et al., 2001; YAMAGUCHI et al., 1998) e, portanto, um excelente antioxidante, além de possuir ação anti-inflamatória, reduzindo um grande número de enzimas implicadas no processo inflamatório (FURSTENAU et al., 2004). Ainda, como o ebselen é uma molécula biodisponível que permeia a barreira hematoencefálica (IMAI et al., 2001), é possível que essa droga atue amplamente através de um efeito direto no sistema nervoso central.

Figura 7. Representação do desenvolvimento temporal do ebselen.



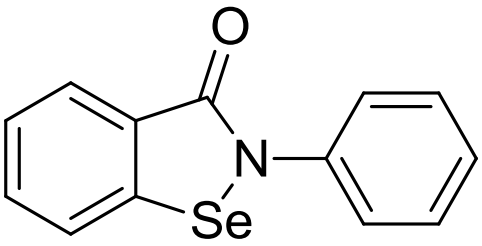
Fonte: Adaptado por PARNHAM, M. J. e SIES (2013). *Biochemical Pharmacology*.

Ainda, o ebselen é um composto que apresenta registro de patentes para o tratamento da diabetes (**Patent DE 4109508**), malária (**Patent DE 3821392**), doenças do pâncreas (**Patent JP 02/083321**), transtorno cerebral (**Patent JP 01/131113**), doenças cardíacas (**Patent JP 01/131114**), doenças hepáticas (**Patent JP 63/27431**) e estresse oxidativo (**Patent DE 3616923**) (KAUR D. et al., 2010). Assim, as evidências sobre a atividade neuroprotetora dessa molécula também são extensas na literatura, em modelos de isquemia cerebral (BORGES et al., 2005; PORCIUNCULA et al., 2001), Parkinson e DA (MARTINI et al., 2018; ROSSATO et al., 2002; SCHEWE, 1995). Em vista dessas características, o composto possui: capacidade de reduzir a toxicidade de outros xenobióticos; propriedades protetoras teciduais, principalmente contra o estresse oxidativo e o potencial anti-inflamatório com vários alvos moleculares, como inibição de lipoxigenases, NO sintase, NADPH oxidase, proteína quinase C entre outros (SCHEWE, 1995). Estudos *in vivo*, reforçam a ação antioxidante e a eficácia neuroprotetora do ebselen em vários modelos de AVC isquêmico (IMAI et al., 2001), evitando a peroxidação lipídica causada pela ativação do sistema glutamatérgico (NOGUEIRA et al., 2002; PORCIUNCULA et al., 2003). Além disso, o ebselen também tem sido administrado com sucesso a humanos, reduzindo os déficits neurológicos causados por aneurismas subaracnóideos hemorrágicos (SAITO et al., 1998; ZHAO e HOLMGREN, 2002).

Entre as drogas antioxidantes, o ebselen parece ser promissor devido à sua baixa toxicidade, o que pode ser explicado, devido ao fato de que o selênio presente em sua molécula não é liberado durante sua biotransformação e assim não é metabolizado pelo

organismo (SCHEWE, 1995). Além da baixa toxicidade, não há relatos de efeitos colaterais relacionados ao uso de ebselen, outra propriedade desta molécula é a estabilidade metabólica, o fato de seus metabólitos terem ação biológica, bem como a redução de peróxidos, a inibição de enzimas e a modulação de processos de transdução de sinais (MUGESH et al., 2001).

Figura 8. Estrutura química do ebselen C13H9NOSe



1.7.2. ESTUDOS CLÍNICOS E O REPOSICIONAMENTO DE DROGAS

Conhecido como um composto selenoorgânico multifuncional (NOGUEIRA et al., 2004), o ebselen há muitos anos encontra-se em evidência por preencher todas as tendências para se tornar uma nova molécula de uso clínico. No ano de 2016, cientistas utilizaram um banco de dados de medicamentos "failed", considerados clinicamente seguros, mas ineficazes para o uso proposto, e apontaram o ebselen como um composto que apresenta formidável benefício durante a fase de uso em modelo animal na pesquisa (NOGUCHI, 2016; NOSENGO, 2016). Onde foi capaz de reproduzir esses efeitos em ensaios clínicos em humanos, como uma possível alternativa para o tratamento do transtorno bipolar (MASAKI et al., 2016; SINGH et al., 2013) e perda auditiva induzida por ruído (MAHADEVAN et al., 2013; WANG, X. et al., 2014b). De fato, os efeitos neuroprotetores do ebselen foram reconhecidos e seus alvos multifatoriais (LUO et al., 2013; PARNHAM, M. J. e SIES, 2013) parecem ser uma vantagem para estratégias terapêuticas prospectivas.

NOSENGO (2016) cita "... esses esforços se inspiram em algumas histórias clássicas de sucesso. Um deles é o sildenafil um medicamento desenvolvido em 1989 para angina e agora comercializado como Viagra e usado para tratar a disfunção erétil. Outra é a azidotimidina, que falhou como droga quimioterápica, mas surgiu na década de 1980 como uma terapia para HIV...".

Sabe-se que em parte, o reposicionamento de drogas é resultado de avanços na tecnologia, onde, estes avanços incluem análises de dados que podem descobrir semelhanças moleculares entre doenças e modelos computacionais que podem prever quais compostos poderiam ser direcionados à outras patologias. Para isso conta-se com sistemas de triagem de alta precisão, no qual pode-se testar rapidamente medicamentos em diferentes linhas celulares e assim iniciar ou não o reposicionamento de uma droga (NOSENGO, 2016).

Outro fator relevante, os gastos e o tempo que a indústria farmacêutica dispõe para colocar um medicamento no mercado, atualmente, levam de 13 a 15 anos e estima-se entre US$ 2 a 3 bilhões em média. Segundo Bernard Munos, membro sênior do conselho do Centro Nacional de Ciências Translacionais Avançadas (CNCTA), medicamentos que foram aprovados e inexplorados ou então, outros milhares que pararam nos testes clínicos, muitos deles, como o ebselen, não necessitam ser submetidos aos testes de fase I, pois apresentam baixos riscos de produzir efeitos colaterais, reduzindo assim os custos de desenvolvimento em comparação com compostos completamente novos. No entanto, segundo Tudor Oprea, pesquisador da Universidade do Novo México, em Albuquerque, esta conduta poderia ser universal, por exemplo, se a dose e o modo de administração de uma droga permanecer semelhantes, a previamente testada, caso contrário, a droga teria que passar, novamente, pela fase I de testes.

Mais recentemente, JAROMIN (2018) desenvolveu um estudo com o ebselen em um sistema de nanoencapsulamento, uma vez que este composto apresenta baixa solubilidade e compostos com essa características requerem um sistema mais eficiente de aplicação. Diante disso, obteve-se neste estudo, uma resposta positiva das nanocápsulas de ebselen contra leveduras de *Candida tropicalis, Candida albicans e Candida parapsilosi,* além disso, o composto foi capaz de suprimir a oxidação lipídica e demonstrou toxicidade mínima em uma linhagem celular. Desta forma, as nanocápsulas de ebselen representam uma alternativa promissora, segura e complementar ao tratamento da candidíase cutânea (JAROMIN, 2018).

1.8. EXERCÍCIO FÍSICO

1.8.1. EFEITO NEUROPROTETOR DO EXERCÍCIO FÍSICO

A prática de exercício físico regular proporciona diversos benefícios, e um deles está associado a aspectos cognitivos, em idosos. Um recente estudo de metanálise sobre os fatores de risco para a DA, em idosos, indicou o sedentarismo como um dos mais importantes entre os riscos modificáveis, correspondendo a 12,7 % de vulnerabilidade populacional em todo o mundo (NORTON et al., 2014). Desta forma, pesquisas acerca desta terapia não farmacológica são desenvolvidas em busca de mecanismos claros sobre de que forma o exercício físico pode prevenir e/ou desacelerar a progressão da demência em idosos (AHLSKOG et al., 2011; HAMER et al., 2010).

Com isso, diferentes modalidades e intensidades de exercícios tem sido estudadas e apresentam importantes contribuições, como por exemplo, um estudo com exercício de corrida voluntária proporcionou o aumento da sobrevivência de neurônios primários e da neurogênese hipocampal (EHNINGER e KEMPERMANN, 2003). Ainda, esta mesma modalidade de exercício, demonstrou aumentar a proliferação celular, a sobrevivência e a maturação de novas células na formação hipocampal (VAN PRAAG, 2008; VAN PRAAG et al., 1999). Essas mudanças neuroplásticas podem ter implicações relevantes no efeito do exercício em contribuir na aprendizagem e na memória, diminuindo os sintomas depressivos, que estão ligados a doenças cognitivas, como na DA (COTMAN e BERCHTOLD, 2007; HILLMAN et al., 2008).

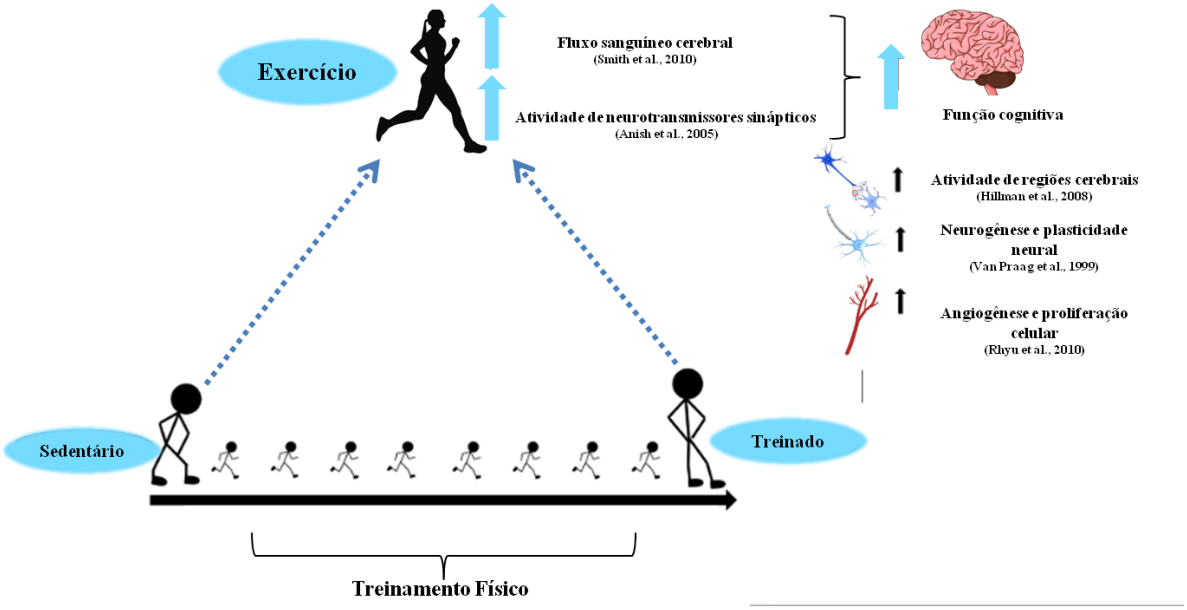
Outra mudança notável induzida pelo treinamento aeróbico, vista no cérebro humano adulto por ressonância magnética, foi o aumento do volume do hipocampo e a reversão do declínio cognitivo de 1 - 2% (ERICKSON et al., 2011). Desta forma, quando o envelhecimento vem acompanhado de doenças neurodegenerativas, que levam à depleção progressiva dos neurônios e à um quadro clínico de declínio cognitivo e perda de memória, o exercício poderia ser uma alternativa terapêutica não – farmacológica que auxiliaria na redução das taxas dos sintomas neuropsiquiátricos (KLEEMEYER et al., 2016).

A DA é um tipo de demência (BISHOP et al., 2010), e a perda neuronal do hipocampo pode ter uma participação importante na fisiopatologia da doença. Curiosamente, indivíduos que não eram dementes mas foram diagnosticados com a neuropatologia da DA, suas células-tronco neurais foram preservadas, quando em comparação com indivíduos com a DA e com a demência (BRILEY et al., 2016). Desta forma o exercício

físico pode ser proposto como uma intervenção de profilaxia para a manutenção da neuroplasticidade e cognição na DA (DUZEL et al., 2016).

Vários mecanismos biológicos têm sido implicados nos efeitos protetores do exercício físico, incluindo o aumento da neurogênese, a indução de angiogênese, o acréscimo da plasticidade sináptica, o aumento da síntese de fatores neurotróficos e de enzimas antioxidantes, além da redução da produção de espécies reativas (PAILLARD et al., 2015; VAYNMAN et al., 2004). Ainda, um aumento do condicionamento aeróbico contribui para o aumento de um melhor desempenho da memória espacial no hipocampo (ERICKSON et al., 2011) (**Figura 8**).

Figura 9. Representação dos Mecanismos fisiológicos do Exercício Físico sobre a cognição.



Fonte: Adaptado de Merege C.A.A et al., 2014. Revista Brasileira de Medicina e Esporte.

1.8.2. EXERCÍCIO DE FORÇA

Evidências aceitas e bem elucidadas sugerem que a neurogênese no adulto é importante para o funcionamento do hipocampo (BAPTISTA AND ANDRADE, 2018; AGER ET AL., 2015). Em roedores, o aprendizado e a memória aumentam a neurogênese, enquanto que fatores que prejudicam a memória, como estresse,

envelhecimento e estados patológicos estão associados a uma diminuição na neurogênese hipocampal (BAPTISTA AND ANDRADE, 2018; AGER ET AL., 2015).

É compreendido que, o exercício de força pode levar a um aumento significativo na proliferação celular, na neurogênese em camundongos e consequentemente, nos processos de memória e aprendizado (VAN PRAAG et al., 1999; VAN PRAAG et al., 2005). No entanto, o impacto do exercício de força nesse processo é pouco compreendido. NOVAES et al. (2014), mostrou pela primeira vez um aumento significativo na proliferação de células e uma diminuição importante em proteínas pró-apoptóticas no hipocampo, após um programa de exercícios de força progressiva em um grupo de mulheres. Em estudos anteriores a esse, o mesmo grupo de autores mostrou que o mesmo programa de exercícios melhorou o desempenho de ratos em diferentes tarefas de memória (CASSILHAS et al., 2012a; CASSILHAS et al., 2012b). Por exemplo, em um dos trabalhos, ratos submetidos a oito semanas de exercício de força passaram mais tempo no quadrante da plataforma na tarefa do Labirinto Aquático de Morris (CASSILHAS et al., 2012b) e apresentaram uma latência maior para ingressar no compartimento aversivo, em uma tarefa de esquiva inibitória comparado com os animais controles. Para reforçar a ideia de que este tipo de exercício pode proteger o cérebro, um estudo mostrou que quatro semanas de exercício de força reduziram a frequência de crises epilépticas em ratos, sugerindo um papel neuroprotetor (PEIXINHO-PENA et al., 2012).

Desse modo, com base na heterogeneidade da etiologia da DA, na necessidade de encontrar novas alternativas para a prevenção e/ou tratamento desta doença e nas propriedades farmacológicas apresentadas pelo composto ebselen e pelo exercício físico de força, este trabalho investigou o potencial uso destas estratégias em modelos de DA esporádica em camundongos.

2. OBJETIVOS

2.1. OBJETIVOS GERAIS

Considerando os aspectos mencionados anteriormente, o principal objetivo deste estudo foi investigar a ação neuroprotetora do composto ebselen e do exercício de força na fisiopatologia da doença de Alzheimer em modelos animais de DA esporádica.

2.2. OBJETIVOS ESPECÍFICOS

- Investigar o efeito de diferentes concentrações de ebselen na atividade das isoformas globulares G1 e G4 da AChE no córtex cerebral e no hipocampo de ratos *in vitro*;
- Investigar a ação neuroprotetora do ebselen nos modelos de DA esporádica induzidos pela escopolamina e por ETZ – i.c.v. em camundongos;
- Avaliar o efeito neuroprotetor do exercício de força, como forma de tratamento não-farmacológico, em camundongos induzidos com ETZ - i.c.v;

3. DESENVOLVIMENTO

O desenvolvimento desta tese está apresentado sob a forma de dois artigos e um manuscrito científico. Os itens Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas encontram-se nos próprios artigos e manuscrito, os quais estão estruturados de acordo com as normas de cada revista onde foram publicados ou submetidos. Em anexo a esta tese encontram-se as autorizações das editoras para reprodução dos artigos científicos e as cartas de aprovação dos projetos de pesquisa pela Comissão de Ética no Uso de Animais (CEUA) da Universidade Federal de Santa Maria.

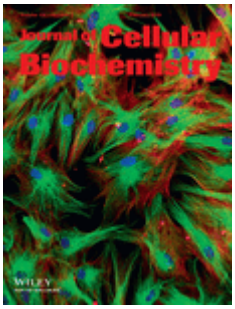
3.1. ARTIGO 1

EBSELEN INIBE A ATIVIDADE DA ISOFORMA GLOBULAR DA ACETILCOLINESTERASE G4 IN VITRO E ATENUA AMNÉSIA INDUZIDA POR ESCOPOLAMINA EM CAMUNDONGOS

EBSELEN INHIBITS THE ACTIVITY OF ACETYLCHOLINESTERASE GLOBULAR ISOFORM G4 IN VITRO AND ATTENUATES SCOPOLAMINE-INDUCED AMNESIA IN MICE

Franciele Martini, Ana Paula Pesarico, César Augusto Brüning, Gilson Zeni,

Cristina Wayne Nogueira



Received: 15 September 2017 | Accepted: 29 January 2018
DOI: 10.1002/jcb.26731

RESEARCH ARTICLE

WILEY Journal of Cellular Biochemistry

Ebselen inhibits the activity of acetylcholinesterase globular isoform G4 in vitro and attenuates scopolamine-induced amnesia in mice

Franciele Martini[1] | **Ana P. Pesarico**[1] | **César A. Brüning**[2] | **Gilson Zeni**[1] | **Cristina W. Nogueira**[1]

[1] Departamento de Bioquímica e Biologia Molecular, Laboratório de Síntese, Reatividade e Avaliação Farmacológica e Toxicológica de Organocalcogênios, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, Brazil

[2] Programa de Pós-Graduação em Bioquímica e Bioprospecção, Centro de Ciências Químicas, Farmacêuticas e de Alimentos (CCQFA), Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brazil

Correspondence
Cristina W. Nogueira, Departamento de Bioquímica e Biologia Molecular, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, CEP 97105–900, RS, Brazil.
Email: criswn@ufsm.br

Funding information
Conselho Nacional de Desenvolvimento Científico e Tecnológico, Grant number: 441405/2014-2; Fundação de Amparo à Pesquisa do Estado do RS (FAPERGS), Grant number: 17/2551-0000; CNPq fellowship, Grant number: 304864/2015-3; CAPES

There is a well-known relationship between the cholinergic system and learning, memory, and other common cognitive processes. The process for researching and developing new drugs has lead researchers to repurpose older ones. This study investigated the effects of ebselen on the activity of acethylcholinesterase (AChE) isoforms in vitro and in an amnesia model induced by scopolamine in Swiss mice. In vitro, ebselen at concentrations equal or higher than 10 μM inhibited the activity of cortical and hippocampal G4/AChE, but not G1/AChE isoform. Treatment of mice with ebselen (50 mg/kg, i.p.) was effective against impairment of spatial recognition memory in both Y-maze and novel object recognition tests induced by scopolamine (1 mg/kg, i.p.). Ebselen (50 mg/kg) inhibited hippocampal AChE activity in mice. The present study demonstrates that ebselen inhibited the G4/AChE isoform in vitro and elicited an anti-amnesic effect in a mouse model induced by scopolamine. These findings reveal ebselen as a potential compound in terms of opening up valid therapeutic avenues for the treatment of memory impairment diseases.

KEYWORDS
acetylcholinesterase, ebselen, memory, neuroprotective, organoselenium, selenium

1 | INTRODUCTION

Cognitive dysfunction is present in neurodegenerative diseases, associated with memory and cognitive impairments, such as Alzheimer's disease (AD), Parkinson's disease, Huntington's disease and multiple sclerosis.[1–3] Studies have shown that the observed pathophysiological changes are similar among the various neurodegenerative diseases at different levels, such as extracellular deposition of amyloid-beta (Aβ) peptide and intracellular deposition of neurofibrillary tangles, resulting in the loss of cholinergic neurons in different regions of the brain.[4,5]

The main alternative therapy for memory loss is to increase the functionality of the cholinergic neurotransmitter system by inhibiting acetylcholinesterase (AChE) activity, the enzyme responsible for the breakdown of acetylcholine (ACh).[6] It is reported in the scientific literature the vital role of the neurotransmitter AChE in central and peripheral

cognitive processes, including learning and memory.[7] For example, the release of ACh, decreased after the loss of cholinergic neurons, results in learning deficits.[6] Thus, the duration of the ACh action depends on the activity of AChE, which hydrolyzes ACh upon its release.[8] This is the therapeutic approach used to treating different types of dementia, AD, traumatic brain injury and delirium, and may be useful in the treatment of schizophrenia.[9] It is also known that AChE is present in the brain as two isoforms G1 and G4, differently distributed in the cell compartments. Whereas the G1 isoform is located in the cytosol, the G4 is found in the plasma membrane.[10] In the brain of mammals the isoform G4 represents 60-90% of the total AChE, depending on the cerebral region.[11] In this context, we wonder whether a selective AChE isoform inhibitor might provide a better cognitive enhancer drug.

The process for researching and developing new drugs has lead researchers to repurpose older ones.[12] This way, ebselen has been run for bipolar disorder[13,14] and type 2 diabetes clinical trials.[15,16] Such story is becoming more common: taking drugs that have been developed for one disorder and repositioning them to tackle another is an important strategy for researchers in industry and academia.[17] Experimentally, extensive work has been done to figure out pharmacological and toxicological properties of ebselen.[18,19] Regarding the effects on the AChE activity, ebselen is a reversible inhibitor of cerebral AChE activity in a mixed type in vitro[20] and in vivo.[21] The chemical structure of ebselen has been also used as a model for anti AD compounds by fusion of ebselen and donepezil, a potent AChE inhibitor.[22,23]

Considering ebselen properties and the importance of cholinergic system to cognitive processes, this study aimed to investigate the effects of ebselen on the activity of AChE isoforms in vitro and in an amnesia model induced by scopolamine in mice.

2 | EXPERIMENTAL PROCEDURE

2.1 | Animals

The in vitro experiments were carried out with male adult Wistar rats (150-200 g), whereas male adult Swiss mice (25-30 g) were used for the in vivo protocol. The animals were maintained at 22-25°C with free access to water and food, under a 12:12 h light/dark cycle with lights turned on at 7.00 a.m.

The present experimental study was approved by the Institutional Ethics Committee on Care and Use of Experimental Animal Resources from the Federal University of Santa Maria, Brazil and registered under the number #7372110915. The procedures in this study were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments.

2.2 | Drugs

Ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one) was prepared and characterized in our laboratory by the method previously described by Engman.[24] Analysis of the ^{1}H NMR and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of ebselen (99.9%) was determined by GC/MS. All other chemical reagents utilized were obtained from standard commercial suppliers.

Ebselen was dissolved in dimethyl sulfoxide (DMSO), for the in vitro experiments, and in 1:4:5 of DMSO, polyethylene glycol and distilled water for the in vivo protocol. Donepezil and scopolamine were dissolved in distilled water and saline, respectively.

2.3 | Experimental design

2.3.1 | In vitro experiments

Ebselen effects on the activity of globular isoforms G1 and G4 of AChE

Tissue preparation of AChE isoforms Rats were killed by decapitation and frontal cortex and hippocampus were quickly removed, placed on ice and homogenized. Tissues were immediately homogenized in cold 30 mmo/l sodium phosphate buffer, pH 7.0 and protease inhibitor, ethylene glycol-bis (β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) (10 mM), (1/10, weight/volume, w/v).

The homogenates were centrifuged at 100 000*g* at 4°C in a Beckman Ultracentrifuge (LE 80) using a fixed angle rotor (80 Ti) for 60 min. The supernatant was collected and stored at 4°C, which constituted the salt soluble (SS or G1) isoform. The pellet was re-suspended in 1% Triton X-100 (10% w/v in 30 mmol/l of sodium phosphate buffer, pH 7.0) and incubated at 4°C for 60 min. These samples were then centrifuged at 100 000*g* at 4°C for 60 min. The supernatant was collected and stored at 4°C, which is the detergent soluble (DS or G4) isoform. This double-extraction method recovered 80-90% of the total AChE activity.[25] The extraction of G1 and G4 isoforms was further confirmed using gel filtration chromatography purification.[26]

Enzyme assay The AChE activity was performed in G1 and G4 isoforms by the method of Ellman's[27] and Das[28] with minor modifications such as the final concentration (1 mmol/l) of substrate, acetylthiocholine iodide and chromophore, 5′,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) (1 mM), which results in a better kinetic profile. The enzyme sample (0.5 μg of protein) was pre-incubated for 2 min at 25°C in the presence of 3, 10, 30, and 50 μM of ebselen and 39, 78, 156, 312, 625, and

1.250 nM of donepezil as a positive control. The enzymatic reaction was initiated by adding the substrate acetylthiocholine iodide (0.8 mM). The enzyme activity was determined spectrophotometrically (Shimadzu, Kyoto, Japan) at 412 nm at an interval of 30 s for 2 min. The assay was run in duplicate for each sample and each experiment was performed three times. The AChE specific activity was expressed in µmoles/min/mg of protein.

2.3.2 | In vivo experiments

The experimental design of this study is depicted in Figure 1. Ebselen or donepezil (positive control) was administered by the intraperitoneal (i.p.) route once a day for 5 days to mice. Scopolamine (i.p.) was administered to mice once a day from the third to the fifth day (Figure 1).[29] Ebselen (50 mg/kg), donepezil (5 mg/kg), and scopolamine (1 mg/kg) doses were chosen based on our previous study[30] and studies by other authors.[31–33] All drugs were administered to mice in a constant volume of 10 ml/kg. Appropriate vehicle-treated groups were also assessed simultaneously.

The animals were randomly assigned in five different groups as following: Group I ($n = 9$): Vehicle (1:4:5 of DMSO, polyethylene glycol and distilled water) + vehicle (saline); Group II ($n = 8$): Ebselen (50 mg/kg) + vehicle (saline), Group III ($n = 9$): Vehicle (1:4:5 of DMSO, polyethylene glycol and distilled water) + scopolamine (1 mg/kg), Group IV ($n = 9$): Ebselen + scopolamine, Group V ($n = 8$): Donepezil (5 mg/kg) + scopolamine.

2.3.3 | Behavioral tests

The locomotor, Y-Maze, and object recognition tests (ORT) were carried out at days 3, 4, and 5 (Figure 1). The number of mice assigned to each experimental group was 8-9, and the same mice performed all behavioral tests. The mouse behavior was recorded by an observer who was blind to the treatment condition to which the animal was exposed.

Object recognition test (ORT)

The object recognition test was performed according to Rosa[34] with some modifications. The behavioral test was performed in a 45 × 45 cm open field surrounded by 30 cm height walls, made of brown plywood. All animals were given a habituation session where they were left to freely exploring the open field for 5 min. No object was placed in the box during the habituation trial. Subsequently, four objects were used: A_1, A_2, B, and C. The "A" objects were two identical triangles, the "B" object was a ball and the "C" object was a rectangle. All objects were made of plastic material, with

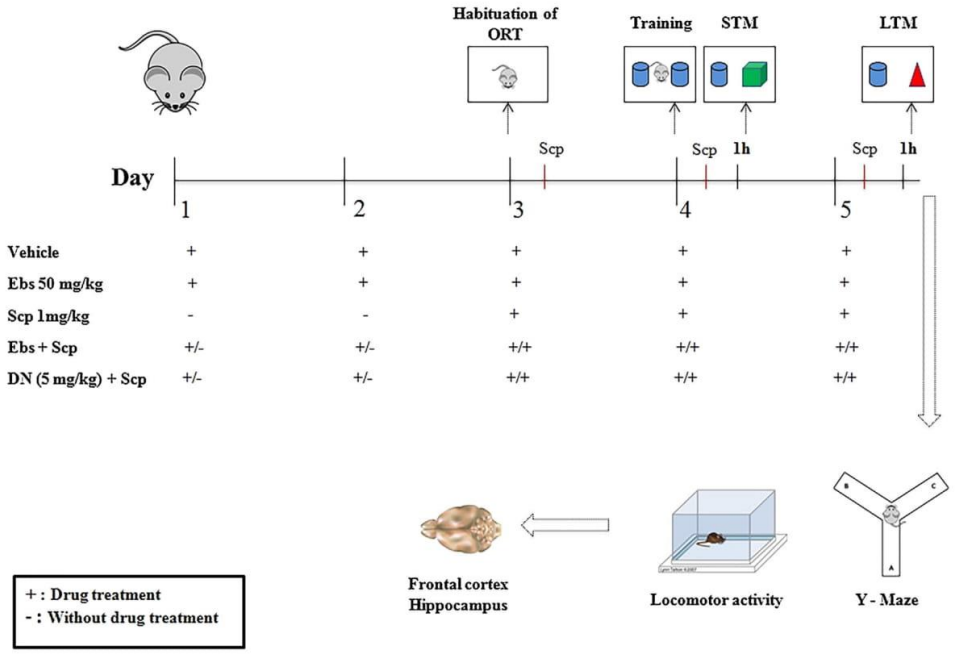


FIGURE 1 Schematic representation of the experimental design. Scopolamine (Scp), Ebselen (Ebs), Donepezil (DN), object recognition test (ORT), short-term memory (STM), long-term memory (LTM)

$10 \times 10\ cm^2$ (length × height). Each object had the pattern of color, as follows: blue, red, and yellow. Twenty-four hour after habituation, training was conducted by placing each individual mouse for 5 min into the field, in which two identical objects (objects A_1 and A_2) were positioned in two adjacent corners, 10 cm from the walls. In a short-term memory (STM), test performed 1.5 h after training; the mouse explored the open field for 5 min in the presence of one familiar (A) and one novel (B) object. All objects presented similar textures, colors, and sizes, but distinctive shapes. The percentage of the total exploration time that the animal spent investigating the novel object was the measure of recognition memory. Between trials the objects were washed with 10% ethanol solution. In a long-term memory (LTM) test, 24 h after training, the same mouse explored the field for 5 min in the presence of a familiar object A and a novel object C. Recognition memory was evaluated in a similar way to that of STM test. Exploration was defined as sniffing or touching the object with the nose and/or forepaws. Data are expressed as the mean ± SEM percentage. Exploratory preference in: training = $(A_2/(A_1 + A_2)) \times 100$; STM = $(B/(A_1 + B)) \times 100$; LTM = $(C/(A_1 + C)) \times 100$.

Y-maze test

The Y-maze is three-arm maze with equal angles between all arms, which were 30 cm long and 5 cm wide with 12 cm high walls. The maze floor and walls were constructed from dark gray, polyvinyl plastic. Mice were initially placed within one arm, and the sequence and number of arm entries were recorded manually for each mouse over an 8 min period. The percentage of trials in which the mouse entered all three arms (ABC, CAB, or BCA but not ABB) was recorded as an alternation to estimate short-term memory. One hour before each test, the mice were given ebselen (50 mg/kg). After 30 min, mice received scopolamine (1 mg/kg) (Figure 1). The Y-maze arms were cleaned with diluted 10% ethanol between tests to remove odors and residues. The alternation score (%) for each mouse was defined as the ratio of the actual number of alternations to the possible number (defined as the total number of arm entries minus two) multiplied by 100 as shown by the following equation: % Alternation = [(Number of alternations)/(Total arm entries − 2)] × 100. The number of arm entries per trial was used as an indicator of locomotor activity.[35]

Spontaneous locomotor test

With the purpose of excluding sedative or motor abnormality the mouse performed spontaneous locomotor test. The animals were exposed to the chamber before testing, and activity was monitored under light and sound-attenuated conditions. Testing took place in a clear acrylic chamber (500 × 480 × 500 mm) equipped with 16 infrared sensors for the automatic recording of horizontal activity (Model EP149, Insight Instruments Ltda, Sao Paulo, Brazil). Each animal initially was placed in the center of the testing chamber and allowed to freely move while being tracked by an automated tracking system. The data (distance, velocity, and crossings) were collected and recorded during 4 min.

2.3.4 | Ex vivo analyses

Tissue preparation of total AChE activity

At the end of behavioral tests, mice were killed by decapitation and the frontal cortex and hippocampus were quickly removed, placed on ice and homogenized 1:20 (w/v) in Medium I (0.32 M sucrose, 5.0 mM HEPES, 0.1 mM ethylenediamine tetraacetic acid disodium salt) buffer. The homogenates were centrifuged at 2400*g* at 4°C for 15 min to yield the low-speed supernatant (S1) fraction that was used in the total AChE assay.

Tissue preparation of AChE isoforms

The tissue preparation of G1 and G4 isoforms was performed as previously described in in vitro experiments.

G1 and G4 isoforms and total AChE activity assays

G1 and G4 isoforms and total AChE activity assays were performed as previously described in in vitro experiments.

Protein quantification

Protein concentration was measured by the method of[36] using bovine serum albumin as the standard.

2.4 | Statistical analysis

All results are given as the mean ± S.E.M. Statistical analyses were performed by using one-way or two-way ANOVA followed by Newman-Keuls to post hoc comparisons. The IC50 was calculated by nonlinear regression analysis (GraphPad Software, San Diego, CA). Correlation analyses were performed using Pearson's correlation coefficient (r) followed by the Gaussian distribution. All analyses were performed by using the STATISTICA (StatSoft, Tulsa, OK). Probability values less than 0.05 ($P < 0.05$) were considered as statistically significant.

3 | RESULTS

3.1 | Ebselen and donepezil effects on the AChE activity in G1 and G4 isoforms in vitro

The one-way ANOVA for G4 isoform of AChE activity revealed a significant inhibitory effect of ebselen at concentrations of 30 and 50 μM in the frontal cortex [$F_{(4,26)} = 29.65$, $P < 0.0001$] (Figure 2A) and at concentrations equal or higher than 10 μM in the hippocampus of rats

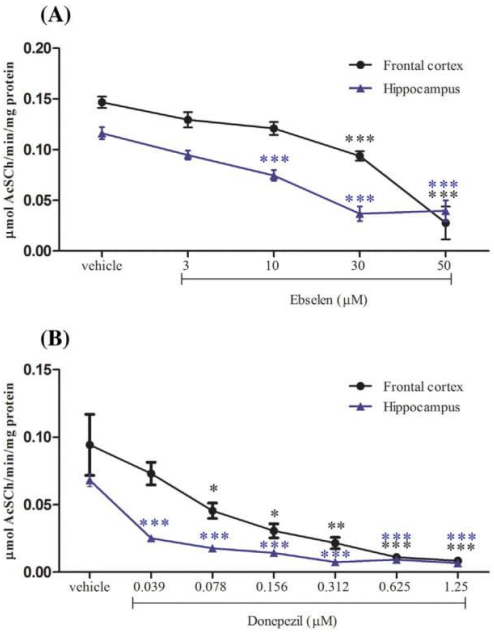


FIGURE 2 Effect of ebselen and donepezil on the AChE G4 isoform activity in the hippocampus and frontal cortex in vitro: ebselen (A) and donepezil (B). Values are expressed as mean ± S.E.M. of four independent experiments, performing in different days, using different animals. AChE activity was expressed as µmol acetyltiocholine/min/mg protein. Asterisks denote the significance levels when compared to the respective vehicle group: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ (one-way ANOVA/Newman-Keuls). Ebs, ebselen; DN, donepezil; AcSCh, acetyltiocholine

$[F_{(4,27)} = 27.88, P < 0.0001]$ (Figure 2A). The IC_{50} and Imax values for AChE/G4 activity of ebselen were: IC_{50} 33.14 (28.93-37.97) µM and Imax of 92 ± 3% in the frontal cortex and IC_{50} 17.11 (13.09-22.37) µM and Imax of 69 ± 11% in the hippocampus of rats.

By contrast, the one-way ANOVA of AChE/G1 activity did not reveal a significant effect of ebselen in the frontal cortex $[F_{(4,25)} = 0.31, P > 0.05]$ (Supplementary Figure S1A) and hippocampus of rats $[F_{(4,31)} = 1.09, P > 0.05]$ (Figure Supplementary S1B).

The one-way ANOVA for G4 isoform of AChE activity revealed a significant inhibitory effect of donepezil at concentrations of 78-1250 nM in the frontal cortex $[F_{(6,30)} = 6.40, P < 0.001]$ (Figure 2B) and at concentrations equal or higher than 39 nM in the hippocampus of rats $[F_{(6,28)} = 95.65, P < 0.0001]$ (Figure 2B). The IC_{50} values for AChE/G4 activity of donepezil were: IC_{50} 53.97 (32.53-89.54) nM in the frontal cortex and IC_{50} 18.73 (9.47-37.00) nM in the hippocampus of rats.

However, one-way ANOVA of AChE/G1 activity did not show a significant effect of donepezil in the frontal cortex $[F_{(6,27)} = 1.75, P > 0.05]$ (Figure Supplementary S2A) and hippocampus of rats $[F_{(6,27)} = 0.70, P > 0.05]$ (Figure Supplementary S2B).

3.2 | Ebselen effect on scopolamine-induced memory impairment of spatial recognition memory in the Y-maze

Figure 3 shows the effect of ebselen treatment on the performance of mice in the Y-maze. The two-way ANOVA of alternations (%) revealed a significant ebselen *x* scopolamine interaction $[F_{(1,28)} = 8.25 \; P < 0.01]$. Post hoc analysis showed that scopolamine decreased the number of correct alternations when compared with those of the vehicle group and treatment of mice with ebselen increased this parameter

Student *t*-test showed that donepezil increased the alternations in mice treated with scopolamine in comparison with those of the scopolamine/vehicle mice ($P < 0.001$).

The scopolamine administration also resulted in an increased number of arm entries compared to those of the control group (Table 1) $[F_{(1,24)} = 8.16 \; P < 0.01]$.

3.3 | Ebselen effect on scopolamine-induced memory impairment in the object recognition test in mice

Figure 4 shows the effect of ebselen treatment on the performance of mice in the object recognition test. There were no significant differences among groups in the time spent exploring any of the two identical objects during training $[F_{(1,28)} = 0.32 \; P > 0.05)$ (Figure 4A). The two-way

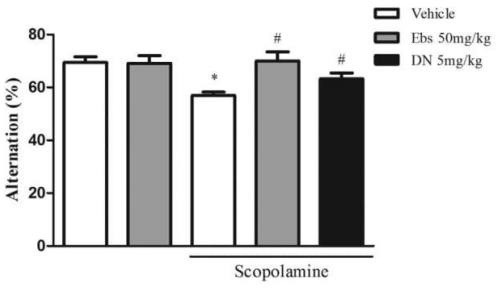


FIGURE 3 Effect of ebselen on scopolamine-induced memory impairment in the Y-Maze test in mice. Values are expressed as mean ± S.E.M. of 9-10 animals. Asterisk denotes the significance levels when compared to the respective vehicle group: $*P < 0.05$. Hashtag denotes the significance levels when compared to the Scopolamine group: $^{\#}P < 0.05$ (two-way ANOVA followed by the Newman Keuls and the Student's *t*-test for donepezil)

TABLE 1 Effects of ebselen on the number of arm entries in the scopolamine-treated mice

Groups	Number of arm entries
Control	31.0 ± 1.5
Ebs	23.8 ± 2.2
Scp	38.2 ± 1.9^{a}
Ebs + Scp	34.6 ± 3.6
DN + Scp	44.1 ± 4.0^{b}

Values are expressed as mean ± S.E.M. of 8-9 animals.
[a]Denotes the significance levels when compared to the respective vehicle group, $P < 0.05$ (two-way ANOVA followed by the Newman Keuls).
[b]Denotes the significance levels when compared to the scopolamine group, $P < 0.05$ the Student's *t*-test. Ebs, ebselen; Scp, scopolamine; DN, donepezil.

ANOVA revealed significant ebselen *x* scopolamine interactions in the STM $[F_{(1,26)} = 9.35 \quad P < 0.01]$ (Figure 4B) and in the LTM $[F_{(1,27)} = 9.30 \quad P < 0.01]$ (Figure 4C). Pos hoc analysis showed that scopolamine decreased the exploratory preference (% time spent exploring a novel object) in the STM and LTM tests when compared with that of the vehicle group and treatment of mice with ebselen increased these parameters.

The Student *t*-test revealed that donepezil increased the exploratory preference in STM ($P < 0.001$; Figure 4B) and LTM ($P < 0.001$; Figure 4C) in scopolamine-treated mice in comparison with that of the scopolamine/vehicle mice.

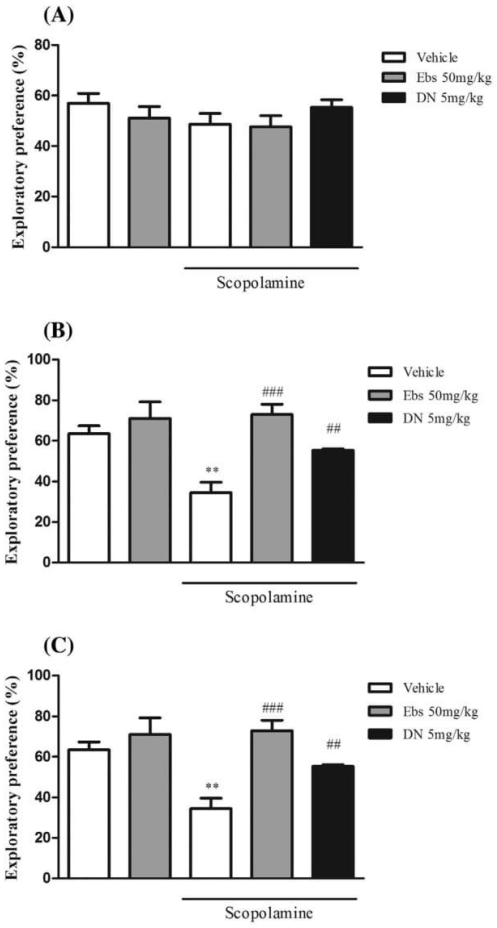


FIGURE 4 Effect of ebselen on scopolamine-induced memory impairment in the mouse object recognition test. Training (percentage of time spent exploring any of two identical objects) (A), STM (percentage of time spent exploring the novel object, test carried out 1 h after training) (B), and LTM (percentage of time spent exploring the novel object, test carried out 24 h after training) (C). Values are expressed as mean ± S.E.M. of 9-10 animals. Asterisks denote the significance levels when compared to the respective vehicle group: $^{**}P < 0.01$. Hashtags denote the significance levels when compared to the scopolamine group: $^{###}P < 0.001$ (two-way ANOVA followed by the Newman Keuls and the Student's *t*-test for donepezil)

3.4 | Ebselen effect on spontaneous locomotor activity of mice treated with scopolamine

Table 2 shows the effect of ebselen, donepezil and scopolamine treatments on the performance of mice in the spontaneous locomotor test. The two-way ANOVA of locomotor activity data revealed that scopolamine increased total distance traveled $[F_{(1,28)} = 10.53, P < 0.01]$ and velocity $[F_{(1,14)} = 4.49, P = 0.05]$ of mice.

The Student *t*-test demonstrated that donepezil did not alter distance ($P > 0.05$), velocity ($P > 0.05$), and crossing ($P > 0.05$) in mice treated with scopolamine in comparison with that of the scopolamine/vehicle mice (Table 2).

3.5 | Ebselen effects on the AChE activity in the frontal cortex and hippocampus of mice treated with scopolamine

Figure 5 shows the effects of ebselen on the AChE activity in the frontal cortex and hippocampus of mice. The two-way ANOVA of the AChE activity revealed a significant ebselen *x* scopolamine interaction $[F_{(1,23)} = 12.78, \ P < 0.01)]$ in the hippocampus (Figure 5B), but not in the frontal cortex $[F_{(1,24)} = 0.43, \ P > 0.05)]$ (Figure 5A). Post hoc analysis showed that scopolamine increased the AChE activity when compared with the vehicle group and treatment of mice with ebselen decreased this parameter.

The Student *t*-test revealed that donepezil inhibited the AChE activity in scopolamine-treated mice when compared to the scopolamine/vehicle mice ($P < 0.01$).

TABLE 2 Effects of ebselen on spontaneous locomotor activity in the scopolamine-treated mice

Groups	Number of crossings	Distance (mm)	Velocity (mm/s)
Control	563.3 ± 68.09	8600 ± 157	39.6 ± 9.1
Ebs	410.4 ± 46.10	6727 ± 105	32.4 ± 5.8
Scp	668.8 ± 38.45	13 140 ± 883[a]	66.5 ± 9.6[a]
Ebs + Scp	511.0 ± 57.08	10 020 ± 119	41.2 ± 6.1
DN + Scp	688.3 ± 65.42	13 620 ± 1280	67.8 ± 6.0

Values are expressed as mean ± S.E.M. of 8-9 animals.
[a]Denotes the significance levels when compared to the respective vehicle group, $P < 0.05$ (two-way ANOVA followed by the Newman Keuls). Ebs, ebselen; Scp, scopolamine; DN, donepezil.

3.6 | Ebselen effects on activities of AChE G1 and G4 isoforms in the frontal cortex and hippocampus of mice treated with scopolamine

Table 3 shows the effect of ebselen on the activities of AChE G1 and G4 isoforms. The two-way ANOVA revealed a significant main effect of ebselen [$F_{(1,20)} = 7.14$, $P = 0.014$] in AChE/G4 activity in hippocampus. Scopolamine increased the hippocampal AChE/G4 activity when compared with that of ebselen/vehicle-treated mice.

The two-way ANOVA of frontal cortical G4 [$F_{(1,24)} = 1.63$ $P > 0.05$] and G1 [$F_{(1,24)} = 0.55$ $P > 0.05$] isoform activities and of hippocampal G1 isoform activity [$F_{(1,24)} = 0.88$ $P > 0.05$] did not reveal a significant ebselen *x* scopolamine interaction.

The Student *t*-test revealed that donepezil did not alter the activities of both AChE isoforms in mice treated with scopolamine when compared to those of the scopolamine/vehicle-treated mice ($P > 0.05$, Table 3).

Pearson's correlation analyses of the scopolamine-treated groups data revealed a significant negative correlation between the hippocampal AChE activity and behavioral performance in the Y-maze ($r = 0.58$, $P = 0.00631$) (Figure 6A), STM ($r = 0.74$, $P = 0.00016$) (Figure 6B) and LTM ($r = 0.46$, $P = 0.040$) (Figure 6C).

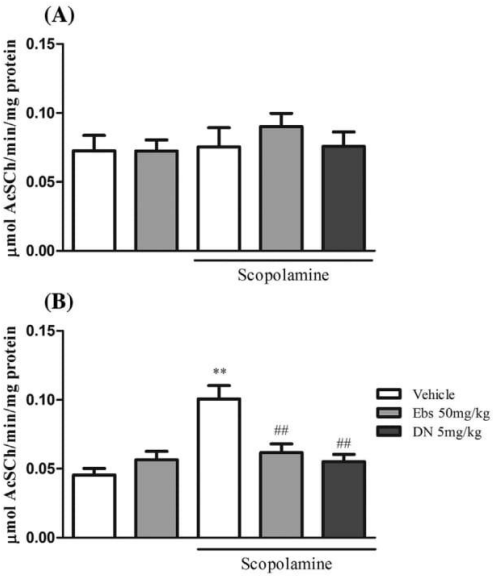


FIGURE 5 Effect of ebselen on the AChE activity in the frontal cortex (A) and hippocampus (B) of mice. Values are expressed as mean ± S.E.M. of 8-9 animals. Asterisks denote the significance levels when compared to the respective vehicle group: $^{**}P < 0.01$. Hashtags denote the significance levels when compared to the Scopolamine group: $^{\#\#}P < 0.01$ (two-way ANOVA followed by the Newman Keuls and the Student's *t*-test for donepezil)

4 | DISCUSSION

The present study demonstrates that ebselen protected against memory impairment induced by scopolamine, characterized in the Y-maze and novel object recognition tests, and inhibited hippocampal AChE activity in mice. In addition, ebselen inhibited the activity of AChE/G4 isoform in the frontal cortex and hippocampus of rats in vitro.

AChE inhibitors offer an evidence-based approach to delay the symptomatic cognitive decline in different neurodegenerative conditions. AChE inhibitors prevent the hydrolysis of ACh and have demonstrated efficacy, first in clinical trials and more in clinical practice.[37] Because cholinergic system plays a critical role in the learning and memory functions[31] the cholinergic deficit is one of the major neuropathological characteristics associated with memory loss and is intimately correlated with the progression of cognitive impairment.[38]

The in vitro results indicate that ebselen, similar to donepezil, inhibited cortical, and hippocampal AChE/G4 activity. Accordingly, AChE inhibitors used in the clinical treatment of AD are selective to G4 isoform that is relatively more related to cognition than G1 isoform.[28]

The G4 isoform of AChE is the major form for metabolizing ACh and is selectively increased in AD.[39] This information suggests that G4 isoform is the physiologically relevant form of AChE at cholinergic synapses, and its inhibition would be expected to prolong the action of ACh. By contrast, the AChE/G1 isoform occurs primarily in the neural cytoplasm, where its inhibition would be unlikely to affect synaptic physiology.[39] In spite of extensive studies on various biochemical aspects of AChE, information on its molecular isoforms and their involvement in learning and memory

TABLE 3 Effects of ebselen on G1 and G4 isoforms of AChE activity in the scopolamine-treated mice

AChE activity (μmoles/min/mg protein)				
	AChE-G4		AChE-G1	
	Frontal cortex	Hippocampus	Frontal cortex	Hippocampus
Control	0.112 ± 0.008	0.143 ± 0.021	0.042 ± 0.005	0.017 ± 0.002
Ebs	0.097 ± 0.009	0.090 ± 0.013[a]	0.031 ± 0.003	0.017 ± 0.002
Scp	0.087 ± 0.016	0.169 ± 0.015[b]	0.042 ± 0.004	0.019 ± 0.001
Ebs + Scp	0.100 ± 0.009	0.141 ± 0.008	0.039 ± 0.004	0.020 ± 0.003
DN + Scp	0.090 ± 0.020	0.133 ± 0.020	0.041 ± 0.001	0.027 ± 0.005

The AChE activity values are expressed as mean ± S.E.M. of 7-8 animals.

[a]Denotes the significance levels when compared to the respective vehicle group, $P < 0.05$ (two-way ANOVA followed by the Newman Keuls).

[b]Denotes the significance levels when compared to the ebselen group, $P < 0.05$ (two-way ANOVA followed by the Newman Keuls and the Student's *t*-test for donepezil).

Ebs, ebselen; DN, donepezil.

functions are quite sparse. The pattern of AChE activity in rat brain areas differs dependent on the sex and age.[10] The loss of the major G4 isoform correlates with the overall loss of AChE activity observed by histochemical staining and is mostly associated with axonal fibers in the cortex.[40] Thus, the loss of G4 may be a consequence of the cellular and axonal degeneration known to occur in the cortex.[41]

In the present study, we used two well-established memory tests, Y-maze and object recognition,[42] to evaluate the effect of ebselen on the memory impairment induced by scopolamine in mice. Scopolamine, an antagonist of cholinergic muscarinic receptors, is effective to induce ACh depletion in the hippocampus and frontal cortex, resulting in the increase of AChE activity and cognitive impairment in the short- and long-term memory in rodents.[43] It is known that scopolamine increases the locomotor activity of animals when administered at systemic doses above 0.056 mg/kg.[44] The findings of the present study demonstrate that ebselen protected against hyperlocomotion induced by scopolamine in mice, suggesting a neuroprotective effect of this compound.

Despite the differences between other[45] and ours experimental protocol such as, the route of administration, dose, species, and memory paradigm, recently Xie et al[45] reported that administration of ebselen in drinking water, for 6 months, improved cognitive impairment in the spatial learning, reduced the level of amyloid-β, inhibited tau hyperphosphorilation, and ameliorated oxidative stress.

In the present study, the administration of ebselen to mice protected against the increase in the hippocampal total AChE activity induced by scopolamine. This result reinforces those previously demonstrated by us, in which ebselen behaviors as a mixed and reversible inhibitor of AChE in vitro.[20] The fact that ebselen inhibited the hippocampal AChE/G4 in vitro but did not alter the activity of this enzyme in the ex vivo protocol could be explained by the time-consuming process with multiple washes and centrifugations, the method used to obtain the isoforms, and the kinetic type of inhibition demonstrated by ebselen in the in vitro experiments.[20] In other words, the process of isoform preparation could wash out this compound and, consequently, its inhibitory effect on G4 isoform of AChE in mice because ebselen is a reversible inhibitor of AChE. Mention should also be made of the donepezil behavior in the G4 isoform of AChE that was similar to that of demonstrated by ebselen either in vitro or ex vivo.

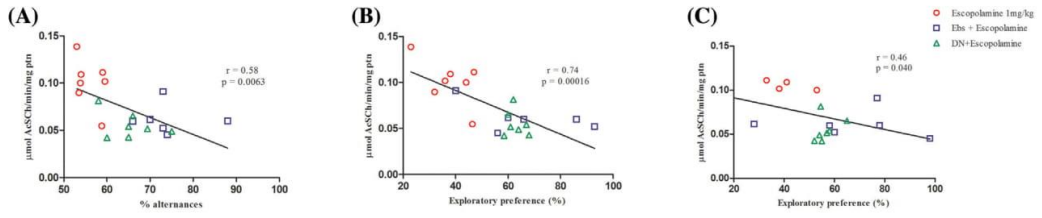


FIGURE 6 Correlation analysis between hippocampal AChE activity and % alternance (Y-maze) (A), hippocampal AChE activity and short-term memory (STM) (B), and hippocampal AChE activity and long-term memory (LTM) (C). Statistical analysis revealed the *r* and *P*-values of 0.58 ($P = 0.0063$) (A); 0.74 ($P = 0.00016$) (B); and 0.46 ($P = 0.040$) (C)

AChE inhibitors (donepezil, galanthamine, and rivastigmine) can cause cognitive improvement, which depends on their level of inhibition of the AChE activity.[46] However, there are many limitations on the use of AChEI, such as cholinergic side effects, hepatotoxicity, or poor bioavailability.[47] Different from this, ebselen is bioavailable, blood-brain barrier permeant and safe based on cellular toxicity[17,48,49] and a large body of evidence has confirmed that ebselen exhibits neuroprotection on preclinical studies.[50] Because ebselen has anti-inflammatory and neuroprotective effects, and inhibits AChE activity and tau phosphorylation, it appears as a potential therapeutic agent for the treatment of diseases in which cognitive function is present or, alternatively, ebselen could be used in drug combination. The combination of two or three drugs has been used to treat patients with AD[51] and other neurodegenerative diseases,[52] this because the pathological processes involve different and inter-related aspects, neurotoxicity, oxidative stress, neuro-inflammation, apoptosis, and cholinergic dysfunction.[52] In this study the effects of ebselen were similar to those of donepezil, the first-line treatment for Alzheimer's and non Alzheimer's dementia. Thus we hypothesize whether ebselen could be, in the future, used as an add-on therapy to donepezilbecause pharmacological strategies based on drug combination could be the only efficient way to treat some diseases.[53]

As a study design limitation we acknowledge the lack of mechanistic insight with regards to the cellular and molecular basis for ebselen beneficial effects on memory. As it is beyond the scope of this study it will be a subject of further studies.

Moreover, the molecular effects of ebselen on muscarinic receptors have been not investigated and deserve further investigation. In fact, the regulation of cognition in short-term memory, which is particularly impaired in AD, occurs within the hippocampus where post-synaptic muscarinic receptors M1 (M1 mAChR) are predominant and play an important role in memory and learning.[54] Based on the beneficial effects of ebselen on memory we wonder whether ebselen acts directly inhibiting the activity of AChE/G4 isoform or indirectly affecting cholinergic receptors. In this context, the contribution of hippocampal AChE/G4 isoform in learning and memory processing in the passive avoidance task[28] and the induction of hippocampal long-term potentiation[55] has been documented.

Furthermore, because we use a chemical model to induce memory impairment, we cannot rule out the possibility that ebselen directly blocks the scopolamine effects or that scopolamine indirectly affects other neurotransmitter systems and that the effects of ebselen on the globular isoforms of AChE may be an indirect consequence.

In conclusion, the present study demonstrates that ebselen is an inhibitor of the AChE/G4 in vitro and elicits an anti-amnesic effect in a scopolamine mouse model. The present findings provide ebselen as a potential compound in terms of opening up valid therapeutic avenues for treating memory impairment. Besides, a new synergic mode of action could be proposed that may boost the therapeutic efficacy of drug.

ACKNOWLEDGMENTS

This work was supported by the Fundação de Amparo à Pesquisa do Estado do RS (FAPERGS) [grant number 17/2551-0000]. Cristina W. Nogueira is recipient of CNPq fellowship (304864/2015-3). Franciele Martini and Ana Paula Pesarico are recipients of research scholarship from CAPES.

CONFLICTS OF INTEREST

The authors declare they have no conflicts of interest to disclose.

COMPLIANCE WITH ETHICAL STANDARDS

The experimental procedures were approved by the Institutional Animal Care and Use Committee of the Federal University of Santa Maria, Brazil and met the requirements of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

ORCID

Cristina W. Nogueira http://orcid.org/0000-0003-2950-3632

REFERENCES

1. Goldman JS, Hahn SE, Catania JW, et al. Genetic counseling and testing for Alzheimer disease: joint practice guidelines of the American College of Medical Genetics and the National Society of Genetic Counselors. *Genet Med*. 2011;13:597–605.
2. Paulsen JS. Cognitive impairment in Huntington disease: diagnosis and treatment. *Curr Neurol Neurosci Rep*. 2011;11:474–483.
3. Schoonheim MM, Popescu V, Rueda Lopes FC, et al. Subcortical atrophy and cognition: sex effects in multiple sclerosis. *Neurology*. 2012;79:1754–1761.
4. Aguzzi A, O'Connor T. Protein aggregation diseases: pathogenicity and therapeutic perspectives. *Nat Rev Drug Discov*. 2010;9:237–248.
5. Husain M, Mehta MA. Cognitive enhancement by drugs in health and disease. *Trends Cogn Sci*. 2011;15:28–36.
6. Rijpma A, Meulenbroek O, Olde Rikkert MG. Cholinesterase inhibitors and add-on nutritional supplements in Alzheimer's disease: a systematic review of randomized controlled trials. *Ageing Res Rev*. 2014;16:105–112.
7. Ballard C, Morris C, Kalaria R, McKeith I, Perry R, Perry E. The k variant of the butyrylcholinesterase gene is associated with reduced phosphorylation of tau in dementia patients. *Dement Geriatr Cogn Disord*. 2005;19:357–360.

8. Eichenbaum H. How does the brain organize memories? *Science*. 1997;277:330–332.
9. Giacobini E. Cholinesterase inhibitors: new roles and therapeutic alternatives. *Pharmacol Res*. 2004;50:433–440.
10. Das A, Dikshit M, Nath C. Profile of acetylcholinesterase in brain areas of male and female rats of adult and old age. *Life Sci*. 2001;68:1545–1555.
11. Descarries L, Aznavour N, Hamel E. The acetylcholine innervation of cerebral cortex: new data on its normal development and its fate in the hAPP(SW,IND) mouse model of Alzheimer's disease. *J Neural Transm (Vienna)*. 2005;112:149–162.
12. Heemskerk J, Tobin AJ, Ravina B. From chemical to drug: neurodegeneration drug screening and the ethics of clinical trials. *Nat Neurosci*. 2002;5:1027–1029.
13. Singh N, Halliday AC, Thomas JM, et al. A safe lithium mimetic for bipolar disorder. *Nat Commun*. 2013;4:1332.
14. Masaki C, Sharpley AL, Godlewska BR, et al. Effects of the potential lithium-mimetic, ebselen, on brain neurochemistry: a magnetic resonance spectroscopy study at 7 tesla. *Psychopharmacology (Berl)*. 2016;233:1097–1104.
15. Mahadevan J, Parazzoli S, Oseid E, et al. Ebselen treatment prevents islet apoptosis, maintains intranuclear Pdx-1 and MafA levels, and preserves beta-cell mass and function in ZDF rats. *Diabetes*. 2013;62:3582–3588.
16. Wang X, Yun JW, Lei XG. Glutathione peroxidase mimic ebselen improves glucose-stimulated insulin secretion in murine islets. *Antioxid Redox Signal*. 2014;20:191–203.
17. Nosengo N. Can you teach old drugs new tricks? *Nature*. 2016;534:314–316.
18. Huther AM, Zhang Y, Sauer A, Parnham MJ. Antimalarial properties of ebselen. *Parasitol Res*. 1989;75:353–360.
19. Nogueira CW, Zeni G, Rocha JB. Organoselenium and organotellurium compounds: toxicology and pharmacology. *Chem Rev*. 2004;104:6255–6285.
20. Martini F, Bruning CA, Soares SM, Nogueira CW, Zeni G. Inhibitory effect of ebselen on cerebral acetylcholinesterase activity in vitro: kinetics and reversibility of inhibition. *Curr Pharm Des*. 2015;21:920–924.
21. Mazzanti CM, Spanevello R, Ahmed M, et al. Pre-treatment with ebselen and vitamin E modulate acetylcholinesterase activity: interaction with demyelinating agents. *Int J Dev Neurosci*. 2009;27:73–80.
22. Luo Z, Sheng J, Sun Y, et al. Synthesis and evaluation of multi-target-directed ligands against Alzheimer's disease based on the fusion of donepezil and ebselen. *J Med Chem*. 2013;56:9089–9099.
23. Luo Z, Liang L, Sheng J, et al. Synthesis and biological evaluation of a new series of ebselen derivatives as glutathione peroxidase (GPx) mimics and cholinesterase inhibitors against Alzheimer's disease. *Bioorg Med Chem*. 2014;22:1355–1361.
24. Engman DM, Krause KH, Blumin JH, Kim KS, Kirchhoff LV, Donelson JE. A novel flagellar Ca2±binding protein in trypanosomes. *J Biol Chem*. 1989;264:18627–18631.
25. Moral-Naranjo MT, Cabezas-Herrera J, Vidal CJ. Molecular forms of acetyl- and butyrylcholinesterase in normal and dystrophic mouse brain. *J Neurosci Res*. 1996;43:224–234.
26. Linardaki ZI, Lamari FN, Margarity M. Saffron (*Crocus sativus L.*) tea intake prevents learning/memory defects and neurobiochemical alterations induced by Aflatoxin B1 exposure in adult mice. *Neurochem Res*. 2017;42:2743–2754.
27. Ellman GL, Courtney KD, Andres V, Jr., Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol*. 1961;7:88–95.
28. Das A, Dikshit M, Nath C. Role of molecular isoforms of acetylcholinesterase in learning and memory functions. *Pharmacol Biochem Behav*. 2005;81:89–99.
29. Budzynska B, Boguszewska-Czubara A, Kruk-Slomka M, et al. Effects of imperatorin on scopolamine-induced cognitive impairment and oxidative stress in mice. *Psychopharmacology (Berl)*. 2015;232:931–942.
30. Costa MD, Gai BM, Acker CI, Souza AC, Brandao R, Nogueira CW. Ebselen reduces hyperglycemia temporarily-induced by diazinon: a compound with insulin-mimetic properties. *Chem Biol Interact*. 2012;197:80–86.
31. Blokland A. Acetylcholine: a neurotransmitter for learning and memory? *Brain Res Brain Res Rev*. 1995;21:285–300.
32. Dong H, Yuede CM, Coughlan CA, Murphy KM, Csernansky JG. Effects of donepezil on amyloid-beta and synapse density in the Tg2576 mouse model of Alzheimer's disease. *Brain Res*. 2009;1303:169–178.
33. Kim SJ, Park C, Han AL, et al. Ebselen attenuates cisplatin-induced ROS generation through Nrf2 activation in auditory cells. *Hear Res*. 2009;251:70–82.
34. Rosa RM, Flores DG, Appelt HR, Braga AL, Henriques JA, Roesler R. Facilitation of long-term object recognition memory by pretraining administration of diphenyl diselenide in mice. *Neurosci Lett*. 2003;341:217–220.
35. Romberg C, Horner AE, Bussey TJ, Saksida LM. A touch screen-automated cognitive test battery reveals impaired attention, memory abnormalities, and increased response inhibition in the TgCRND8 mouse model of Alzheimer's disease. *Neurobiol Aging*. 2013;34:731–744.
36. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976;72:248–254.
37. Grossberg GT. Cholinesterase inhibitors for the treatment of Alzheimer's disease: getting on and staying on. *Curr Ther Res Clin Exp*. 2003;64:216–235.
38. Hasselmo ME. The role of acetylcholine in learning and memory. *Curr Opin Neurobiol*. 2006;16:710–715.
39. Siek GC, Katz LS, Fishman EB, Korosi TS, Marquis JK. Molecular forms of acetylcholinesterase in subcortical areas of normal and Alzheimer disease brain. *Biol Psychiatry*. 1990;27:573–580.
40. Mesulam MM. Cholinergic pathways and the ascending reticular activating system of the human brain. *Ann NY Acad Sci*. 1995;757:169–179.
41. Davis KL, Yamamura HI. Cholinergic underactivity in human memory disorders. *Life Sci*. 1978;23:1729–1733.
42. Wolf A, Bauer B, Abner EL, Ashkenazy-Frolinger T, Hartz AM. A comprehensive behavioral test battery to assess learning and memory in 129s6/Tg2576 mice. *PLoS ONE*. 2016;11:e0147733.
43. Ishola IO, Tota S, Adeyemi OO, Agbaje EO, Narender T, Shukla R. Protective effect of *Cnestis ferruginea* and its active constituent on scopolamine-induced memory impairment in mice: a behavioral and biochemical study. *Pharm Biol*. 2013;51:825–835.
44. Chintoh A, Fulton J, Koziel N, Aziz M, Sud M, Yeomans JS. Role of cholinergic receptors in locomotion induced by scopolamine and oxotremorine-M. *Pharmacol Biochem Behav*. 2003;76:53–61.

45. Xie Y, Tan Y, Zheng Y, Du X, Liu Q. Ebselen ameliorates beta-amyloid pathology, tau pathology, and cognitive impairment in triple-transgenic Alzheimer's disease mice. *J Biol Inorg Chem*. 2017;22:851–865.
46. Loizzo MR, Tundis R, Menichini F, Menichini F. Natural products and their derivatives as cholinesterase inhibitors in the treatment of neurodegenerative disorders: an update. *Curr Med Chem*. 2008; 15:1209–1228.
47. Rountree SD, Chan W, Pavlik VN, Darby EJ, Siddiqui S, Doody RS. Persistent treatment with cholinesterase inhibitors and/or memantine slows clinical progression of Alzheimer disease. *Alzheimers Res Ther*. 2009;1:7.
48. Parnham MJ, Sies H. The early research and development of ebselen. *Biochem Pharmacol* 2013;86:1248–1253.
49. Noguchi N. Ebselen, a useful tool for understanding cellular redox biology and a promising drug candidate for use in human diseases. *Arch Biochem Biophys*. 2016;595:109–112.
50. Singh N, Sharpley AL, Emir UE, et al. Effect of the putative lithium mimetic ebselen on brain Myo-inositol, sleep, and emotional processing in humans. *Neuropsychopharmacology*. 2016;41:1768–1778.
51. Parsons CG, Danysz W, Dekundy A, Pulte I. Memantine and cholinesterase inhibitors: complementary mechanisms in the treatment of Alzheimer's disease. *Neurotox Res*. 2013;24:358–369.
52. Sharma M, Tiwari M, Tiwari RK. Hyperhomocysteinemia: impact on neurodegenerative diseases. *Basic Clin Pharmacol Toxicol*. 2015;117:287–296.
53. Cacabelos R, Cacabelos P, Torrellas C, Tellado I, Carril JC. Pharmacogenomics of Alzheimer's disease: novel therapeutic strategies for drug development. *Methods Mol Biol*. 2014;1175: 323–556.
54. Drever BD, Riedel G, Platt B. The cholinergic system and hippocampal plasticity. *Behav Brain Res*. 2011;221:505–514.
55. Appleyard ME. Acetylcholinesterase induces long-term potentiation in CA1 pyramidal cells by a mechanism dependent on metabotropic glutamate receptors. *Neurosci Lett*. 1995;190:25–28.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Martini F, Pesarico AP, Brüning CA, Zeni G, Nogueira CW. Ebselen inhibits the activity of acetylcholinesterase globular isoform G4 in vitro and attenuates scopolamine-induced amnesia in mice. *J Cell Biochem*. 2018;119: 5598–5608. https://doi.org/10.1002/jcb.26731

3.2. ARTIGO 2

O COMPOSTO MULTIFUNCIONAL EBSELEN REVERTE O COMPROMETIMENTO DA MEMÓRIA, APOPTOSE E ESTRESSE OXIDATIVO EM UM MODELO DE DOENÇA DE ALZHEIMER ESPORÁDICA EM CAMUNDONGOS

A MULTIFUNCTIONAL COMPOUND EBSELEN REVERSES MEMORY IMPAIRMENT, APOPTOSIS AND OXIDATIVE STRESS IN A MOUSE MODEL OF SPORADIC ALZHEIMER'S DISEASE

Franciele Martini, Suzan Gonçalves Rosa, Isabella Pregardier Klann, Bruna da Cruz Weber Fulco, Fabiano Barbosa Carvalho, Francine Luciano Rahmeier, Marilda da Cruz Fernandes, Cristina Wayne Nogueira

Journal of Psychiatric Research 109 (2019) 107–117

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/jpsychires

A multifunctional compound ebselen reverses memory impairment, apoptosis and oxidative stress in a mouse model of sporadic Alzheimer's disease

Franciele Martini[a], Suzan Gonçalves Rosa[a], Isabella Pregardier Klann[a], Bruna Cruz Weber Fulco[a], Fabiano Barbosa Carvalho[b], Francine Luciano Rahmeier[b], Marilda Cruz Fernandes[b], Cristina Wayne Nogueira[a,*]

[a] Laboratório de Síntese, Reatividade e Avaliação Farmacológica e Toxicológica de Organocalcogênios, Departamento de Bioquímica e Biologia Molecular, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, CEP 97105-900, Santa Maria, Rio Grande do Sul, Brazil
[b] Laboratório de Patologia da Fundação, Universidade Federal de Ciências da Saúde de Porto Alegre, CEP 90050-170, Porto Alegre, Rio Grande do Sul, Brazil

ARTICLE INFO

Keywords:
Ebselen
Apoptosis
Memory
Selenium
Neuroprotective
Organoselenium

ABSTRACT

Alzheimer 's disease (AD) is characterized by progressive cognitive decline including memory impairment, cortical dysfunction, and neuropsychiatric disturbances. The drug discovery to treat AD consists to develop compounds able to act in multiple molecular targets involved in the pathogenesis of the disease and the re-positioning of old drugs for new application. This way, the intracerebroventricular (icv) injection of streptozotocin (STZ) has been used as a metabolic model of sporadic AD. The aim of the present study was to investigate whether ebselen (1–10 mg/kg), a multifunctional selenoorganic compound, ameliorates memory impairment, hippocampal oxidative stress, apoptosis and cell proliferation in a mouse model of sporadic AD induced by icv STZ (3 mg/kg, 1 μl/min). The administration of ebselen (10 mg/kg, i.p.) reversed memory impairment and hippocampal oxidative stress, by increasing the activities of antioxidant enzymes and the level of a non-enzymatic antioxidant defense, in Swiss mice administered with icv STZ. The anti-apoptotic property of ebselen was demonstrated by its effectiveness against the increase in the ratios of Bax/Bcl-2, cleaved PARP/PARP and the cleaved caspase-3 levels in the hippocampus of icv STZ mice. Although ebselen reversed memory impairment, it was ineffective against the reduction in the number of BrdU positive cells induced by icv STZ. In conclusion, the multifunctional selenoorganic compound ebselen was effective to reverse memory impairment, hippocampal oxidative stress and apoptosis in a mouse model of sporadic AD induced by icv STZ.

1. Introduction

Alzheimer's disease (AD), the most common form of dementia, is characterized by progressive cognitive decline including memory impairment, cortical dysfunction, and neuropsychiatric disturbances (Paulsen, 2011; Schoonheim et al., 2012). To date, an estimated 35 million people are suffering from AD, and this number is projected to grow to 106.8 million people by 2050 (Sosa-Ortiz et al., 2012). Apart from the slow and progressive degeneration process, the neuropathologic hallmarks of AD are senile plaques and neurofibrillary tangles, along with neuronal and synaptic loss, astrocytic and microglial changes (Selkoe and Hardy, 2016; Walsh and Selkoe, 2004). The development of an appropriate animal model of AD has been difficult, as the etiology of this neurodegenerative disorder is complex and multifactorial (Jack et al., 2018). This way, the intracerebroventricular (icv) injection of streptozotocin (STZ) has been used as a metabolic model of sporadic AD (Grieb, 2016). This model produces central insulin resistance, glucose hypometabolism (Shoham et al., 2003), oxidative stress (Ishrat et al., 2006), inflammation (Shoham et al., 2003), neurodegeneration, and memory impairment (Santos et al., 2012).

Regarding AD therapy, over the last decade, more than 50 candidates have successfully passed phase II clinical trials, but none has passed phase III. Therefore, some major trends in AD drug discovery would be the development of compounds acting on the main stages of the pathogenesis of the disease; the design of drugs acting on multiple molecular targets involved in the pathogenesis of the disease and the

* Corresponding author. Departamento de Bioquímica e Biologia Molecular, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, CEP 97105-900, RS, Brazil.
E-mail address: criswn@ufsm.br (C.W. Nogueira).

https://doi.org/10.1016/j.jpsychires.2018.11.021
Received 26 July 2018; Received in revised form 24 October 2018; Accepted 21 November 2018

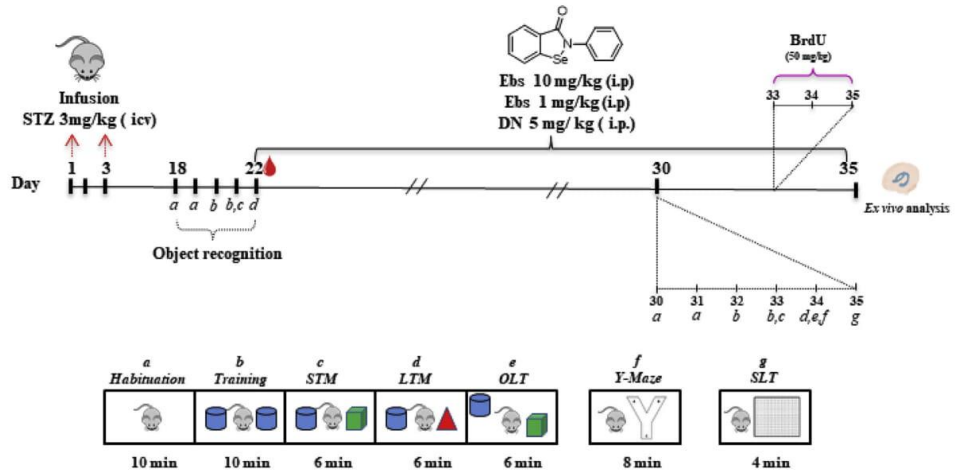


Fig. 1. Schematic representation of the experimental design of this study. Ebs - ebselen, DN - donepezil, STZ - streptozotocin, BrdU- bromodeoxyuridine, STM - short-term memory, LTM-long-term memory, OLT - object location test and SLT - spontaneous locomotor test.

repositioning of old drugs for new (anti-Alzheimer's) application (Bachurin et al., 2017). Ebselen fulfills all these trends because it is a multifunctional selenoorganic compound (Nogueira et al., 2004) currently undergoing clinical trials for cerebral ischemia (Gabryel and Malecki, 2006), bipolar disorder (Masaki et al., 2016; Singh et al., 2013), and noise-induced hearing loss (Mahadevan et al., 2013; Wang et al., 2014). Moreover, the effectiveness of ebselen has been already proven in a transgenic model (Xie et al., 2017), a model of familial AD. Therefore, the aim of the present study was to investigate whether ebselen ameliorates memory impairment, oxidative stress, apoptosis and cell proliferation in a metabolic model of sporadic AD induced by icv STZ.

2. Experimental procedures

2.1. Animals

The experiments were carried out using male adult Swiss mice (25–35 g) obtained from our breeding colony. The mice were housed in cages (5 mice per cage), with free access to food and water. The animals were kept in an air-conditioned room (22 ± 2 °C) under a 12:12 h light/dark cycle, with lights turned on at 7:00 a.m. The experimental procedures of this study were approved by the Institutional Ethics Committee on Care and Use of Experimental Animal Resources from the Federal University of Santa Maria-RS - Brazil (#7372110915). All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments.

2.2. Chemicals

Ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one) was prepared and characterized in our laboratory by the method previously described by Engman and Hallberg (1989). Analyses of the ^{1}H NMR and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of ebselen (99.9%) was determined by gas chromatography-mass spectrometry (GC/MS). Streptozotocin (STZ) and 5-bromo-deoxyuridine (BrdU) were obtained from Sigma (St. Louis, MO, USA). Donepezil was purchased from a local pharmacy (Santa Maria, Brazil). All other chemical reagents utilized were obtained from standard commercial suppliers. Ebselen was dissolved in 1: 4: 5 of dimethyl sulfoxide (DMSO), polyethyleneglycol and distilled water. Donepezil and BrdU were dissolved in saline solution. All drugs were administered to mice in a volume of 10 ml/kg. Appropriate vehicle-treated groups were also assessed simultaneously.

2.3. Intracerebroventricular (icv) injection of STZ

Adult male Swiss mice were anaesthetized by the intraperitoneal (ip) route with ketamine at a dose of 100 mg/kg and xylazine at a dose of 5 mg/kg for icv injections. The head of mouse was placed in the stereotaxic apparatus and a midline sagittal incision was made in the scalp. The stereotaxic coordinates for the lateral ventricle were measured accurately as anterio-posterior −0.8 mm, lateral 1.5 mm and dorso-ventral, −4.0 mm relative to bregma and ventral from dura with the tooth bar set at 0 mm. Through a skull hole, a 28-gauge Hamilton® syringe of 10 µl attached to a stereotaxic apparatus and piston of the syringe was lowered manually into lateral ventricle (Kroon and Riley, 1986). STZ (3 mg/kg, icv, 1 µl/min) was dissolved in citrate buffer (3 mg/ml, pH 4.4) (Tiwari et al., 2009) just prior to administration. STZ was injected, on alternate days (days 1 and 3), using a Hamilton microsyringe in a volume of 5 µl/injection in the lateral cerebral ventricle of mice (Deshmukh et al., 2009; Sharma et al., 2010).

After surgery, the animals took approximately 1–2 h to recover from anesthesia. The mice were kept in a well-ventilated room at 25 ± 2 °C in individual cages and provided with free access to food and water until they regained full consciousness. Food and water were placed inside the cage for 2–3 days so that the animals could easily access it without any physical trauma due to head injury.

2.4. Experimental protocol

The experimental design of this study is depicted in Fig. 1. Firstly, in order to validate memory impairment induced by icv STZ, mice performed the object recognition test on days 18–22. At the 22 day of protocol experimental, mice received ebselen at the dose of 1 or 10 mg/kg (ip), or positive control, donepezil, at the dose of 5 mg/kg (ip), once a day for 14 days (35 day) (Blokland, 1995). Regarding the doses of ebselen used in this experimental protocol, the highest dose was selected based on a previously published study (Unsal et al., 2016), which revealed 10 mg/kg (ip) as an effective dose against icv STZ induced oxidative stress and apoptosis in Sprague-Dawley rats. The dose of 1 mg/kg ebselen was carried out in order to investigate whether a lower dose would be effective in this experimental protocol.

The animals (n = 72) were randomly assigned in eight different

groups (n = 9/group) as following: ***Group I***- Sham: mice sham-operated injected with vehicle of STZ and treated with vehicle of ebselen; ***Groups II*** and ***III*** - Sham + ebselen: mice sham-operated injected with vehicle of STZ and treated with ebselen at a dose of 1 or 10 mg/kg, respectively. ***Group IV*** -Sham + donepezil: mice sham-operated injected with vehicle of STZ and treated with donepezil at a dose of 5 mg/kg. ***Group V***- STZ; mice injected with STZ (3 mg/kg, icv) and treated with vehicle of ebselen; ***Groups VI*** and ***VII*** - STZ + ebselen: mice injected with STZ and treated with ebselen at a dose of 1 or 10 mg/kg; ***Group VIII***- STZ + donepezil: mice injected with STZ and treated with donepezil at a dose of 5 mg/kg.

At day 22, which corresponds to the end of validation of memory impairment model, glycemia of animals was measured to rule out the effect of hyperglycemia on behavioral tests and *ex vivo* analyses.

At the end of behavioral tests, mice were immediately killed by cervical dislocation and samples of hippocampus were excised and stored at −80 °C for *ex vivo* analyses (Fig. 1).

2.5. BrdU protocol

A new set of animals, that did not perform the behavioral tests, was used (n = 40) to carry out this experiment. The protocol was performed according to Kim and Sung (2017) and Kee et al. (2002), with some modifications. BrdU was given to mice at a dose of 50 mg/kg once daily for three consecutive days (days 33–35). The animals were euthanized 24 h after the last BrdU injection (Fig. 1).

2.6. Behavioral tests

On days 30–35, the animals performed the behavioral tests as shown in Fig. 1: days 30 and 31- habituation to object recognition test (ORT) arena; days 32 and 33- training of test, day 33 - mice performed training and after that they were subjected to short-term memory test (STM); day 34 - mice performed LTM test (long-term memory), object location test (OLT) and Y-maze test; day 35- mice performed spontaneous locomotor test (SLT) and after that the animals were euthanized.

2.6.1. Object recognition test (ORT)

The object recognition test was performed according to Rosa et al. (2003) with some modifications. The behavioral test was performed in a 45 × 45 cm open field surrounded by 30 cm height walls, made of brown plywood. All animals were given a habituation session where they were left to freely exploring the open field for 10 min. No object was placed in the box during the habituation trial. Subsequently, four objects were used: A1, A2, B and C. The “A” objects were two identical double Lego colorful toys; the “B” object was a double Lego colorful toy “C” object was a double Lego colorful toy. All objects were made of plastic material, with 10 cm × 10 cm (length × height) and presented similar textures, colors (blue, red and yellow), and sizes, but distinctive shapes.

Twenty-four and 48 h after habituation two trainings were carried out by placing each individual mouse for 10 min into the field, in which two identical objects (objects A1 and A2) were positioned in two adjacent corners, 10 cm from the walls. In a STM test given 1.5 h after the last training, the mice explored the open field for 6 min in the presence of one familiar (A) and one novel (B) object. The percentage of the total exploration time that the animal spent investigating the novel object was the measure of recognition memory. Between trials the objects were washed with 10% ethanol solution. In a LTM test given 24 h after the last training, the same mice explored the field for 6 min in the presence of a familiar object A and a novel object C. Recognition memory was evaluated as for the STM test. Exploration was defined as sniffing or touching the object with the nose and/or forepaws. Exploratory preference in: training = (A2/(A1 + A2)) x 100; STM = (B/(A1 + B)) x 100; LTM = (C/(A1 + C)) x 100.

2.6.2. Object location test (OLT)

This test was performed according to Dao et al. (2013) with some modifications, in the same apparatus used in the ORT. The training was carried out by placing each mouse into the field where two objects (objects A1 and C; double Lego colorful toy) were positioned in two adjacent corners, 10 cm from the walls. Thus, 2 h after the session training, object C was moved to a location that was diagonally opposite to object A1, and the animals were allowed to explore the objects during 6 min and the evaluation of the exploration was done as described the steps above in section 2.6.1.

2.6.3. Y- maze test

The Y- maze has three-arm maze with equal angles between all arms, which were 30 cm long and 5 cm wide with 12 cm high walls. The maze floor and walls were constructed from dark grey, polyvinyl plastic. Mice were initially placed within one arm, and the sequence and number of arm entries were recorded manually for each mouse over an 8-min period. The percentage of trials in which the mice entered all three arms (ABC, CAB, or BCA but not ABB) was recorded as an alternation to estimate short-term memory. The Y- maze arms were cleaned with diluted 10% ethanol between tests to remove odors and residues. The alternation score (%) for each mouse was defined as the ratio of the actual number of alternations to the possible number (defined as the total number of arm entries minus two) multiplied by 100 as shown by the following equation: % Alternation = [(Number of alternations)/(Total arm entries − 2)] × 100. The number of arm entries per trial was used as an indicator of locomotor activity (Gotz and Ittner, 2008).

2.6.4. Spontaneous locomotor test (SLT)

With the purpose of excluding sedative or motor abnormality, the mouse performed spontaneous locomotor test. The animals were exposed to the chamber and activity was monitored under light and sound-attenuated conditions. Testing took place in a clear acrylic chamber (500 × 480 × 500 mm) equipped with 16 infrared sensors for the automatic recording of horizontal activity (Model EP149, Insight Instruments Ltda, Sao Paulo, BR). Each animal initially was placed in the center of the testing chamber and allowed to freely move while being tracked by an automated tracking system. The data (distance, speed and crossings) were collected and recorded during 4 min.

2.7. Ex vivo analyses

2.7.1. Glycemia

The glucose levels were determined in portable ACCU-CHEK ACTIVE glucose. The glucose level was expressed as mg dl^{-1}.

2.7.2. Parameters of oxidative stress

The hippocampus samples of mice from all experimental groups were homogenized in 50 mM Tris HCl at pH 7.4, 1:10 (w/v), and centrifuged at 2500 g for 10 min at 4 °C to yield a low-speed supernatant (S1) fraction. S1 was used for the determination of oxidative stress parameters.

2.7.2.1. Malondialdehyde levels (MDA). Briefly, an aliquot of S1 was added to NaOH 3 M and incubated at 60 °C during 30 min. After, 6% H_3PO_4 and 0.8% thiobarbituric acid (TBA) were added to the system and the mixture was heated at 90 °C for 2 h. Following, 10% SDS and *n*-butanol were added to extract the TBA-malondialdehyde (MDA) product, which was analyzed on Shimadzu®HPLC equipment. The analytical column was a Phenomenex® ODS-$2C_{18}$ reverse-phase (250 mm × 4.6 mm, 5 µm; 100 Å, Allcrom, BR) and the mobile phase was ultrapure water and methanol (50:50; v/v). The HPLC analysis was performed under isocratic conditions at a 0.6 ml/min flow rate and UV detector set at 532 nm with a 20 µl sample volume injection (Grotto et al., 2007). The results were expressed as nmol MDA/mg protein.

2.7.2.2. Catalase (CAT) activity. The CAT activity was spectrophotometrically assayed by monitoring the H_2O_2 consumption at 240 nm according to Aebi (1984). The enzymatic reaction was performed by the addition of an S1 aliquot and the substrate (H_2O_2) at a concentration of 0.3 mM in a medium containing 50 mM phosphate buffer, pH 7.0. The enzymatic activity was expressed in units (1 U decomposes 1 μmol of H_2O_2/min at pH 7.0 and at 25 °C)/mg protein.

2.7.2.3. Superoxide dismutase (SOD) activity. The SOD activity was measured spectrophotometrically according toMisra and Fridovich (1972). This method is based on the capacity of SOD in inhibiting autoxidation of epinephrine to epinechrome. In this assay, S1 diluted 1:10 (v/v) was added in a 50 mmol/L Na_2CO_3 buffer pH 10.3 and the enzymatic reaction was initiated by adding epinephrine. The color reaction was measured at 480 nm and the results were express in units (1 U decomposes 1 μmol of epinephrine/min at pH 7 and at 25 °C)/mg protein.

2.7.2.4. Glutathione S-transferase (GST) activity. The GST activity was assayed spectrophotometrically at 340 nm by the method ofHabig et al. (1974). The reaction mixture contained an aliquot of S_1, 0.1 M potassium phosphate buffer pH 7.4, 100 mM glutathione (GSH) and 100mM1-Chloro-2,4-dinitrobenzene (CDNB), which was used as substrate. The enzymatic activity was expressed as nmol CDNB conjugated min/mg/protein.

2.7.2.5. Total non-protein sulfhydryl (NPSH) levels. The NPSH levels were determined by the method of Ellman et al. (1961). S_1 was mixed (1:1) with 10% trichloroacetic acid. After the centrifugation (2500 g for 10 min), the protein pellet was discarded and free – SH groups were determined in the clear supernatant. An aliquot of supernatant was added in 1M potassium phosphate buffer (pH 7.4) and 10 mM 5.5′-dithiobis – (2 – nitrobenzoic acid). The color reaction was measured at 412 nm. NPSH levels were expressed as nmol NPSH/g tissue.

2.7.2.6. Protein quantification. The protein concentration was measured by the method described byBradford (1976), using bovine serum albumin (1 mg/ml) as the standard.

2.7.3. BrdU immunohistochemistry

The animals (n = 6–9) were deeply anaesthetized and perfused with saline followed by cold 4% paraformaldehyde (PFA). Brains were removed, fixed in 4% PFA and embedded in paraffin. For BrdU immunohistochemistry, 5 μm brain sections were cut through the hippocampus, and slices were first deparaffinized (40 min at 80 °C) and rinsed in xylol. Sections were then rinsed in phosphate-buffered saline (PBS, pH 7.00) and the blockade of non-specific proteins were made with 1% of bovine serum albumin (BSA) diluted in PBS. Afterward, the blockade of endogenous peroxidase were made with 5% H_2O_2 in methanol (3 times) and washed in 0.05% Triton X-100 diluted in PBS, and then were incubated with a mouse monoclonal anti-BrdU antibody (RPN 202 Kit, GR Healthcare®, 1:100) diluted in DNAse-1 overnight at 4 °C. Subsequently, the sections were incubated with a secondary antibody peroxidase anti-mouse-IgG, followed by tertiary antibody (both Spring®) 40 min each, at room temperature. The immunohistochemical reaction was revealed by 0.06% 3,3-diaminobenzidine (DAB) in PBS for 5 min. After being rinsed in distilled water, sections were counterstained with hematoxylin for 5 s, dehydrated in ethanol and mounted on slides using Entellan. For each brain, BrdU-positive cells were identified by their brown stain and were counted visually using a *Leica Application Suite X* microscope at 40× objective magnification. The number of stained BrdU cells was determined through mean of 10 cuts per animal with an interval of 50 μm in the dentate gyrus area of hippocampus. Data were expressed as total number of BrdU positive cells in the dentate gyrus.

2.7.4. Western blot assay

For the western blot analyses, the contents of proteins were not determined in the samples from mice of icv STZ treated with ebselen (1 mg/kg) and its respective control group because this dose of ebselen was not effective in all behavioral tests.

Samples of hippocampus (n = 5–6 animals/group) were homogenized in Radioimmunoprecipitation assay buffer (RIPA buffer) solution containing 150 mM NaCl, 1.0% IGEPAL® CA-630, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris, pH 8.0, in the presence of commercial phosphates and protein inhibitor cocktail (Sigma-Aldrich Company, St. Louis, Missouri, United States). Tissue extracts were diluted to a final protein concentration of 2 μg/μl. The samples (40 μg of protein) and pre stained molecular weight standards (Sigma-Aldrich Company, St. Louis, Missouri, United States) were separated on 10% resolving with 4% concentrating SDS-PAGE electrophoresis gels. Proteins were transferred to nitrocellulose membrane using Transfer-Blot® Turbo™ Transfer System (1.0 A; 45 min for proteins above 25 kDa or 5 min for proteins below 25 kDa). After blocking with 3% bovine serum albumin solution, the blots were incubated overnight at 4 °C with rabbit anti-poly (ADP-ribose) polymerase (PARP) (1:1000); rabbit cleaved caspase- 3 (caspase- 3) (1:1000); and rabbit (Bcl - 2) (1:1000) obtained from Cell Signaling Technology, Beverly, MA, USA and rabbit (Bax) (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, US). Mouse anti-β-actin (1:5000, abcam) was stained as additional control of the protein loading. After primary antibodies incubation, membranes were washed and incubated with secondary antibodies conjugated with horseradish peroxidase (Bio-Rad Laboratories, Hercules, CA, USA) for 1 h at room temperature and developed with chemiluminescence kit (Amersham, São Paulo/Brazil). Optical density (OD) of the Western blotting bands was quantified using Image J (NIH, Bethesda, MD, USA) software for Windows. Each value was derived from the ratio between arbitrary units obtained by the protein band and the respective β-actin band.

2.8. Statistical analysis

All experimental results are presented as the mean ± S.E.M. Normality of data was analyzed using a D'Agostino and Pearson omnibus normality test. Student *t*-test was used to compare STZ and vehicle groups in the object recognition test (in the validation model of memory impairment). Statistical comparisons among experimental groups (STZ x ebselen) and (STZ x donepezil) in behavioral tests and parameters of oxidative stress were performed by two-way analysis of variance followed by the Newman–Keuls test when appropriate. Immunohistochemistry and western blot data were statistically compared by one-way analysis of variance followed by the Newman–Keuls test when appropriate. All analyses were performed by using the STATISTICA for Windows software Version 7 (Stat Soft, Oklahoma, USA) by an investigator blinded to treatment. A value of $P < 0.05$ was considered to be significant.

3. Results

3.1. STZ induced memory impairment in mice

Fig. 2 shows the effect of STZ injection on the performance of mice in the object recognition test. The Student's *t*-test revealed that STZ decreased the exploratory preference (% time spent exploring a novel object) of mice when compared with that of the vehicle-treated group in the STM ($P = 0.006$ and LTM ($P = 0.0001$) tests.

By contrast, the insert of Fig. 2 demonstrates that STZ did not alter the time spent exploring any of the two identical objects in training ($P = 0.480$).

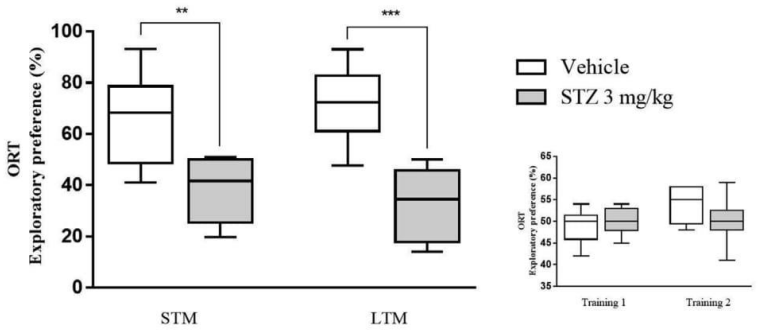


Fig. 2. Effects of STZ on the mouse performance in the ORT. Insert shows the performance of mice in training (percentage of time spent exploring any of two identical objects). Values are expressed as mean ± S.E.M. of 9–10 animals. Asterisks denote the significance levels when compared with the respective vehicle group: (**) $P < 0.01$ and (***) $P < 0.001$ (Student's *t*-test). STM - short-term memory; LTM - long-term memory.

3.2. Ebselen reversed memory impairment induced by STZ in the mouse ORT

Fig. 3 shows the effect of ebselen treatment on the performance of mice in the ORT. There were no significant differences among groups in the time spent exploring any of the two identical objects in trainings 1 and 2 [$F_{(2,48)} = 0.896$, $P = 0.415$](Fig. 3A).

The two-way ANOVA of percentage of exploratory preference revealed a significant ebselen × STZ interaction in the STM [$F_{(2,48)} = 5.38$, $P = 0.007$] and in the LTM [$F_{(2,48)} = 5.64$, $P = 0.006$]. Post-hoc analyses demonstrated that both doses of ebselen reversed memory impairment induced by STZ of mice in the STM (Fig. 3B), but only the highest dose of ebselen increased the percentage of exploratory preference of mice in the LTM (Fig. 3C).

The inserts of figures demonstrate the effects of donepezil, the positive control, in the training session (Fig. 3A), STM (Fig. 3B) and LTM (Fig. 3C). There were no significant differences among groups in the time spent exploring any of the identical objects in trainings 1 and 2 [$F_{(1,32)} = 0.479$, $P = 0.493$]. The two-way ANOVA of percentage of exploratory preference revealed a significant donepezil × STZ interaction in the STM [$F_{(1,32)} = 41.34$, $P = 0.0001$], but not in the LTM [$F_{(1,32)} = 2.61$, $P = 0.115$]. Post hoc analysis showed that donepezil at a dose of 5 mg/kg reversed memory impairment induced by STZ in mice only in the STM (Fig. 3C).

3.3. Ebselen reversed memory impairment induced by STZ in the mouse OLT

Fig. 4 shows the effect of ebselen treatment on the performance of mice in the OLT. The two-way ANOVA of percentage of exploratory preference revealed a significant ebselen × STZ interaction in the OLT [$F_{(2,48)} = 4.44$, $P = 0.016$]. Post hoc comparisons showed that STZ decreased the percentage of exploratory preference of mice in the OLT when compared with that of the vehicle-treated group, and ebselen at a dose of 10 mg/kg reversed this parameter.

The insert of Fig. 4 demonstrates the effect of the positive control, donepezil, in the OLT. The two-way ANOVA of percentage of exploratory preference indicated a significant donepezil × STZ interaction in the OLT [$F_{(1,32)} = 14.04$, $P = 0.0007$]. Post hoc analysis showed that donepezil at a dose of 5 mg/kg reversed the STZ– induced mouse memory impairment in the OLT.

3.4. Ebselen reversed memory impairment induced by STZ in the mouse Y - maze

Fig. 5 shows the effect of ebselen treatment on the performance of mice in the Y- maze test. The two-way ANOVA of alternations (%) indicated a significant ebselen × STZ interaction [$F_{(2,48)} = 3.21$, $P = 0.049$]. Post hoc comparisons showed that STZ decreased the percentage of alternations of mice in the Y-maze test when compared with that of the vehicle-treated group, and ebselen at both doses reversed this parameter.

The insert of Fig. 5 demonstrates the effect of the positive control, donepezil, in the Y - maze test. The two-way ANOVA of alternations (%) demonstrated a significant donepezil × STZ interaction in the Y - maze [$F_{(1,32)} = 11.13$, $P = 0.002$]. Post hoc analysis showed that donepezil at a dose of 5 mg/kg reversed STZ – induced mouse memory impairment in the Y-maze test.

3.5. Ebselen and STZ did not alter the mouse spontaneous locomotor activity

Table 1 shows the effect of ebselen treatment or STZ on the mouse spontaneous locomotor activity. The two-way ANOVA of crossings [$F_{(2,42)} = 0.077$, $P = 0.92$], distance travelled [$F_{(2,42)} = 0.596$, $P = 0.55$] and speed [$F_{(2,42)} = 0.031$, $P = 0.96$] revealed that there was not a statistically significant ebselen × STZ interaction. Neither STZ nor ebselen changed the number of crossings, distance travelled and speed of mice.

The two-way ANOVA of crossings [$F_{(1,28)} = 0.77$, $P = 0.78$], distance travelled [$F_{(1,28)} = 2.01$, $P = 0.16$] and speed [$F_{(1,28)} = 0.055$, $P = 0.81$] did not show a statistically significant donepezil × STZ interaction. The data on spontaneous locomotor activity parameters were similar in all experimental groups (Table 1).

3.6. Ebselen reversed oxidative stress induced by STZ in hippocampus of mice

Fig. 6A–E shows the ebselen effects on parameters of oxidative stress in the mouse hippocampus. The two-way ANOVA of MDA levels in the mouse hippocampus [$F_{(2,42)} = 3.25$, $P = 0.048$] revealed a significant ebselen × STZ interaction. Post hoc analyses showed that STZ increased MDA levels when compared with those of the vehicle-treated group. Treatment of mice with ebselen was effective against this parameter (Fig. 6A) only at the dose of 10 mg/kg.

The two-way ANOVA of CAT activity revealed a significant ebselen × STZ interaction [$F_{(2,36)} = 5.38$, $P = 0.008$]. Post hoc analysis showed that STZ decreased the CAT activity when compared with that of the vehicle-treated group, and ebselen at the highest dose was effective against this parameter (Fig. 6B).

The two-way ANOVA of SOD activity showed a significant ebselen × STZ interaction [$F_{(2,42)} = 13.94$, $P = 0.0001$]. Post hoc analysis showed that STZ decreased the SOD activity when compared with the vehicle-treated group and treatment of mice with ebselen reversed this parameter only at the dose of 1 mg/kg (Fig. 6C).

The two-way ANOVA of GST activity indicated a significant ebselen × STZ interaction [$F_{(2,36)} = 3.58$, $P = 0.037$]. Pos hoc analysis showed that STZ increased the GST activity when compared with the vehicle-treated group and treatment of mice with ebselen was effective against this parameter at both doses tested (Fig. 6D).

The two-way ANOVA of NPSH levels revealed a significant

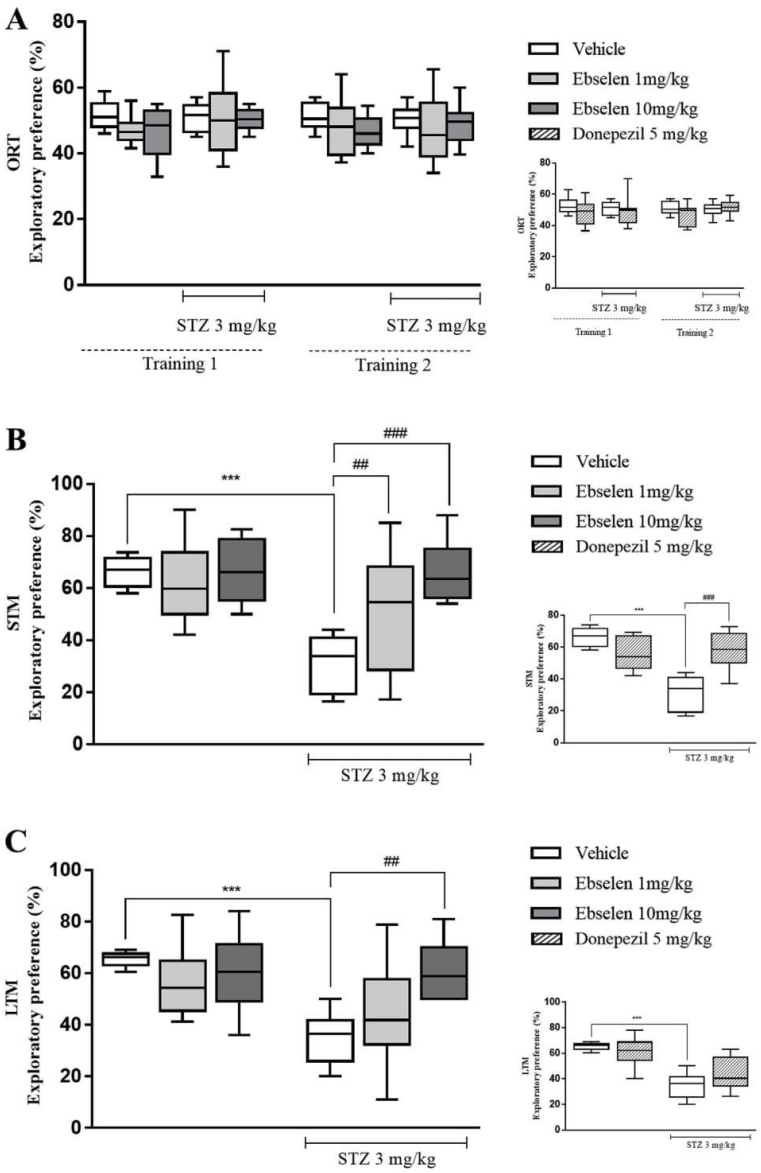


Fig. 3. Effects of ebselen on training sessions (A), STM (B) and LTM (C) in the ORT of mice treated with STZ. Inserts show effects of donepezil treatment (5 mg/kg) on the performance of mice in training sessions (A), STM (B) and LTM (C). Values are expressed as mean ± S.E.M. of 9 animals. Asterisk denotes the significance levels when compared with the respective vehicle group: (***) $P < 0.001$. Hashtag denotes the significance levels when compared with the STZ group: ($^{\#\#}$) $P < 0.01$ and ($^{\#\#\#}$) $P < 0.001$ (two-way ANOVA followed by the Newman Keuls). STM - short-term memory; LTM - long-term memory.

ebselen × STZ interaction [$F_{(2,30)} = 4.23$, $P = 0.024$]. Pos hoc analysis showed that STZ decreased the NPSH levels when compared with the vehicle-treated group and treatment of mice with ebselen reversed this parameter only at the dose of 10 mg/kg (Fig. 6E).

The inserts of Fig. 6 A-E demonstrate the effect of positive control, donepezil, in the parameters of oxidative stress. The two-way ANOVA of MDA levels indicated a significant donepezil × STZ interaction [$F_{(1,28)} = 4.62$, $P = 0.040$]. Post hoc analysis showed that treatment of mice with donepezil at a dose of 5 mg/kg was effective against the increase in MDA levels (Fig. 6A) induced by STZ in the mouse hippocampus.

The two-way ANOVA of CAT activity did not reveal a significant effect of donepezil [$F_{(1,24)} = 0.378$, $P = 0.544$] (Fig. 6B).

The two-way ANOVA revealed a significant donepezil × STZ interaction in the SOD activity [$F_{(1,24)} = 8.37$, $P = 0.007$]. Post hoc analysis showed that STZ decreased the SOD activity when compared with the vehicle-treated group and treatment of mice with donepezil at a dose of 5 mg/kg reversed this parameter in the hippocampus (Fig. 6C).

The two-way ANOVA revealed a significant donepezil × STZ interaction in the GST activity [$F_{(1,24)} = 8.02$, $P = 0.009$]. Pos hoc analysis

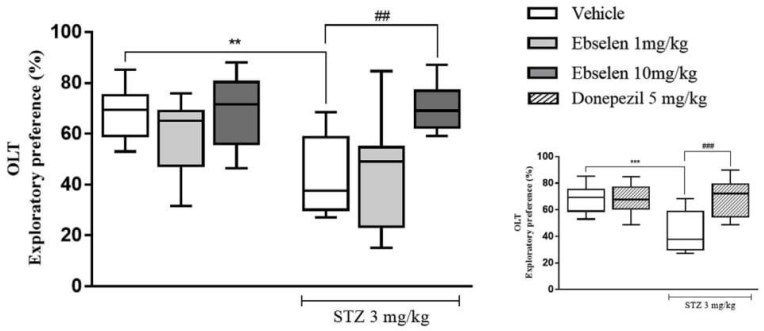


Fig. 4. Effects of ebselen on the OLT of mice treated with STZ. Insert shows the effect of donepezil treatment (5 mg/kg). Values are expressed as mean ± S.E.M. of 9 animals. Asterisk denotes the significance levels when compared with the respective vehicle group: (**) P < 0.01 and (***) P < 0.001. Hashtag denotes the significance levels when compared with the STZ, vehicle treated group (##) P < 0.01 and (###) P < 0.001 (two-way ANOVA followed by the Newman Keuls). LTM - long-term memory.

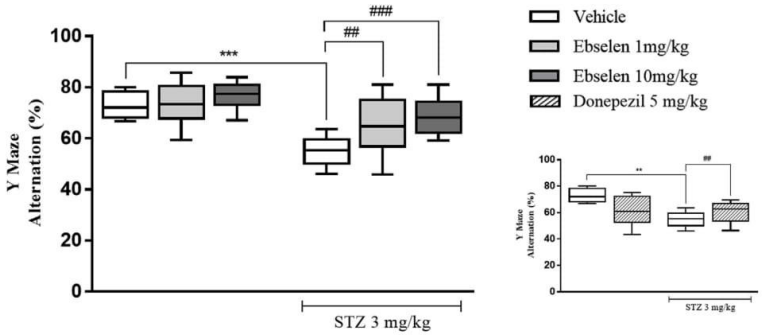


Fig. 5. Effects of ebselen on Y- maze of mice treated with STZ. Insert shows the effect of donepezil treatment (5 mg/kg). Values are expressed as mean ± S.E.M. of 9 animals. Asterisk denotes the significance levels when compared with the respective vehicle group: (**) P < 0.01 and (***) P < 0.001. Hashtag denotes the significance levels when compared with the STZ, vehicle treated group (##) P < 0.01 and (###) P < 0.001 (two-way ANOVA followed by the Newman Keuls).

Table 1
Ebselen and icv STZ effects on the mouse spontaneous locomotor test.

Groups	Number of Crossings	Distance (mm)	Speed (mm/s)
Vehicle	607.0 ± 55.86, *p = 0.775*	10060 ± 803, *p = 0.847*	46.0 ± 2.0, *p = 0.658*
Ebs 1 mg/kg	632.5 ± 34.78, *p = 0.937*	10560 ± 729, *p = 0.849*	52.0 ± 3.0, *p = 0.815*
Ebs 10 mg/kg	552.0 ± 44.38, *p = 0.996*	9605 ± 703,*p = 0.831*	48.0 ± 3.2, *p = 0.838*
DN 5 mg/kg	645.5 ± 49.30, *p = 0.951*	10990 ± 526, *p = 0.460*	45.8 ± 2.2, *p = 0.904*
STZ	530.0 ± 65.14, *p = 0.775*	9428 ± 950, *p = 0.847*	43.3 ± 4.9, *p = 0.658*
STZ + Ebs1 mg/kg	615.0 ± 45.94, *p = 0.981*	11640 ± 525, *p = 0.811*	49.8 ± 1.5, *p = 0.609*
STZ + Ebs10 mg/kg	669.0 ± 53.94, *p = 0.934*	11698 ± 869, *p = 0.931*	44.2 ± 4.5, *p = 0.944*
STZ + DN 5 mg/kg	647.5 ± 51.69, *p = 0.915*	10770 ± 960, *p = 0.597*	46.4 ± 4.7, *p = 0.833*

Values are expressed as mean ± S.E.M. of 8–9 animals. Data analysis was carried out through two-way analysis of variance ANOVA followed by the Newman-Keul's test. P values of *per se* groups (Ebs 1 and 10 mg/kg, DN) were obtained by comparing these groups with the vehicle group, and those of STZ-treated groups (STZ + ebselen (1 and 10) and STZ + DN) were obtained by comparing with the STZ group. Ebs – ebselen; DN – donepezil; STZ – streptozotocin.

showed that STZ increased the GST activity when compared with the vehicle-treated group and treatment of mice with donepezil at a dose of 5 mg/kg reversed this parameter (Fig. 6D).

The two-way ANOVA revealed a significant donepezil × STZ interaction in the NPSH levels [$F_{(1,20)} = 4.36$, P = 0.049]. Post hoc analysis showed that STZ decreased the NPSH levels when compared with the vehicle-treated group and treatment of mice with donepezil at a dose of 5 mg/kg increased this parameter in the hippocampus (Fig. 6E).

3.7. Icv STZ did not alter blood glucose levels

Table 2 shows the effect of icv STZ on glycemia. An unpaired Student's *t*-test revealed that icv STZ did not alter glycemia in mice when compared with that of the vehicle group (P = 0.610).

3.8. Ebselen did not restore reduced cell proliferation induced by STZ in the mouse dentate gyrus of hippocampus

Fig. 7 shows the ebselen effect on the number of BrdU positive cells, a marker of cell proliferation, in the dentate gyrus area of hippocampus. The one-way ANOVA revealed the reduction on the number of BrdU positive cells in the dentate gyrus area of hippocampus of animals injected with STZ when compared with those of the vehicle-treated group [$F_{(4,34)} = 21.69$, P = 0.0001]. By contrast, one-way ANOVA showed that neither ebselen nor donepezil restored cell proliferation in the dentate gyrus area of hippocampus (P = 0.210).

3.9. Ebselen reversed the levels of apoptotic proteins altered by STZ in the hippocampus of mice

The Bax/Bcl-2 and PARP cleaved/PARP ratios; and the protein levels of cleaved caspase- 3 in the hippocampus of mice are shown in Fig. 8A–C. There was a significant increase in the ratio of Bax/Bcl-2 [F

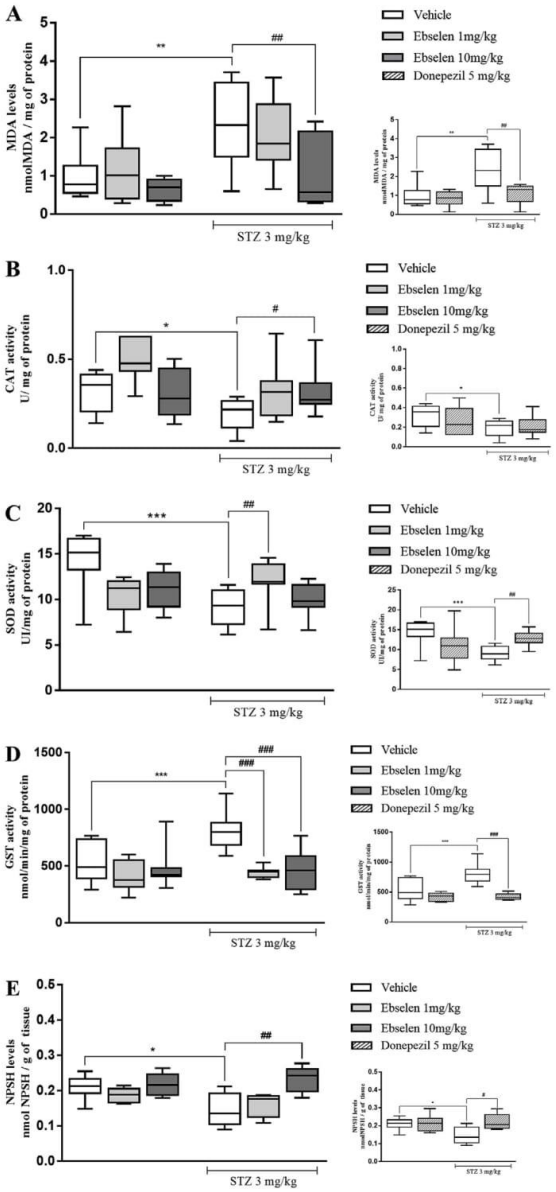


Fig. 6. Effects of ebselen on hippocampal MDA levels (A), CAT activity (B), SOD activity (C), GST activity (D) and NPSH levels (E) of mice treated with STZ. Inserts show the effects of donepezil treatment (5 mg/kg). Values are expressed as mean ± S.E.M. of 8 (MDA), 7 (CAT, SOD and GST) and 6 (NPSH) animals. Asterisk denotes the significance levels when compared with the respective vehicle treated group: (*) $P < 0.05$, (**) $P < 0.01$ and (***) $P < 0.001$. Hashtag denotes the significance levels when compared with the STZ, vehicle treated group ($^{\#}$) $P < 0.05$, ($^{\#\#}$) $P < 0.01$ and ($^{\#\#\#}$) $P < 0.001$ (two-way ANOVA followed by the Newman Keuls).

$_{(5,29)} = 14.59$, $P = 0.0001$] and cleaved PARP/PARP [$F_{(5,29)} = 12.28$, $P = 0.0001$] and in the level of cleaved caspase- 3 [$F_{(5,31)} = 5.16$, $P = 0.002$] in the hippocampus of mice injected with STZ when compared to those of the vehicle-treated group. Ebselen and donepezil reversed this increase to the levels of control group.

The levels of proteins Bax [$F_{(5,30)} = 14.59$, $P = 0.0001$] and Bcl-2 [$F_{(5,30)} = 3.96$, $P = 0.0087$] separately plotted are depicted in Fig. 1S.

4. Discussion

The results of the present study demonstrate that the multifunctional compound ebselen reversed memory impairment and modulated oxidative stress and the levels of proteins related to apoptosis in a mouse model of sporadic AD induced by icv STZ.

Animal models are critical to drug discovery and provide a basic platform to investigate new therapies. The icv injection of STZ is an established, standardized and reproducible approach to metabolic sporadic AD (Salkovic-Petrisic et al., 2013). STZ alters the function of enzymes involved in the brain glucose metabolism (Hoyer and Lannert, 2007), stimulates oxidative stress (Agrawal et al., 2011; Ishrat et al., 2009; Javed et al., 2012), the apoptotic pathway (Agrawal et al., 2011) and the release of cytotoxic factors, which can lead to neuronal death (Bhalala, 2015). Although icv STZ is a non-transgenic metabolic model of sporadic AD, which resembles features in brains of AD patients, we should acknowledge the lack of mechanistic explanation of icv STZ action as a limitation of this model (Grieb, 2016).

In the present study, the icv injection of STZ besides causing memory impairment and oxidative stress, it increased the hippocampal levels apoptotic markers and decreased neurogenesis in the dentate gyrus area of hippocampus. Despite differences between STZ doses, some authors Mishra et al. (2018); Kraska et al. (2012) have reported that icv STZ induced severe atrophy of the area around the lateral ventricles after 21 days and lesions extended posterior to the dentate gyrus, and neurodegenerative lesions associated with inflammation and oxidative stress (Kraska et al., 2012). The STZ model has also behavioral consequences, such as memory and learning impairment (Mehla et al., 2013). In fact, our findings demonstrate that icv STZ decreased mouse performance in the STM, LTM, OLT and Y-maze tests, reflecting loss of memory.

Scientists have used a database of 'failed' drugs, found to be safe but ineffective for their proposed use, to identify ebselen, a compound which displays formidable benefit during the animal model phase of research (Noguchi, 2016; Nosengo, 2016) and have been able to reproduce these effects in human clinical trials, as a possible alternative to treat bipolar disorder (Masaki et al., 2016; Singh et al., 2013), and noise-induced hearing loss (Mahadevan et al., 2013; Wang et al., 2014). In fact, ebselen neuroprotective effects have been recognized (Singh et al., 2016) and its multifactorial targets (Luo et al., 2013; Parnham and Sies, 2013) seem to be an advantage for prospective therapeutic strategies. Besides, the effectiveness of ebselen has been already proven in a transgenic model of AD (Xie et al., 2017). Widely used transgenic mouse AD models have provided valuable insights into the molecular mechanisms underlying the memory decline; however, due to the particular β -amyloid-related gene manipulation, they resemble the familial but not the sporadic AD form. Despite the differences in the experimental protocol (dose, species, time of administration and route) between the Xie et al. (2017) study and our present study, the demonstration that ebselen improved the cognitive impairment in spatial learning, reduced β-amyloid level and inhibited tau hyperphosphorylation in a model of familial AD motivated us to investigate this compound in a model that mimics a specific endophenotype, such as STZ icv-treated animals which develop insulin resistant brain state.

The findings of the present study reproduced well-known properties of ebselen, such as antioxidant, anti-apoptotic, neuroprotective and memory enhancing (Singh et al., 2016) in a mouse model of sporadic AD induced by icv STZ. Ebselen administration reversed hippocampal oxidative stress induced by icv STZ by increasing the activities of antioxidant enzymes and the levels of a non-enzymatic antioxidant defense. The anti-apoptotic property of ebselen was demonstrated in this study by its effectiveness against the increase in the ratios of Bax/Bcl-2,

Table 2
Effect of icv STZ on glycemia of mice.

Groups	Glycemia (mg/dl)
Vehicle	107.7 ± 4.86
STZ	104.8 ± 2.67

Values are expressed as mean ± S.E.M. of 9 animals. Data analysis was carried out through unpaired Student's *t*-test. STZ – streptozotocin.

cleaved PARP/PARP and in the levels of caspase-3 in the hippocampus of mice experimentally induced by icv STZ. Apoptosis inhibition depends partly on the balance between the levels of Bcl-2 and Bax, increased apoptotic frequency is associated with decline in Bcl-2 expression (Upadhyay and Kamp, 2003). In other words, the decrease of Bcl-2/Bax ratio inhibits the DNA repair capacity with consequent disassembly and cell death (Pollack et al., 2002). Moreover, the increase of Bax/Bcl-2 ratio found in the hippocampus of icv STZ mice, can upregulate caspase-3 and increase apoptosis (Salakou et al., 2007). Another characteristic event of apoptosis is the proteolytic cleavage of PARP, a nuclear enzyme involved in DNA repair, DNA stability, and transcriptional regulation. Caspases, in particular caspase-3 and -7, cleave the 116-kDa form of PARP-1 to generate cleaved fragments (Wei and Shi, 2013). In the present study, ebselen reversed caspase- 3 activation and induction of PARP cleavage in the hippocampus of icv STZ mice. These findings, taken together, reinforce the anti-apoptotic action of ebselen in a sporadic model of AD.

The general consensus in the scientific literature is that adult hippocampal neurogenesis plays a vital role in the long-term spatial memory (Snyder et al., 2005) and that reduction of adult neurogenesis produces behavioral disturbances that lead to learning and memory impairment (Cameron and Glover, 2015). Whether decreased neurogenesis is just a neuroanatomical manifestation of the AD or functionally contributes to memory impairment remains uncertain, but it is known that neurogenesis can be regulated by numerous factors associated with behavioral intervention and cognitive states (Baptista and Andrade, 2018). In fact, behavioral interventions, including hippocampus dependent learning, environmental enrichment and voluntary running, can increase the rate of neurogenesis and BrdU positive cells (Deng et al., 2010).

Moreover, it is plausible that interventions that improve neurogenesis may be useful to treat hippocampal dysfunctions found in AD. Evidence found in transgenic mouse models of AD indicates that intrahippocampal transplantation of human neural stem cells improved cognition by enhancing synaptogenesis (Ager et al., 2015). Even though promising results highlight transplantation therapy as a possible intervention for AD it is not clear the role new neurons play in the functional activity of the mature brain and whether these cells display any clinical relevance (Apple et al., 2017).

In the present study, icv STZ reduced the neuronal cell proliferation in the dentate gyrus area of hippocampus, which helps to explain the memory impairment of STZ mice; however, neither ebselen nor donepezil was effective against this decrease even that they reversed memory impairment. This result leads us to believe that the multi target profile of ebselen is behind its memory enhancing effect demonstrated in this study.

Because ebselen is a bioavailable molecule that permeates the blood-brain barrier (Imai et al., 2001) it is possible that this drug acts largely through a direct central nervous system effects. Regarding the concentration of ebselen in plasma, a single oral dose of 100 mg/kg ebselen in rats produces serum values of 4–5 μM (Salom et al., 2004), whereas 1 mg/kg of ebselen (iv) reached 12 μg/ml in rat plasma (Imai et al., 2001). Moreover, data on ebselen pharmacokinetic reveal that the selenium portion was not bioavailable and, therefore, ebselen did not enter the selenium pool of the body. On the contrary, it was metabolized and excreted explaining its low toxicity (Parnham and Sies, 2000). Considering that ebselen has been reported to have antioxidant (Wang et al., 2014), anti-inflammatory (Xie et al., 2017) and neuroprotective (Singh et al., 2016) actions and that it inhibits the activity of acetyl cholinesterase (Martini et al., 2018) and phosphorylation of tau (Mahadevan et al., 2013), we may have found a new role for ebselen to treat diseases in which cognitive function is present.

This study has some limitations that have to be pointed out as follow, because neuronal atrophy is the main hallmark of AD (Fu et al., 2017), we acknowledge the lack of this end point measurement as a weakness of this study. The use of BrdU, a marker of cell proliferation; reflects on new neurons and glial cells. Although it is known that the granular layer of dentate gyrus consists predominantly of neurons to affirm that BrdU-positive cells are related to neuronal proliferation, it would be required a BrdU/NeuN co-labeling. There is no doubt that icv STZ is an experimental model to study metabolic sporadic AD and potential therapies. Nevertheless it is still an open question how is the STZ

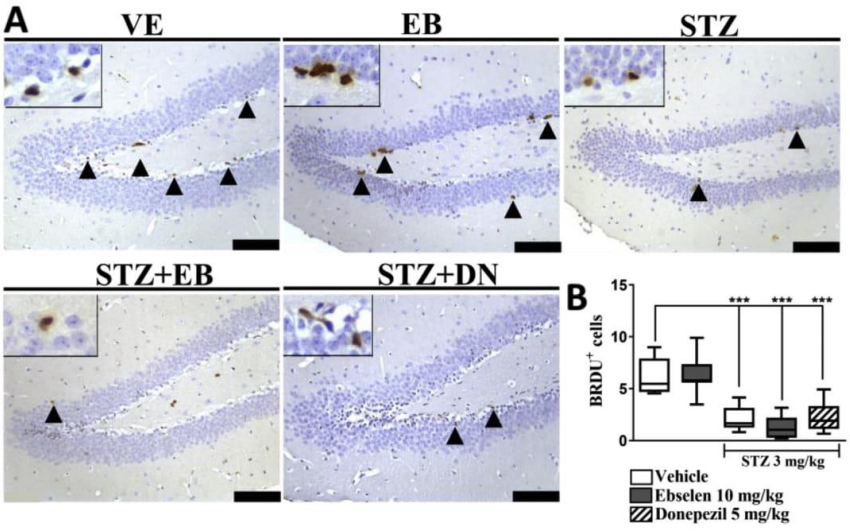


Fig. 7. Effect of ebselen on the number of BrdU positive cells in the dentate gyrus area of hippocampus in mice treated with STZ. Photographs are representation of qualitative immunohistochemistry (A) and $BrdU^+$ cells (B). Values are expressed as mean ± S.E.M. of 6–9 animals. Asterisk denotes the significance levels when compared with the respective vehicle treated group: (***) $P < 0.001$ (one -way ANOVA followed by the Newman Keuls).

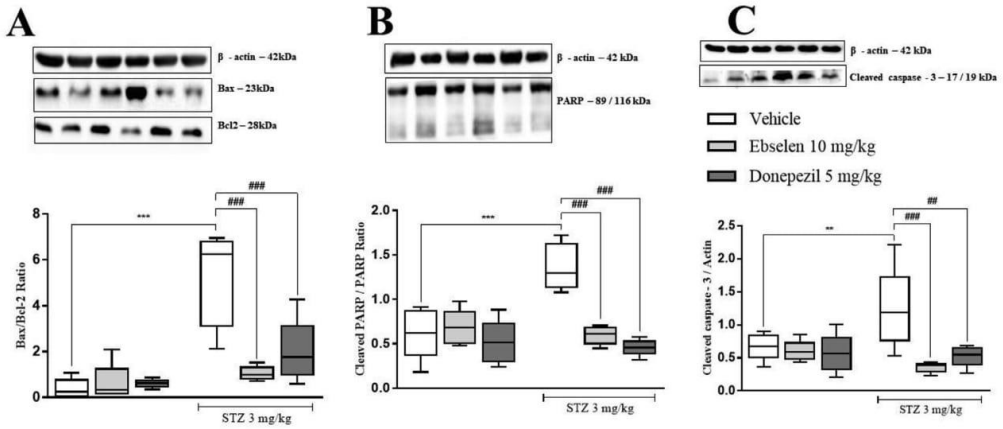


Fig. 8. Effect of ebselen on the hippocampal ratios of Bax/Bcl 2 (A) and cleaved PARP/PARP (C) and the levels of cleaved caspase- 3 (D) in mice treated with STZ. Values are expressed as mean ± S.E.M. of 5–6 animals. Asterisk denotes the significance levels when compared with the respective vehicle group: (*) $P < 0.05$, (**) $P < 0.01$ and (***) $P < 0.001$. Hashtag denotes the significance levels when compared with the STZ, vehicle treated group ($^{\#}$) $P < 0.05$, ($^{\#\#}$) $P < 0.01$ and ($^{\#\#\#}$) $P < 0.001$ (one -way ANOVA followed by the Newman Keuls).

mechanistic action, such as how is the brain insulin receptor desensitization or is brain glucose hypometabolism secondary effect or primary cause of AD (Grieb, 2016), these answers would help to converge the data obtained in this model to human sporadic AD. Moreover, it should be acknowledged the limitations of this model to mimic other relevant end points of sporadic AD.

Finally, in this study, most of ebselen effects, such as reversion of memory impairment, antioxidant and anti-apoptotic, were similar to those elicited by donepezil, the first-line treatment for Alzheimer's disease and non Alzheimer's dementia. Because long-term use of donepezil could be limited by increased vagal tone side effects associated to bradycardia, anorexia, abdominal pain, nausea and diarrhea (Turon-Estrada et al., 2003), drugs with fewer side effects would likely have a lower rate of people stop taking their prescribed drug.

In conclusion, the multifunctional selenoorganic compound, ebselen, was effective to reverse memory impairment, oxidative stress and apoptosis in a mouse model of metabolic sporadic AD induced by icv STZ.

Conflicts of interest

The authors declare they have no conflicts of interest to disclose.

Funding Information/Acknowledgements

We gratefully acknowledge Fundação de Amparo a Pesquisa do Estado do Rio Grande do Sul (FAPERGS, grant number 17/2551- 0000), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES/PROEX #23038.005848/2018-31) for the financial support. F.M. and B.W.C.F. (#88887.212657/2018-00) are recipients of CAPES fellowships. C.W.N (#304864/2015-3) is recipient of CNPq fellowship.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychires.2018.11.021.

References

Aebi, H., 1984. [13] Catalase in vitro. Methods Enzymol. 105, 121–126.
Ager, R.R., Davis, J.L., Agazaryan, A., Benavente, F., Poon, W.W., LaFerla, F.M., Blurton-Jones, M., 2015. Human neural stem cells improve cognition and promote synaptic growth in two complementary transgenic models of Alzheimer's disease and neuronal loss. Hippocampus 25 (7), 813–826.
Agrawal, R., Tyagi, E., Shukla, R., Nath, C., 2011. Insulin receptor signaling in rat hippocampus: a study in STZ (ICV) induced memory deficit model. Eur. Neuropsychopharmacol : J Eur. Coll. Neuropsychopharmacol. 21 (3), 261–273.
Apple, D.M., Solano-Fonseca, R., Kokovay, E., 2017. Neurogenesis in the aging brain. Biochem. Pharmacol. 141, 77–85.
Bachurin, S.O., Bovina, E.V., Ustyugov, A.A., 2017. Drugs in clinical trials for Alzheimer's disease: the major trends. Med. Res. Rev. 37 (5), 1186–1225.
Baptista, P., Andrade, J.P., 2018. Adult hippocampal neurogenesis: regulation and possible functional and clinical correlates. Front. Neuroanat. 12, 44.
Bhalala, O.G., 2015. The emerging impact of microRNAs in neurotrauma pathophysiology and therapy. In: Kobeissy, F.H. (Ed.), Brain Neurotrauma: Molecular, Neuropsychological, and Rehabilitation Aspects. Boca Raton (FL).
Blokland, A., 1995. Acetylcholine: a neurotransmitter for learning and memory? Brain research. Brain Res. Rev. 21 (3), 285–300.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
Cameron, H.A., Glover, L.R., 2015. Adult neurogenesis: beyond learning and memory. Annu. Rev. Psychol. 66, 53–81.
Dao, A.T., Zagaar, M.A., Levine, A.T., Salim, S., Eriksen, J.L., Alkadhi, K.A., 2013. Treadmill exercise prevents learning and memory impairment in Alzheimer's disease-like pathology. Curr. Alzheimer Res. 10 (5), 507–515.
Deng, W., Aimone, J.B., Gage, F.H., 2010. New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? Nat. Rev. Neurosci. 11 (5), 339–350.
Deshmukh, R., Sharma, V., Mehan, S., Sharma, N., Bedi, K.L., 2009. Amelioration of intracerebroventricular streptozotocin induced cognitive dysfunction and oxidative stress by vinpocetine – a PDE1 inhibitor. Eur. J. Pharmacol. 620 (1–3), 49–56.
Ellman, G.L., Courtney, K.D., Andres Jr., V., Feather-Stone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88–95.
Engman, L., Hallberg, A., 1989. Expedient synthesis of ebselen and related compounds. J. Org. Chem. 54 (12), 2964–2966.
Fu, H., Rodriguez, G.A., Herman, M., Emrani, S., Nahmani, E., Barrett, G., Figueroa, H.Y., Goldberg, E., Hussaini, S.A., Duff, K.E., 2017. Tau pathology induces excitatory neuron loss, grid cell dysfunction, and spatial memory deficits reminiscent of early Alzheimer's disease. Neuron 93 (3) 533-541 e535.
Gabryel, B., Malecki, A., 2006. Ebselen attenuates oxidative stress in ischemic astrocytes depleted of glutathione. Comparison with glutathione precursors. Pharmacol. Rep. : PR 58 (3), 381–392.
Gotz, J., Ittner, L.M., 2008. Animal models of Alzheimer's disease and frontotemporal dementia. Nat. Rev. Neurosci. 9 (7), 532–544.
Grieb, P., 2016. Intracerebroventricular streptozotocin injections as a model of Alzheimer's disease: in search of a relevant mechanism. Mol. Neurobiol. 53 (3), 1741–1752.
Grotto, D., Santa Maria, L.D., Boeira, S., Valentini, J., Charao, M.F., Moro, A.M., Nascimento, P.C., Pomblum, V.J., Garcia, S.C., 2007. Rapid quantification of malondialdehyde in plasma by high performance liquid chromatography-visible detection. J. Pharmaceut. Biomed. Anal. 43 (2), 619–624.
Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249 (22), 7130–7139.
Hoyer, S., Lannert, H., 2007. Long-term abnormalities in brain glucose/energy

metabolism after inhibition of the neuronal insulin receptor: implication of tau-protein. J. Neural. Transm. Suppl. (72), 195–202.
Imai, H., Masayasu, H., Dewar, D., Graham, D.I., Macrae, I.M., 2001. Ebselen protects both gray and white matter in a rodent model of focal cerebral ischemia. Stroke 32 (9), 2149–2154.
Ishrat, T., Khan, M.B., Hoda, M.N., Yousuf, S., Ahmad, M., Ansari, M.A., Ahmad, A.S., Islam, F., 2006. Coenzyme Q10 modulates cognitive impairment against intracerebroventricular injection of streptozotocin in rats. Behav. Brain Res. 171 (1), 9–16.
Ishrat, T., Parveen, K., Khan, M.M., Khuwaja, G., Khan, M.B., Yousuf, S., Ahmad, A., Shrivastav, P., Islam, F., 2009. Selenium prevents cognitive decline and oxidative damage in rat model of streptozotocin-induced experimental dementia of Alzheimer's type. Brain Res. 1281, 117–127.
Jack Jr., C.R., Bennett, D.A., Blennow, K., Carrillo, M.C., Dunn, B., Haeberlein, S.B., Holtzman, D.M., Jagust, W., Jessen, F., Karlawish, J., Liu, E., Molinuevo, J.L., Montine, T., Phelps, C., Rankin, K.P., Rowe, C.C., Scheltens, P., Siemers, E., Snyder, H.M., Sperling, R., Contributors, 2018. NIA-AA Research Framework: toward a biological definition of Alzheimer's disease. Alzheimer's Dementia : J. Alzheimer's Assoc. 14 (4), 535–562.
Javed, H., Khan, M.M., Ahmad, A., Vaibhav, K., Ahmad, M.E., Khan, A., Ashafaq, M., Islam, F., Siddiqui, M.S., Safhi, M.M., Islam, F., 2012. Rutin prevents cognitive impairments by ameliorating oxidative stress and neuroinflammation in rat model of sporadic dementia of Alzheimer type. Neuroscience 210, 340–352.
Kee, N., Sivalingam, S., Boonstra, R., Wojtowicz, J.M., 2002. The utility of Ki-67 and BrdU as proliferative markers of adult neurogenesis. J. Neurosci. Methods 115 (1), 97–105.
Kim, T.W., Sung, Y.H., 2017. Regular exercise promotes memory function and enhances hippocampal neuroplasticity in experimental autoimmune encephalomyelitis mice. Neuroscience 346, 173–181.
Kraska, A., Santin, M.D., Dorieux, O., Joseph-Mathurin, N., Bourrin, E., Petit, F., Jan, C., Chaigneau, M., Hantraye, P., Lestage, P., Dhenain, M., 2012. In vivo cross-sectional characterization of cerebral alterations induced by intracerebroventricular administration of streptozotocin. PloS One 7 (9), e46196.
Kroon, J.P., Riley, A.L., 1986. A microcomputer-based system for stereotaxic coordinates in the rat brain. Physiol. Behav. 38 (4), 593–596.
Luo, Z., Sheng, J., Sun, Y., Lu, C., Yan, J., Liu, A., Luo, H.B., Huang, L., Li, X., 2013. Synthesis and evaluation of multi-target-directed ligands against Alzheimer's disease based on the fusion of donepezil and ebselen. J. Med. Chem. 56 (22), 9089–9099.
Mahadevan, J., Parazzoli, S., Oseid, E., Hertzel, A.V., Bernlohr, D.A., Vallerie, S.N., Liu, C.Q., Lopez, M., Harmon, J.S., Robertson, R.P., 2013. Ebselen treatment prevents islet apoptosis, maintains intranuclear Pdx-1 and MafA levels, and preserves beta-cell mass and function in ZDF rats. Diabetes 62 (10), 3582–3588.
Martini, F., Pesarico, A.P., Bruning, C.A., Zeni, G., Nogueira, C.W., 2018. Ebselen inhibits the activity of acetylcholinesterase globular isoform G4 in vitro and attenuates scopolamine-induced amnesia in mice. J. Cell. Biochem. 119 (7), 5598–5608.
Masaki, C., Sharpley, A.L., Godlewska, B.R., Berrington, A., Hashimoto, T., Singh, N., Vasudevan, S.R., Emir, U.E., Churchill, G.C., Cowen, P.J., 2016. Effects of the potential lithium-mimetic, ebselen, on brain neurochemistry: a magnetic resonance spectroscopy study at 7 tesla. Psychopharmacology 233 (6), 1097–1104.
Mehla, J., Pahuja, M., Gupta, Y.K., 2013. Streptozotocin-induced sporadic Alzheimer's disease: selection of appropriate dose. J. Alzheim. Dis. : JAD 33 (1), 17–21.
Mishra, S.K., Singh, S., Shukla, S., Shukla, R., 2018. Intracerebroventricular streptozotocin impairs adult neurogenesis and cognitive functions via regulating neuroinflammation and insulin signaling in adult rats. Neurochem. Int. 113, 56–68.
Misra, H.P., Fridovich, I., 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247 (10), 3170–3175.
Noguchi, N., 2016. Ebselen, a useful tool for understanding cellular redox biology and a promising drug candidate for use in human diseases. Arch. Biochem. Biophys. 595, 109–112.
Nogueira, C.W., Zeni, G., Rocha, J.B., 2004. Organoselenium and organotellurium compounds: toxicology and pharmacology. Chem. Rev. 104 (12), 6255–6285.
Nosengo, N., 2016. Can you teach old drugs new tricks? Nature 534 (7607), 314–316.
Parnham, M., Sies, H., 2000. Ebselen: prospective therapy for cerebral ischaemia. Expet Opin. Invest. Drugs 9 (3), 607–619.
Parnham, M.J., Sies, H., 2013. The early research and development of ebselen. Biochem. Pharmacol. 86 (9), 1248–1253.
Paulsen, J.S., 2011. Cognitive impairment in Huntington disease: diagnosis and treatment. Curr. Neurol. Neurosci. Rep. 11 (5), 474–483.
Pollack, M., Phaneuf, S., Dirks, A., Leeuwenburgh, C., 2002. The role of apoptosis in the normal aging brain, skeletal muscle, and heart. Ann. N. Y. Acad. Sci. 959, 93–107.
Rosa, R.M., Flores, D.G., Appelt, H.R., Braga, A.L., Henriques, J.A., Roesler, R., 2003. Facilitation of long-term object recognition memory by pretraining administration of diphenyl diselenide in mice. Neurosci. Lett. 341 (3), 217–220.
Salakou, S., Kardamakis, D., Tsamandas, A.C., Zolota, V., Apostolakis, E., Tzelepi, V., Papathanasopoulos, P., Bonikos, D.S., Papapetropoulos, T., Petsas, T., Dougenis, D., 2007. Increased Bax/Bcl-2 ratio up-regulates caspase-3 and increases apoptosis in the thymus of patients with myasthenia gravis. In Vivo 21 (1), 123–132.
Salkovic-Petrisic, M., Knezovic, A., Hoyer, S., Riederer, P., 2013. What have we learned from the streptozotocin-induced animal model of sporadic Alzheimer's disease, about the therapeutic strategies in Alzheimer's research. J. Neural. Transm. 120 (1), 233–252.
Salom, J.B., Perez-Asensio, F.J., Burguete, M.C., Marin, N., Pitarch, C., Torregrosa, G., Romero, F.J., Alborch, E., 2004. Single-dose ebselen does not afford sustained neuroprotection to rats subjected to severe focal cerebral ischemia. Eur. J. Pharmacol. 495 (1), 55–62.
Santos, T.O., Mazucanti, C.H., Xavier, G.F., Torrao, A.S., 2012. Early and late neurodegeneration and memory disruption after intracerebroventricular streptozotocin. Physiol. Behav. 107 (3), 401–413.
Schoonheim, M.M., Popescu, V., Rueda Lopes, F.C., Wiebenga, O.T., Vrenken, H., Douw, L., Polman, C.H., Geurts, J.J., Barkhof, F., 2012. Subcortical atrophy and cognition: sex effects in multiple sclerosis. Neurology 79 (17), 1754–1761.
Selkoe, D.J., Hardy, J., 2016. The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Mol. Med. 8 (6), 595–608.
Sharma, U., Sahu, R., Roy, A., Golwala, D., 2010. In vivo antidiabetic and antioxidant potential of stephania hernandifolia in streptozotocin-induced-diabetic rats. J. Young Pharm. : JYP 2 (3), 255–260.
Shoham, S., Bejar, C., Kovalev, E., Weinstock, M., 2003. Intracerebroventricular injection of streptozotocin causes neurotoxicity to myelin that contributes to spatial memory deficits in rats. Exp. Neurol. 184 (2), 1043–1052.
Singh, N., Halliday, A.C., Thomas, J.M., Kuznetsova, O.V., Baldwin, R., Woon, E.C., Aley, P.K., Antoniadou, I., Sharp, T., Vasudevan, S.R., Churchill, G.C., 2013. A safe lithium mimetic for bipolar disorder. Nat. Commun. 4, 1332.
Singh, N., Sharpley, A.L., Emir, U.E., Masaki, C., Herzallah, M.M., Gluck, M.A., Sharp, T., Harmer, C.J., Vasudevan, S.R., Cowen, P.J., Churchill, G.C., 2016. Effect of the putative lithium mimetic ebselen on brain myo-inositol, sleep, and emotional processing in humans. Neuropsychopharmacology : Official Pub. Am. Coll. Neuropsychopharmacol. 41 (7), 1768–1778.
Snyder, J.S., Hong, N.S., McDonald, R.J., Wojtowicz, J.M., 2005. A role for adult neurogenesis in spatial long-term memory. Neuroscience 130 (4), 843–852.
Sosa-Ortiz, A.L., Acosta-Castillo, I., Prince, M.J., 2012. Epidemiology of dementias and Alzheimer's disease. Arch. Med. Res. 43 (8), 600–608.
Tiwari, V., Kuhad, A., Bishnoi, M., Chopra, K., 2009. Chronic treatment with tocotrienol, an isoform of vitamin E, prevents intracerebroventricular streptozotocin-induced cognitive impairment and oxidative-nitrosative stress in rats. Pharmacol. Biochem. Behav. 93 (2), 183–189.
Turon-Estrada, A., Lopez-Pousa, S., Gelada-Batlle, E., Garre-Olmo, J., Lozano-Gallego, M., Hernandez-Ferrandiz, M., Fajardo-Tibau, C., Morante-Munoz, V., Vilalta-Franch, J., 2003. Tolerance and adverse events of treatment with acetylcholinesterase inhibitors in a clinical sample of patients with very slight and mild Alzheimer s disease over a six-month period. Rev. Neurol. 36 (5), 421–424.
Unsal, C., Oran, M., Albayrak, Y., Aktas, C., Erboga, M., Topcu, B., Uygur, R., Tulubas, F., Yanartas, O., Ates, O., Ozen, O.A., 2016. Neuroprotective effect of ebselen against intracerebroventricular streptozotocin-induced neuronal apoptosis and oxidative stress in rats. Toxicol. Ind. Health 32 (4), 730–740.
Upadhyay, D., Kamp, D.W., 2003. Asbestos-induced pulmonary toxicity: role of DNA damage and apoptosis. Exp. Biol. Med. 228 (6), 650–659.
Walsh, D.M., Selkoe, D.J., 2004. Deciphering the molecular basis of memory failure in Alzheimer's disease. Neuron 44 (1), 181–193.
Wang, X., Yun, J.W., Lei, X.G., 2014. Glutathione peroxidase mimic ebselen improves glucose-stimulated insulin secretion in murine islets. Antioxidants Redox Signal. 20 (2), 191–203.
Wei, Q., Shi, F., 2013. Cleavage of poly (ADP-ribose) polymerase-1 is involved in the process of porcine ovarian follicular atresia. Anim. Reprod. Sci. 138 (3–4), 282–291.
Xie, Y., Tan, Y., Zheng, Y., Du, X., Liu, Q., 2017. Ebselen ameliorates beta-amyloid pathology, tau pathology, and cognitive impairment in triple-transgenic Alzheimer's disease mice. J. Biol. Inorg. Chem. : JBIC : Pub. Soc. Biol. Inorg. Chem. 22 (6), 851–865.

3.3. MANUSCRITO I

O EXERCÍCIO DE FORÇA SUPRIME O PREJUÍZO NA MEMÓRIA ESPACIAL INDUZIDA PELA STREPTOZOTOCINA E MODULA A VIA DE SINALIZAÇÃO CAMK - II/CREB NO HIPOCAMPO DE CAMUNDONGOS

STRENGTH EXERCISE SUPPRESSES STRETOZOTOCIN -INDUCED SPATIAL MEMORY IMPAIRMENT AND MODULATES BDNF/ERK - CAMK - II/CREB SIGNALING PATHWAY IN THE HIPPOCAMPUS OF MICE

Franciele Martini, Marlon Régis Leite, Suzan Gonçalves Rosa, Isabella Pregardier Klann, Cristina Wayne Nogueira

Strength exercise suppresses STZ-induced spatial memory impairment and modulates BDNF/ERK - CAMK - II/CREB signaling pathway in the hippocampus of mice

Franciele Martini[a], Marlon Régis Leite[a], Suzan Gonçalves Rosa[a], Isabella Pregardier Klann[a], Cristina Wayne Nogueira[a]

[a]Laboratório de Síntese, Reatividade e Avaliação Farmacológica e Toxicológica de Organocalcogênios, Departamento de Bioquímica e Biologia Molecular, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, CEP 97105-900, Santa Maria, Rio Grande do Sul, Brasil.

*Correspondence should be sent to:

Cristina Wayne Nogueira

Departamento de Bioquímica e Biologia Molecular, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, CEP 97105-900, RS, Brazil.

E-mail: criswn@ufsm.br

ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that has generated scientific interest in part as a reflection of its prevalence in the population. Evidence provided by studies with animal models and humans indicate that physical exercise promotes neuroplasticity and improves cognitive function. The aim of this study was to investigate the effects of 4-week strength exercise on the hippocampal protein contents and memory performance in mice subjected to a model of sporadic AD induced by streptozotocin (STZ). STZ (3 mg/kg, i.c.v.) was injected to adult male Swiss mice. Mice started the exercise training at the 21st day after induction with STZ and spent 4 weeks (5 day/week) in this training. Each training session consisted of 1 series of 10 repetitions of climbing a ladder (1 m high, with 1.5 cm grids). Mice performed the Morris Water Maze (MWM) test and samples of hippocampus were used to determine protein contents of BDNF/ERK - CAMK - II/CREB signaling pathway. Strength exercise was effective against the decrease in the time spent and distance traveled in the target quadrant induced by STZ. The strength exercise was effective against the reduction of *m*BDNF, TrkB and NeuN hippocampal protein levels in STZ-injected mice. The decrease in the hippocampal ratio of *p*ERK/ERK, *p*CAMKII/CAMKII and *p*CREB/CREB induced by STZ was reversed by strength exercise. Strength exercise modulated the levels of Bcl2 and Bax in the hippocampus of STZ-injected mice. The present study demonstrates that strength exercise suppressed STZ-induced spatial memory impairment and modulated the BDNF/ERK - CAMK - II/CREB signaling pathway in the hippocampus of mice.

Keywords: strength; exercise; memory; BDNF/ERK - CAMK - II/CREB

1. INTRODUCTION

Alzheimer's disease (AD) is the most prevalent type of dementia due to the increasing aging process of society [1]. Overall, 135 million people are estimated to be living with dementia by 2050, which will lead to an increase in the social, physical and economic burden for both patients and caregivers, becoming a major public health problem [2]. The complexity of AD, which involves multiple phenotypes, makes the discovery of new treatments even more difficult [3].

Considering that a healthy lifestyle improves mental activity and reduces the risk of various diseases (Khodadadi et al., 2018), neural repair and synaptic plasticity in the central nervous system (CNS) can be improved through non-pharmacological interventions [4]. Physical exercise is one of the most important neuroprotective, noninvasive and non-pharmacological interventions [5,6] in addition to be affordable and inexpensive for the population [7]. Physical exercise has been proposed as one of the ways to counteract neurological and cognitive disorders in animal models [8-10] and human beings [11]. Moreover, treadmill running reduced amyloid plaque and improved the mouse performance in the Morris Water Maze in a transgenic model of AD [12]. To date, the frequent practice of physical activity has been related to the lower AD risk and delay or slowing down the progression of the disease [13,14].

Although, the majority of the molecular targets involved in the positive effects of exercise in the CNS are still unknown [15,16], the mitogenic signaling of activated protein kinases (MAPK), multiple transducing agents that participate in neural plasticity [17,18], are activated by physical exercise [13]. Many of these changes in signaling have been observed in hippocampal formation, a region of the brain linked to learning, memory and emotional processes [19,20] and highly susceptible to damage in neurodegenerative diseases.

Considering that little is known about the effects of strength exercise in animal models of AD, the aim of this study was to investigate the effects of 4-week strength exercise on the hippocampal protein contents and memory performance in mice subjected to a model of sporadic AD induced by streptozotocin (STZ).

2. EXPERIMENTAL PROCEDURES

2.1. Animals

The experiments were carried out using male adult Swiss mice (25–35g) obtained from our breeding colony. The mice were housed in cages (5 mice per cage), with free access to food and water. The animals were kept in an air-conditioned room (22 ± 2ºC) under a 12:12 h light/dark cycle, with lights turned on at 7:00 a.m. The experimental procedures of this study were approved by the Institutional Ethics Committee on Care and Use of Experimental Animal Resources from the Federal University of Santa Maria-RS - Brazil (#7372110915). All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments.

2.2. Chemicals

STZ (3 mg/kg) was dissolved in citrate buffer (3 mg/ml, pH 4.4) [21] just prior to administration. The drug was administered by the intracerebroventricular (i.c.v) route in a volume of 5 µl/injection, at 1µl/min, in the lateral cerebral ventricles of mice. Appropriate vehicle-treated group was also assessed simultaneously.

2.3 Experimental protocol

The experimental design of this study is shown in **Fig. 1**. The animals were randomly assigned in three different groups as following: ***Group I*** – Vehicle: mice sham-operated injected with vehicle and that did not perform strength exercise (n=9 animals); ***Groups II*** – STZ: mice sham-operated injected with STZ and that did not perform strength exercise (n=8 animals). ***Group III*** – STZ + EXE: mice injected with STZ and that performed strength exercise (n=9 animals). In order to follow the principles of the 3Rs (Replacement, Reduction and Refinement), the design experimental of this study did not assign mice to the exercise group. The reduction of this group of animals is consistent with the scientific aim of this study.

After exercise protocol, the mice performed the Morris Water Maze test. After that, the mice were immediately killed by cervical dislocation and samples from the hippocampus were excised and stored at - 80 ^{o}C for western blot analyses.

2.3.1 Injection of STZ - i.c.v.

Adult male Swiss mice were anesthetized intraperitoneally (i.p) with ketamine (100 mg/kg) and xylazine (5 mg/kg) for i.c.v injections. The head of mouse was placed in the stereotaxic apparatus and a midline sagittal incision was made in the scalp. The stereotaxic coordinates for the lateral ventricle were measured accurately as anterio-posterior -0.8 mm, lateral 1.5 mm and dorso-ventral, -4.0 mm relative to bregma and ventral from dura with the tooth bar set at 0 mm. Through a skull hole, a 28-gauge Hamilton® syringe of 10 µl attached to a stereotaxic apparatus and piston of the syringe was lowered manually into lateral ventricle [22]. STZ (3 mg/kg, i.c.v., 1µl/min) was injected, on alternate days (days 1 and 3, Fig. 1), using a Hamilton microsyringe in a volume of 5 µl/injection in lateral cerebral ventricle of mice [23,24].

After surgery, the animals took approximately 1-2 h to recover from anesthesia. The mice were kept in a well-ventilated room at 25 ± 2 ºC in individual cages and provided with free access to food and water until they regained full consciousness. Food and water were placed inside the cage for 2-3 days so that the animals could easily access it without any physical trauma due to head injury.

2.3.2 Exercise training protocol

Mice started the exercise training at the 21st day after induction with STZ (Fig. 1). The animals spent 4 weeks (5 days a week) in this strength exercise training. The training consisted of climbing a ladder 1 m high, with 1.5 cm grids. Initially, the animals gained familiarity with the ladder climbing weightless for 5 days during the adaptation period. The initial weight adhered to the tail of each animal was 10% of their body weight and progressively increased up to 60% after 5 weeks (week 1: adaptation period, week 2: 10% of body weight, week 3: 20% of body weight, week 4: 40% of body weight; week 5: 60% of body weight). Each training session consisted of 1 series of 10 repetitions successful starts during the 4 weeks of exercise, with a rest of 1 min between sets[25]. To reduce stress, outsource stimuli such as food reward and electrical stimulation were not given to mice during exercise climbing stairs. In addition, the intensity was carefully adjusted for each animal in each exercise session.

2.3.3 Morris Water Maze test

The MWM test records the learning ability and visual spatial memory of the animals [26]. This test was carried out using a MWM device, including a circular swimming pool (180 cm in diameter, 60 cm height), of black color, filled to a depth of 25 cm with water at 22 ± 1 ° C. Little light was used for illumination and the room had sound insulation. Several visual cues were present. The pool had four quadrants with

four starting lines named north (N), east (E), south (S) and west (W), and a submerged platform (10 cm in diameter) centrally located 1 cm below the water in the N quadrant, painted black, was placed inside the target quadrant of this pool, 2 cm below the surface of the water. The platform was held in a constant position throughout the test. Under normal conditions, animals quickly learn to swim directly toward the platform and reach it in a shorter time. The procedure was performed on five consecutive days [27]. Each mouse underwent four (N-E-S-W) tests in the first 4 days of the test. The maximum time for the attempt was 120 s. If the mouse found the platform hidden within 120 s, it was held for an additional 20 s and then removed. The mouse that could not find the platform hidden during the designated time was gently guided to the platform and kept the control for 20 s. The mean escape latency time that is the time spent by each mouse to find the hidden platform was recorded for each mouse during each test performed during the four test days and was used as an index of acquisition or learning [28]. On the fifth day, the mice were subjected to a probe test session where the platform was removed from the pool and each mice was allowed to explore the pool for 60 s.The time spent by each mouse and the distance traveled in the target quadrant in which the hidden platform was previously placed was recorded as an indicator of recovery or memory [28,29].

2.3.4 Western blot assay

Samples of hippocampus (n=5-6 animals/group) were homogenized in Radioimmunoprecipitation assay buffer (RIPA buffer) solution containing 150 mM NaCl, 1.0% IGEPAL® CA-630, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris, pH 8.0, in the presence of commercial phosphatase and protein inhibitor cocktail (Sigma-Aldrich Company, St. Louis, Missouri, United States). The protein

concentration was determined by protein assay kit (Bio-Rad Laboratories, Hercules, CA). Tissue extracts were diluted to a final protein concentration 2 μg/μl. The samples (40 μg of protein) and pre stained molecular weight standards (Sigma-Aldrich Company, St. Louis, Missouri, United States) were separated on 10% resolving with 4% concentrating SDS-PAGE electrophoresis gels. Proteins were transferred to nitrocellulose membrane using Transfer-Blot® Turbo™ Transfer System (1.0 A; 45 min for proteins above 25 kDa or 5 min for proteins below 25 kDa). After blocking with 3% bovine serum albumin solution, the blots were incubated overnight at 4 °C with primary antibodies as shown in Table 1. β-actin was stained as additional control of the protein loading. After primary antibodies incubation, membranes were washed and incubated with secondary antibodies conjugated with horseradish peroxidase (Bio-Rad Laboratories, Hercules, CA, USA) for 1 h at room temperature and developed with chemiluminescence kit (Amersham, São Paulo/Brazil). Optical density (OD) of the Western blotting bands was quantified using Image J (NIH, Bethesda, MD, USA) software for Windows. Each value was derived from the ratio between arbitrary units obtained by the protein band and the respective β-actin band.

2.4. Statistical analysis

All experimental results are presented as the mean ± S.E.M. Normality of data was analyzed using a D'Agostino and Pearson omnibus normality test. Comparisons between experimental groups on escape latency were performed using repeated measures analysis (ANOVA) followed by the Newman - Keuls test. Other behavioral results and Western blot data were analyzed by one-way ANOVA followed by the Newman-Keuls test. All analyzes were performed using STATISTICA software for Windows version 7 (Stat Soft, Oklahoma, USA) by investigator blind to treatment. Values of probability less than 0.05 ($P < 0.05$) were considered statistically significant.

3. RESULTS

Strength exercise ameliorates spatial memory of STZ- injected mice

Fig. 2 A-D shows that the mice that performed strength exercise had a decrease in escape latency and an increase in the time spent and the distance traveled in the target quadrant in the MWM test. An average of the trials performed each day for each group was recorded. The latency to reach the platform was similar in all groups, on the first day of training. From the second day of training, the mice of all groups reached the platform in a shorter period of time. Fig. 2A shows the representative path traveled by mice in the labyrinth during training days.

Fig. 2B shows the decrease in escape latency of the animals over the training days. The repeated measures analysis of variance revealed that mice from the STZ-EXE group performed better over the days when compared to those of the STZ group [$F_{(2.95)} = 3.63$, $P < 0.001$]. On the day of the probe test, the STZ group showed a significant decrease in time spent and distance traveled in the target quadrant, where the platform was previously located, when compared to the other groups. The mice from the STZ-EXE group showed an increase in the distance traveled ([$F_{(2.23)} = 11.04$, $P < 0.0001$], Fig. 2C) and in the time spent in the target quadrant ([$F_{(2.23)} = 10.08$, $P < 0.0001$], Fig. 2D) when compared to those of the STZ group.

Strength exercise has neuroprotective and anti-apoptotic effects in hippocampus of STZ- injected mice

Representative qualitative images of *m*-BDNF, TrκB, *p*CAMKII/CAMKII, *p*ERK/ERK, *p*CREB/CREB western blotting analyses are shown in Fig. 3 and 4.

The hippocampal protein levels of *m*BDNF (Fig. 3A) and TrkB (Fig. 3B) were reduced in STZ-SED mice when compared to those of the vehicle group. The strength exercise increased *m*BDNF [$F_{(2,15)} = 20.34$, $P < 0.0001$] and TrkB levels [$F_{(2,14)} = 13.36$, $P < 0.0001$] in the hippocampus of mice experimentally induced with STZ.

A statistically significant increase in the ratios of *p*ERK/ERK [$F_{(2,15)} = 10.52$, $P < 0.001$ Fig.3C], *p*CAMKII/CAMKII [$F_{(2,15)} = 4.62$, $P < 0.01$ Fig. 3D] and *p*CREB/CREB [$F_{(2,16)} = 7.73$, $P < 0.001$ Fig. 3E] was found in the hippocampus of STZ-EXE mice when compared to those of the STZ group.

Fig. 4B shows the reduction of protein content of NeuN in the hippocampus of STZ-SED mice when compared to those of the vehicle group and strength exercise was effective against this decrease [$F_{(2,17)} = 25.27$, $P < 0.0001$].

Fig. 4C and 4D show a significant increase in the protein levels of Bcl_2 [$F_{(2,15)} = 8.87$, $P < 0.01$] and a decrease in those of Bax [$F_{(2,16)} = 5.87$, $P < 0.05$] in the hippocampus of STZ mice and strength exercise was effective against the dysregulation of these protein levels induced by STZ.

4. DISCUSSION

The present study demonstrates the effectiveness of a strength exercise strategy against spatial memory impairment in a mouse model of sporadic AD induced by STZ. Moreover, to the best of our knowledge, this is the first study in which strength exercise suppressed STZ-induced memory impairment and modulated the BDNF/ERK - CAMK - II/CREB signaling pathway in the hippocampus of mice.

As memory impairment and dementia are significant features of neurodegenerative diseases, more specifically of AD, it is not surprising that much of the published scientific literature on cognitive enhancement under any neurological conditions is associated with animal models of AD [30,31]. In addition, this is

consistent with clinical studies exploring the effects of new compounds on animal models of AD [31]. To help understanding whether pharmacotherapy is the only viable option for managing AD, in this study we turned our attention to the strength exercise as a noninvasive and non-pharmacological intervention in a mouse model of sporadic AD.

Overall exercise has positive effects on brain functions and in disorders associated with neurodegenerative processes, such as Alzheimer's and Parkinson's disease models [32-35]. In agreement with Sahay et al. [36] results, our behavioral data demonstrate that 4-week strength exercise increased performance of mice in a memory task. Although in the Sahay et al. [36] study, the hippocampal neurogenesis and memory increment were demonstrated in a mouse model of voluntary exercise.

The findings of our study indicate that the integrity of *m*BDNF signaling was affected in the hippocampus of STZ-injected mice and strength exercise promoted an increase in *m*BDNF and TrκB levels, and in downstream proteins, such as *p*ERK / ERK and *p*CAMK-II/CAMK-II ratios, which could lead to increased levels of NeuN in the hippocampus of mice. It has been demonstrated that the integrity of BDNF signaling is affected in neurodegenerative events [37] and that deficient conversion of pro-BDNF to *m*BDNF and its signaling, via TrκB, contributes to cognitive dysfunctions in cases of AD [38].

In addition, CREB, a cyclic AMP responsive element binding protein, is well known to play a key role in mediating synaptic plasticity and promoting neuronal survival in the brain [39-41]. Accordingly, the *p*-CREB/*t*-CREB ratio was increased in response to strength exercise in hippocampus of mice injected with STZ. The suppressive effect of CREB activation on cell death is linked to CREB-mediated transcription of Bcl_2, an anti-apoptotic gene, and CREB-activated cell survival against

stress stimuli is linked to CREB phosphorylation through of MAPK-dependent form and CaMK- II [39,42,41].

The findings of this study show that strength exercise promoted an anti-apoptotic effect because it reduced the protein levels of Bax and increased the levels of Bcl_2, in addition to trigger the activation of the CREB and BDNF pathway mediated by CaMK-II. Collectively these findings suggest that strength exercise induced a cooperative activation of CREB in a manner dependent of ERK and CaMK- II.

It is important to note that the decrease of apoptotic pathway activation, demonstrated in this study, may also be involved with the increase in the levels of NeuN. A plausible explanation to this seems to be that new neurons can be produced because *m*BDNF signaling is involved with both survival and neurogenesis; or an increase in the cellular survival would be a consequence of anti-apoptotic effects of strength exercise. However, these hypotheses should be interpreted with caution, because we did not determine specific markers of neurogenesis in this study.

The present study demonstrates, to the best of our knowledge, for the first time that strength exercise suppressed memory impairment and modulated the hippocampal BDNF/ERK - CAMK - II/ CREB signaling pathway in a mouse model of AD induced by STZ. In addition, the beneficial effects of strength exercise seem to recruit anti apoptotic mechanisms, as seen in the hippocampal levels of Bax and Bcl_2.

Acknowledgements

We gratefully acknowledge Universidade Federal de Santa Maria (UFSM), Fundação de Amparo a Pesquisa do Estado do Rio Grande do Sul (FAPERGS, grant number 17/2551- 0000) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES/PROEX #23038.005848/2018-31) for the financial support. C.W.N is recipient of CNPq fellowship (#304864/2015-3).

Conflict of interest: The authors declare that they have no conflict of interest.

5. REFERENCES

World Health Organization, Ageing and Health, Fact Sheet N°404, September 2015. www.who.int/mediacentre/factsheets/fs404/en/. Accessed June 15, 2018.

1. Ebrahimi K, Majdi A, Baghaiee B, Hosseini SH, Sadigh-Eteghad S (2017) Physical activity and beta-amyloid pathology in Alzheimer's disease: A sound mind in a sound body. EXCLI journal 16:959-972. doi:10.17179/excli2017-475
2. Mavros Y, Gates N, Wilson GC, Jain N, Meiklejohn J, Brodaty H, Wen W, Singh N, Baune BT, Suo C, Baker MK, Foroughi N, Wang Y, Sachdev PS, Valenzuela M, Fiatarone Singh MA (2017) Mediation of Cognitive Function Improvements by Strength Gains After Resistance Training in Older Adults with Mild Cognitive Impairment: Outcomes of the Study of Mental and Resistance Training. Journal of the American Geriatrics Society 65 (3):550-559. doi:10.1111/jgs.14542
3. Yuede CM, Timson BF, Hettinger JC, Yuede KM, Edwards HM, Lawson JE, Zimmerman SD, Cirrito JR (2018) Interactions between stress and physical activity on Alzheimer's disease pathology. Neurobiology of stress 8:158-171. doi:10.1016/j.ynstr.2018.02.004
4. Mattson MP (2012) Energy intake and exercise as determinants of brain health and vulnerability to injury and disease. Cell metabolism 16 (6):706-722. doi:10.1016/j.cmet.2012.08.012
5. Ma CL, Ma XT, Wang JJ, Liu H, Chen YF, Yang Y (2017) Physical exercise induces hippocampal neurogenesis and prevents cognitive decline. Behavioural brain research 317:332-339. doi:10.1016/j.bbr.2016.09.067
6. Sajadi A, Amiri I, Gharebaghi A, Komaki A, Asadbeigi M, Shahidi S, Mehdizadeh M, Soleimani Asl S (2017) Treadmill exercise alters ecstasy- induced long- term potentiation disruption in the hippocampus of male rats. Metabolic brain disease 32 (5):1603-1607. doi:10.1007/s11011-017-0046-9
7. Kramer AF, Erickson KI, Colcombe SJ (2006) Exercise, cognition, and the aging brain. Journal of applied physiology 101 (4):1237-1242. doi:10.1152/japplphysiol.00500.2006
8. Ahlskog JE, Geda YE, Graff-Radford NR, Petersen RC (2011) Physical exercise as a preventive or disease-modifying treatment of dementia and brain aging. Mayo Clinic proceedings 86 (9):876-884. doi:10.4065/mcp.2011.0252
9. Ferreira-Vieira TH, Bastos CP, Pereira GS, Moreira FA, Massensini AR (2014) A role for the endocannabinoid system in exercise-induced spatial memory enhancement in mice. Hippocampus 24 (1):79-88. doi:10.1002/hipo.22206
10. Gomez-Pinilla F, Hillman C (2013) The influence of exercise on cognitive abilities. Comprehensive Physiology 3 (1):403-428. doi:10.1002/cphy.c110063
11. Pietrelli A, Lopez-Costa J, Goni R, Brusco A, Basso N (2012) Aerobic exercise prevents age-dependent cognitive decline and reduces anxiety-related behaviors in middle-aged and old rats. Neuroscience 202:252-266. doi:10.1016/j.neuroscience.2011.11.054
12. Zhao G, Liu HL, Zhang H, Tong XJ (2015) Treadmill exercise enhances synaptic plasticity, but does not alter beta-amyloid deposition in hippocampi of aged APP/PS1 transgenic mice. Neuroscience 298:357-366. doi:10.1016/j.neuroscience.2015.04.038

13. Abd El-Kader SM, Al-Jiffri OH (2016) Aerobic exercise improves quality of life, psychological well-being and systemic inflammation in subjects with Alzheimer's disease. African health sciences 16 (4):1045-1055. doi:10.4314/ahs.v16i4.22
14. Bagyinszky E, Giau VV, Shim K, Suk K, An SSA, Kim S (2017) Role of inflammatory molecules in the Alzheimer's disease progression and diagnosis. Journal of the neurological sciences 376:242-254. doi:10.1016/j.jns.2017.03.031
15. Andel R, Crowe M, Pedersen NL, Fratiglioni L, Johansson B, Gatz M (2008) Physical exercise at midlife and risk of dementia three decades later: a population-based study of Swedish twins. The journals of gerontology Series A, Biological sciences and medical sciences 63 (1):62-66
16. Geda YE, Roberts RO, Knopman DS, Christianson TJ, Pankratz VS, Ivnik RJ, Boeve BF, Tangalos EG, Petersen RC, Rocca WA (2010) Physical exercise, aging, and mild cognitive impairment: a population-based study. Archives of neurology 67 (1):80-86. doi:10.1001/archneurol.2009.297
17. Kaminska B (2005) MAPK signalling pathways as molecular targets for anti-inflammatory therapy--from molecular mechanisms to therapeutic benefits. Biochimica et biophysica acta 1754 (1-2):253-262. doi:10.1016/j.bbapap.2005.08.017
18. Layden MJ, Johnston H, Amiel AR, Havrilak J, Steinworth B, Chock T, Rottinger E, Martindale MQ (2016) MAPK signaling is necessary for neurogenesis in Nematostella vectensis. BMC biology 14:61. doi:10.1186/s12915-016-0282-1
19. Hopkins ME, Davis FC, Vantieghem MR, Whalen PJ, Bucci DJ (2012) Differential effects of acute and regular physical exercise on cognition and affect. Neuroscience 215:59-68. doi:10.1016/j.neuroscience.2012.04.056
20. Kesner RP, Lee I, Gilbert P (2004) A behavioral assessment of hippocampal function based on a subregional analysis. Reviews in the neurosciences 15 (5):333-351
21. Tiwari V, Kuhad A, Bishnoi M, Chopra K (2009) Chronic treatment with tocotrienol, an isoform of vitamin E, prevents intracerebroventricular streptozotocin-induced cognitive impairment and oxidative-nitrosative stress in rats. Pharmacology, biochemistry, and behavior 93 (2):183-189. doi:10.1016/j.pbb.2009.05.009
22. Kroon JP, Riley AL (1986) A microcomputer-based system for stereotaxic coordinates in the rat brain. Physiology & behavior 38 (4):593-596
23. Deshmukh R, Sharma V, Mehan S, Sharma N, Bedi KL (2009) Amelioration of intracerebroventricular streptozotocin induced cognitive dysfunction and oxidative stress by vinpocetine -- a PDE1 inhibitor. European journal of pharmacology 620 (1-3):49-56. doi:10.1016/j.ejphar.2009.08.027
24. Sharma U, Sahu R, Roy A, Golwala D (2010) In vivo Antidiabetic and Antioxidant Potential of Stephania hernandifolia in Streptozotocin-Induced-Diabetic Rats. Journal of young pharmacists : JYP 2 (3):255-260. doi:10.4103/0975-1483.66803
25. Kim HJ, So B, Choi M, Kang D, Song W (2015) Resistance exercise training increases the expression of irisin concomitant with improvement of muscle function in aging mice and humans. Experimental gerontology 70:11-17. doi:10.1016/j.exger.2015.07.006
26. D'Hooge R, De Deyn PP (2001) Applications of the Morris water maze in the study of learning and memory. Brain research Brain research reviews 36 (1):60-90
27. Gupta R, Gupta LK (2012) Improvement in long term and visuo-spatial memory following chronic pioglitazone in mouse model of Alzheimer's disease. Pharmacology, biochemistry, and behavior 102 (2):184-190. doi:10.1016/j.pbb.2012.03.028
28. Singh B, Sharma B, Jaggi AS, Singh N (2013) Attenuating effect of lisinopril and telmisartan in intracerebroventricular streptozotocin induced experimental dementia of Alzheimer's disease type: possible involvement of PPAR-gamma agonistic property. Journal of the renin-angiotensin-aldosterone system : JRAAS 14 (2):124-136. doi:10.1177/1470320312459977

29. Blokland A, Geraerts E, Been M (2004) A detailed analysis of rats' spatial memory in a probe trial of a Morris task. Behavioural brain research 154 (1):71-75. doi:10.1016/j.bbr.2004.01.022
30. Hugo J, Ganguli M (2014) Dementia and cognitive impairment: epidemiology, diagnosis, and treatment. Clinics in geriatric medicine 30 (3):421-442. doi:10.1016/j.cger.2014.04.001
31. LaFerla FM, Green KN (2012) Animal models of Alzheimer disease. Cold Spring Harbor perspectives in medicine 2 (11). doi:10.1101/cshperspect.a006320
32. Asl NA, Sheikhzade F, Torchi M, Roshangar L, Khamnei S (2008) Long-term regular exercise promotes memory and learning in young but not in older rats. Pathophysiology : the official journal of the International Society for Pathophysiology 15 (1):9-12. doi:10.1016/j.pathophys.2007.10.002
33. Hoveida R, Alaei H, Oryan S, Parivar K, Reisi P (2011) Treadmill running improves spatial memory in an animal model of Alzheimer's disease. Behavioural brain research 216 (1):270-274. doi:10.1016/j.bbr.2010.08.003
34. Cotman CW, Berchtold NC (2002) Exercise: a behavioral intervention to enhance brain health and plasticity. Trends in neurosciences 25 (6):295-301
35. Lee JM, Park JM, Song MK, Oh YJ, Kim CJ, Kim YJ (2017) The ameliorative effects of exercise on cognitive impairment and white matter injury from blood-brain barrier disruption induced by chronic cerebral hypoperfusion in adolescent rats. Neuroscience letters 638:83-89. doi:10.1016/j.neulet.2016.12.018
36. Sahay A, Scobie KN, Hill AS, O'Carroll CM, Kheirbek MA, Burghardt NS, Fenton AA, Dranovsky A, Hen R (2011) Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. Nature 472 (7344):466-470. doi:10.1038/nature09817
37. von Bohlen Und Halbach O, von Bohlen Und Halbach V (2018) BDNF effects on dendritic spine morphology and hippocampal function. Cell and tissue research. doi:10.1007/s00441-017-2782-x
38. Greenberg ME, Xu B, Lu B, Hempstead BL (2009) New insights in the biology of BDNF synthesis and release: implications in CNS function. The Journal of neuroscience : the official journal of the Society for Neuroscience 29 (41):12764-12767. doi:10.1523/JNEUROSCI.3566-09.2009
39. Davis S, Vanhoutte P, Pages C, Caboche J, Laroche S (2000) The MAPK/ERK cascade targets both Elk-1 and cAMP response element-binding protein to control long-term potentiation-dependent gene expression in the dentate gyrus in vivo. The Journal of neuroscience : the official journal of the Society for Neuroscience 20 (12):4563-4572
40. Mabuchi T, Kitagawa K, Kuwabara K, Takasawa K, Ohtsuki T, Xia Z, Storm D, Yanagihara T, Hori M, Matsumoto M (2001) Phosphorylation of cAMP response element-binding protein in hippocampal neurons as a protective response after exposure to glutamate in vitro and ischemia in vivo. The Journal of neuroscience : the official journal of the Society for Neuroscience 21 (23):9204-9213
41. Kwon MS, Seo YJ, Choi SM, Choi HW, Jung JS, Park SH, Suh HW (2007) The differential effects of single or repeated restraint stress on kainic acid-induced neuronal death in the hippocampal CA3 region: the role of glucocorticoid and various signal molecules. Journal of neurochemistry 103 (4):1530-1541. doi:10.1111/j.1471-4159.2007.04865.x
42. Finkbeiner S (2000) CREB couples neurotrophin signals to survival messages. Neuron 25 (1):11-14

Figure Captions

Fig. 1. Experimental design of this study.

Fig. 2. Effects of strength exercise on path traveled (A); escape latency (B); distance traveled (C) and time spent (D) in the target quadrant in the MWM test of STZ- injected mice. Values are expressed as mean ± S.E.M. of 8-9 animals. Asterisk denotes the significance levels when compared to the vehicle group: (*) $P < 0.05$; (**) $P < 0.01$. Delta denotes the significance levels when compared to the STZ group: ($^{\delta\delta}$) $P < 0.01$. ($^{\delta\delta\delta}$) $P < 0.001$ (one -way ANOVA followed by the Newman Keuls).

Fig. 3. Effects of strength exercise on protein contents of *m*BDNF (A), TrkB (B), *p*ERK/ERK (C), *p*CAMKII/CAMKII (D) and *p*CREB/CREB (E) in the hippocampus of STZ-injected mice. Values are expressed as mean ± S.E.M. of 5 - 6 animals. Asterisk denotes the significance levels when compared to the vehicle group: (*) $P < 0.05$, (**) $P < 0.01$. Delta denotes the significance levels when compared to the STZ group: ($^{\delta}$) $P < 0.05$; ($^{\delta\delta}$) $P < 0.01$ and ($^{\delta\delta\delta}$) $P < 0.001$ (one - way ANOVA followed by the Newman Keuls). Photographs are representative images of one mouse of each group of qualitative Western blotting analyses normalized to β-actin protein.

Fig. 4. Effects of strength exercise on protein contents of NeuN (A), Bax (B) and Bcl2 (C) in the hippocampus of STZ-injected mice. Values are expressed as mean ± S.E.M. of 5 - 6 animals. Asterisk denotes the significance levels when compared to the vehicle group: (*) $P < 0.05$, (**) $P < 0.01$. Delta denotes the significance levels when compared to the STZ group: ($^{\delta}$) $P < 0.05$; ($^{\delta\delta}$) $P < 0.01$ and ($^{\delta\delta\delta}$) $P < 0.001$ (one - way ANOVA followed by the Newman Keuls). Photographs are representative images of one mouse of each group of qualitative Western blotting analyses normalized to β-actin protein.

Fig. 1.

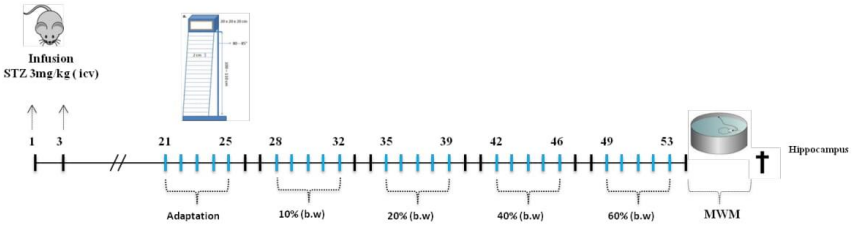

Infusion
STZ 3mg/kg (icv)
1
3
21
25
28
32
35
39
42
46
49
53
Hippocampus
Adaptation
10% (b.w)
20% (b.w)
40% (b.w)
60% (b.w)
MWM

Fig. 2.

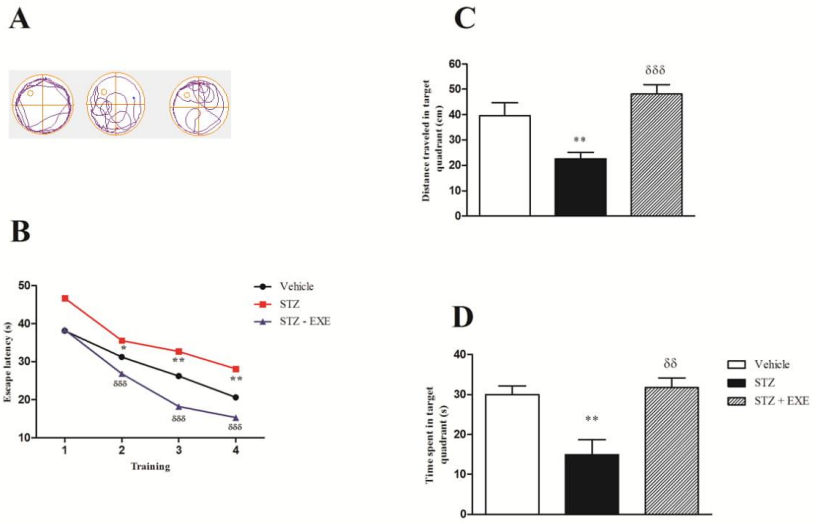

A
B
C
D
Escape latency (s)
Training
Vehicle
STZ
STZ - EXE
Distance traveled in target quadrant (cm)
Time spent in target quadrant (s)
STZ + EXE

Fig. 3.

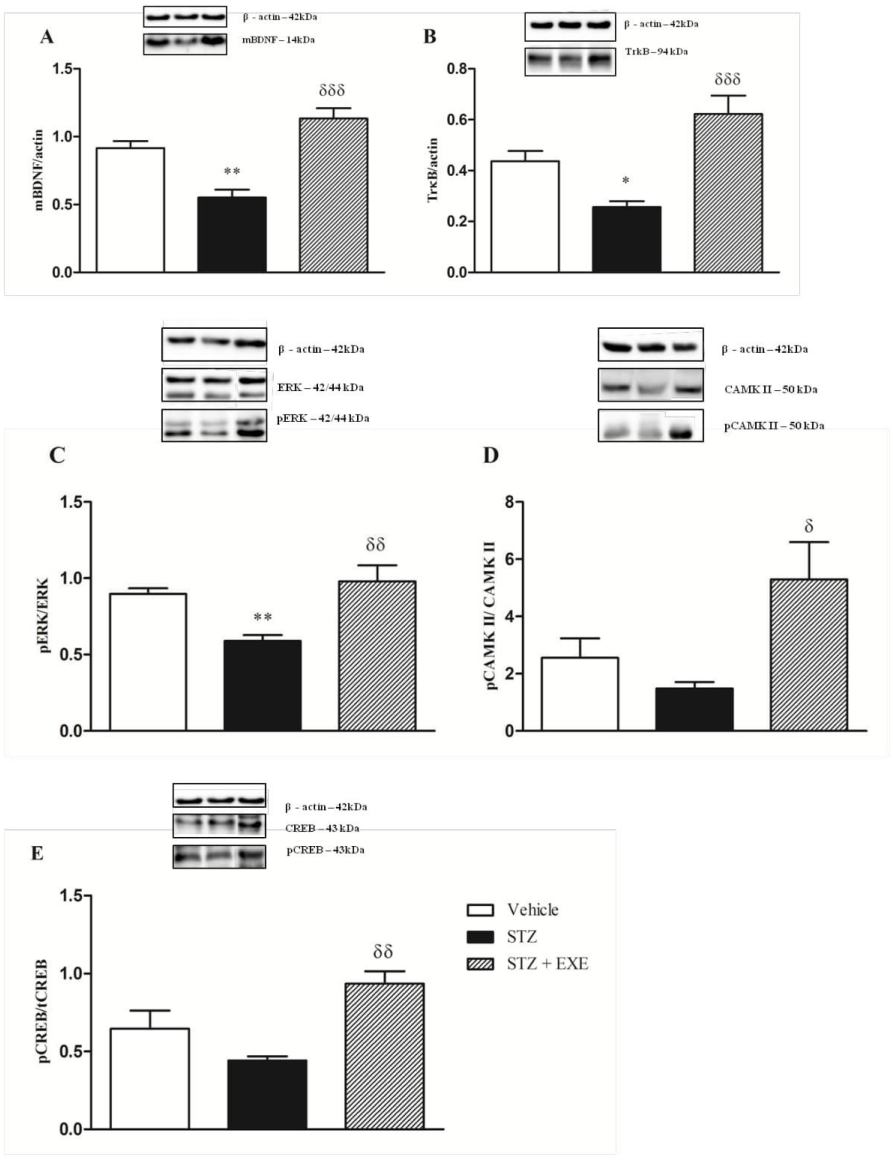

A
β - actin – 42kDa
mBDNF – 14kDa
mBDNF/actin
1.5
1.0
0.5
0.0
**
δδδ
B
β - actin – 42kDa
TrkB – 94 kDa
TrkB/actin
0.8
0.6
0.4
0.2
0.0
*
δδδ
β - actin – 42kDa
ERK – 42/44 kDa
pERK – 42/44 kDa
C
pERK/ERK
1.5
1.0
0.5
0.0
**
δδ
β - actin – 42kDa
CAMK II – 50 kDa
pCAMK II – 50 kDa
D
pCAMK II/ CAMK II
8
6
4
2
0
δ
β - actin – 42kDa
CREB – 43 kDa
pCREB – 43kDa
E
pCREB/CREB
1.5
1.0
0.5
0.0
δδ
Vehicle
STZ
STZ + EXE

Fig. 4.

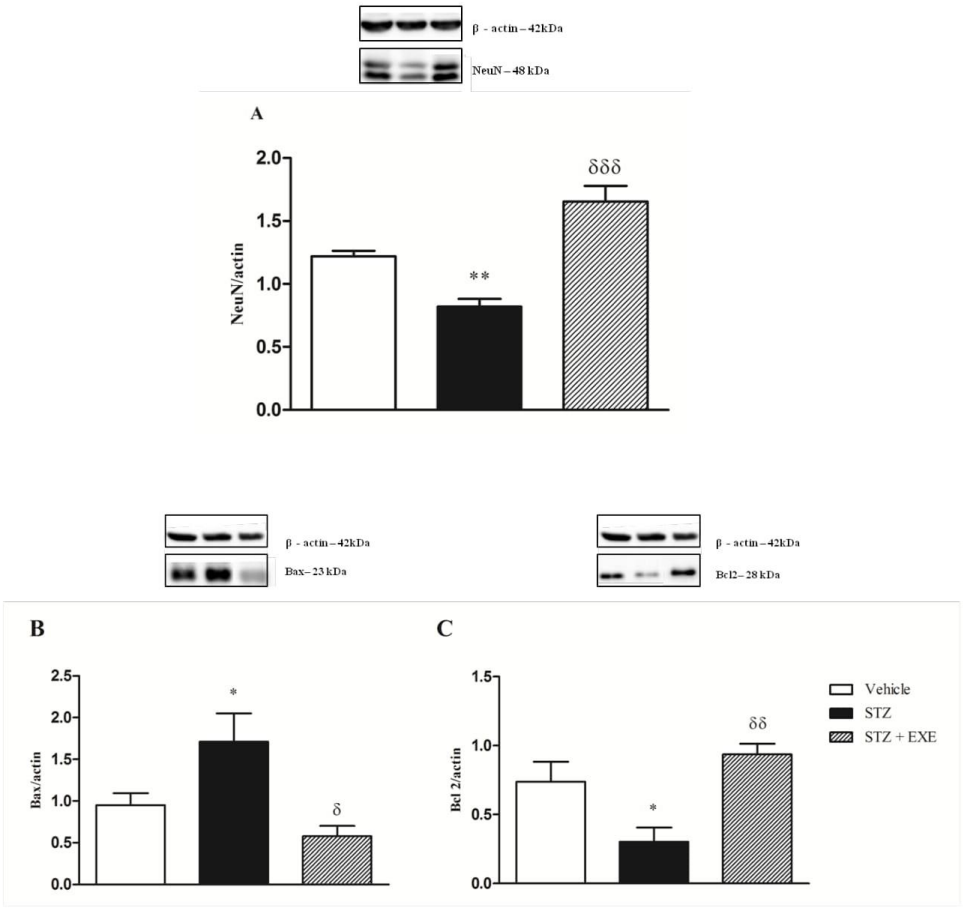

β - actin – 42kDa
NeuN – 48 kDa
A
NeuN/actin
2.0
1.5
1.0
0.5
0.0
**
δδδ
β - actin – 42kDa
Bax – 23 kDa
β - actin – 42kDa
Bcl2 – 28 kDa
B
Bax/actin
2.5
2.0
1.5
1.0
0.5
0.0
*
δ
C
Bcl 2/actin
1.5
1.0
0.5
0.0
*
δδ
Vehicle
STZ
STZ + EXE

Table 1. List of primary antibodies and their dilutions

Antibody	Molecular Weight (kDa)	Type	Company	Dilution
mBDNF	14	rabbit	Abcam	1:1000
TrκB	94	rabbit	Abcam	1:1000
*p*ERK	42/44	rabbit	Abcam	1:1000
ERK	42/44	rabbit	Cell Signaling Technology	1:1000
*p*CAMKII	50	rabbit	Cell Signaling Technology	1:1000
CAMKII	50	rabbit	Cell Signaling Technology	1:1000
pCREB	43	rabbit	Cell Signaling Technology	1:1000
CREB	43	rabbit	Cell Signaling Technology	1:1000
NeuN	48/46	mouse	Millipore	1:1000
Bax	23	rabbit	Cell Signaling Technology	1:1000
Bcl2	28	rabbit	Cell Signaling Technology	1:1000
β-actin	42	mouse	Abcam	1:5000

BDNF (brain-derived neurotrophic factor); TrκB (tropomyosin-related kinase B); ERK (extracellular element binding protein); CREB (cAMP-response element binding protein) (κ-opioid receptor); CAMK II (Ca^{2+} /calmodulin-dependent protein kinase II); NeuN (neuronal nuclear protein).

4. DISCUSSÃO

A DA é uma doença heterogênea e com patofisiologia complexa, a qual se manifesta em múltiplos sintomas fisiológicos, comportamentais e funcionais, que juntos, levam à incapacidade (ZUCCHELLA et al., 2018) . Sendo um dos principais problemas de saúde em indivíduos idosos, leva a deterioração progressiva da cognição, funcionamento diário da atividade e comportamento que, em conjunto, levam à falta de um tratamento modificador e eficaz do percurso da doença (ZUCCHELLA et al., 2018). Os inibidores das colinesterases e a memantina, um antagonista não-competitivo do receptor NMDA, são os únicos fármacos sintomáticos comercialmente disponíveis, no entanto seu impacto clínico permanece modesto e controverso (BIRKS, J. e CRAIG, 2006; BIRKS, J. S. et al., 2015).

Neste sentido, além dos diferentes efeitos farmacológicos do composto orgânico de selênio, ebselen, como antinflamatório, antioxidante, neuroprotetor, estudos têm demonstrado um grande interesse por drogas multifuncionais.

O ebselen é um composto que apresenta benefícios em diferentes modelos animais de estudo(NOGUCHI, 2016; NOSENGO, 2016), dentre seus efeitos, foi capaz de reproduzir esses efeitos em ensaios clínicos com humanos, como uma possível alternativa para o tratamento de transtorno bipolar (MASAKI et al., 2016; SINGH et al., 2013) para perda auditiva induzida por ruído(MAHADEVAN et al., 2013; WANG, X. et al., 2014b). De fato, os efeitos neuroprotetores do ebselen foram reconhecidos e seus alvos multifatoriais parecem ser uma vantagem para as perspectivas terapêuticas (LUO et al., 2013; PARNHAM, M. J. e SIES, 2013; SINGH et al., 2016). Além disso, nenhum evento foi relatado com relação à efeitos colaterais e toxicidade, nas doses administradas de ebselen (SINGH et al., 2016).

De modo geral, os resultados que aqui foram apresentados mostram que o composto ebselen é capaz de melhorar a memória de reconhecimento, localização e memória espacial. Observou-se que, o ebselen corroborou positivamente no desempenho da memória em modelo de amnésia induzido por escopolamina e no modelo de DA esporádica induzido por ETZ i.c.v. (**artigo 1 e artigo 2**).

Inicialmente, observamos que a hipótese colinérgica para a demência sugere que esta, com déficits na memória, no aprendizado e mudanças no comportamento, seja causada, pelo menos em parte, por um decréscimo nos níveis de ACh no cérebro (BALLARD et al., 2005b). Assim, uma redução na atividade da enzima AChE foi demonstrada no córtex

cerebral e no hipocampo de pacientes afetados por doenças neurodegenerativas (FISHMAN et al., 1986), sugerindo que alterações em sua atividade pode estar associada com as alterações cognitivas características de doenças neurodegenerativas (CUMMINGS et al., 2014; LAW et al., 2001). Por outro lado, a degeneração dos terminais nervosos colinérgicos em regiões cerebrais específicas resulta numa redução da forma globular tetramétrica, isoforma G4, associada à membrana da AChE (YOUNKIN et al., 1986). Baseado nesses relatos, inibidores reversíveis das colinesterases têm sido usados como estimuladores cognitivos no tratamento terapêutico da DA (GREIG et al., 2001).

O envolvimento do ebselen com o sistema colinérgico também já foi evidenciado em outros estudos. Nosso grupo de pesquisa reforçou as evidências sobre o envolvimento do ebselen na atividade da AChE, assim este composto foi caracterizado como um inibidor misto e reversível da AChE *in vitro* (MARTINI et al., 2015). Conforme os resultados obtidos no **artigo I,** o ebselen inibiu a atividade da AChE/G4 hipocampal *in vitro.* No entanto, não foi capaz de alterar a atividade desta enzima *ex vivo*, o que poderia ser explicado pelo método utilizado, caracterizado por um processo demorado de múltiplas lavagens e centrifugações e o ebselen apresentar uma inibição cinética reversível, como foi caracterizado anteriormente *in vitro* (MARTINI et al., 2015).

Ainda, os resultados apresentados no **artigo 1** primeiramente estenderam os estudos sobre a forma em que o composto ebselen atua sobre as isoformas da AChE – G1 e G4. Desta forma, o **artigo 1** revelou pela primeira vez que o ebselen inibe a atividade da isoforma AChE/G4, no córtex e no hipocampo de ratos *in vitro*. Já *ex vivo*, inibiu a fração G4 no córtex cerebral e a G1 no hipocampo. Além disso, o ebselen mostrou um efeito neuroprotetor em um modelo de amnésia induzida pela escopolamina, protegendo desses efeitos deletérios nos testes comportamentais. Somado a isso, este composto apresentou uma inibição na atividade da AChE, aos níveis do controle, no hipocampo dos camundongos, neste mesmo modelo. Por essa razão, nossos resultados sugerem que este composto foi capaz de modular a disfunção na neurotransmissão colinérgica induzida pela escopolamina (**artigo 1**). Ainda, estes resultados reforçam àqueles previamente demonstrados pelo nosso grupo, os quais revelam o ebselen como um inibidor misto e reversível da AChE *in vitro* (MARTINI et al., 2015). Interessantemente, os inibidores da AChE utilizados no tratamento clínico da DA são seletivos para a isoforma G4 que está, relativamente, mais relacionada com a cognição quando comparado com a isoforma G1 (DAS et al., 2005). Esta informação sugere que a isoforma G4 é a forma fisiologicamente

relevante da AChE nas sinapses colinérgicas, e espera-se que a sua inibição prolongue a ação da ACh.

Em conjunto, os resultados obtidos e descritos no **artigo 1** são relevantes por apresentarem os primeiros passos para a compreensão do efeito neuroprotetor do ebselen na DA. Uma vez que, o possível mecanismo envolvido no efeito do ebselen em melhorar o desempenho cognitivo dos camundongos nos testes TRO e no labirinto em Y podem ser atribuídos a uma modulação da neurotransmissão colinérgica, vista no **artigo 1**, no qual o fato da atividade da AChE cerebral ter aumentado em decorrência da injeção de escopolamina nos animais, leva à uma diminuição dos níveis de ACh na fenda sináptica, prejudicando assim a memória destes. No entanto, esta hipótese não nos permite afirmar se o ebselen poderia aumentar os níveis do neurotransmissor ACh, uma vez que este ensaio não foi realizado, uma limitação deste estudo.

Os resultados apresentados no **artigo 1** foram promissores e trazem indícios do papel neuroprotetor do ebselen na prevenção e progressão da em um modelo animal da DA. Contudo, para mimetizar uma situação onde o prejuízo na memória e no aprendizado, assim como as disfunções bioquímicas fossem mais consistentes e bem estabelecidas, delineamos o protocolo 2 desta tese utilizando o modelo de DA esporádica, induzida com ETZ i.c.v. em camundongos, e assim avaliamos o efeito terapêutico do ebselen (**artigo 2**).

Um ponto importante à destacar-se, no **artigo 1** utilizamos a dose de 50 mg/kg, pois doses mais baixas testadas no protocolo 1, da escopolamina, não foram encontrados resultados eficazes do ebselen em cinco dias de tratamento. Além disso, visto que a molécula de ebselen caracteriza-se como lipossolúvel, tivemos alguns impasses para encontrar um veículo ideal para diluição da droga. Desta forma, após várias tentativas e diversos veículos utilizados, conseguimos modificar o protocolo 1 e assim, obter doses mais baixas no protocolo 2.

O protocolo experimental utilizado no **artigo 2** visou um tratamento terapêutico e repetido com doses mais baixas de ebselen, sendo 1 e 10 mg/kg. Durante os 21 dias após a indução com a ETZ, os grupos diferentes (Sham e ETZ) foram monitorados em seus pesos e sua alimentação e durante este período não observou-se sinais de infecção e perda de peso devido à cirurgia. Ao final dos 21 dias também não se notou alterações da glicemia, no qual foi monitorado no dia 21 pós-operatório, eliminando qualquer indício de hiperglicemia, um resultado já esperado, uma vez que utilizamos doses subdiabetogênicas.

Certamente, para a realização do tratamento de uma doença, é necessário que a mesma esteja instituída. Logo, para verificar se a ETZ induziu um prejuízo na cognição dos

animais e posteriormente tentar reverter este dano com ebselen, realizou-se o TRO, 21 dias após a injeção da ETZ (**artigo 2**). Corroborando com dados prévios da literatura, o desempenho cognitivo dos animais neste teste confirmou que a ETZ causou um déficit na memória dos camundongos (AGRAWAL et al., 2011; HOYER e LANNERT, 2007; JAVED et al., 2012).

Desta forma, observou-se que o tratamento com ebselen, em doses baixas, foi eficaz em bloquear este efeito da ETZ no TRO e no Y-maze, dois testes consolidados para a identificação do comportamento do tipo Alzheimer. (**Artigo 2**). Estes resultados, em conjunto com aqueles apresentados no **artigo 1**, são relevantes por apresentarem os primeiros passos para a compreensão do efeito neuroprotetor do ebselen em modelos de DA esporádica, também, reforçam a ideia de que o ebselen preserva a memória e é capaz de restaurar as habilidades cognitivas dos animais. Ainda, dados anteriores apontam que o ebselen apresenta efeito neuroprotetor apenas em doses mais elevadas, ou então em tratamentos mais longos, como foi visto por XIE et al. (2017) utilizando um modelo de DA em camundongos transgênicos (MARTINI et al., 2018). É importante salientar também que nenhum dos tratamentos alterou a atividade locomotora dos animais observada no monitor de atividades, o que descarta que o comportamento dos camundongos possa ter sido influenciado por alterações na atividade locomotora.

Ainda no **artigo 2**, tendo em vista a ação antioxidante, já reportada, do ebselen e as evidências que têm apontado que o estresse oxidativo desempenha um papel – chave na patogênese de doenças neurodegenerativas acredita-se que os compostos que apresentam ação antioxidantes sejam capazes de proteger células neuronais sadias da morte ou do dano induzido pelo estresse oxidativo, que consequentemente desencadeia à problemas cognitivos (GOLDMAN et al., 2018). Esta propriedade antioxidante foi evidenciada no **artigo 2**, em que o composto foi efetivo em reverter todas os parâmetros de estresse oxidativo alterados pela ETZ. Assim, sabe –se que o efeito antioxidante do ebselen também esteve envolvido no seu efeito neuroprotetor contra o dano oxidativo induzido pela ETZ, uma vez que o composto preveniu o tecido cerebral do aumento das ER, assim como modulou a atividade das enzimas antioxidantes (**Artigo 2**).

Além disso, a via apoptótica é regulada principalmente pelas proteínas da família Bcl-2, incluindo a proteína pró-apoptótica, Bax e a proteína anti-apoptótica, Bcl-2. O equilíbrio entre as duas classes de proteínas determina o destino das células, inclusive a sobrevivência de neurônios (UPADHYAY et al., 2003). Assim, nesta tese, elucidamos o envolvimento do ebselen na via apoptótica, demonstrado pela efetividade em reverter o

aumento nas razões de Bax/Bcl-2, PARP clivada/PARP e nos níveis de caspase-3 no hipocampo de camundongos induzidos por ETZ i.c.v. Desta forma, a diminuição da relação Bcl-2 / Bax inibe a capacidade de reparo do DNA com consequente inativação de morte celular (POLLACK et al., 2002). Notavelmente, o ebselen reverteu a ativação de caspase-3 e a indução de clivagem da PARP no hipocampo dos camundongos, reforçando a ação anti-apoptótica do ebselen em um modelo de DA esporádica. Ainda, com relação a via apoptótica, vale ressaltar que, no **artigo 2** realizamos uma marcação por imunohistoquímica da caspase -3 nas regiões do giro denteado, CA1 e CA3 do hipocampo dos camundongos e obtivemos um aumento da ETZ na região CA1 e o ebselen foi capaz de reverter este aumento, reforçando seu papel anti-apoptótico (**Anexo D**). As propriedades antioxidantes do ebselen são em parte responsáveis pela proteção exercida por este composto frente à neurodegeneração, uma vez que o EO está associado à morte celular (**Artigo 2**). A atenuação da perda e morte celular também deve ser atribuída ao seu efeito anti-inflamatório.

Além disso, injeção i.c.v. de ETZ também reduziu a proliferação de células neuronais na área do giro denteado do hipocampo, o que nos esclarece, em parte, o comprometimento da memória dos animais induzidos com ETZ, no entanto, nem o ebselen nem o controle positivo, donepezila, foram eficazes frente à essa diminuição, mesmo que eles apresentem efeito em reverter o prejuízo da memória. No entanto, nos questionamos sobre este resultado, uma vez que nos leva a sugerir que o perfil multi-alvo do ebselen poderia estar envolvido na melhora da memória. No entanto, mais estudos precisam ser feitos para elucidar nossa hipótese.

Como amplamente discutido anteriormente a propriedade multi-alvo de uma molécula tem sido considerada a melhor alternativa terapêutica de doenças multipatogênicas e complexas, como é o caso da DA (NOSENGO, 2016). O principal desafio no desenvolvimento de fármacos multi-alvo é ele prevenir ou impactar em diferentes mecanismos celulares e grande parte dessas alterações moleculares foi demonstrada em ambos os **artigos 1** e **2.** De fato, o ebselen nos demonstrou ter amplo espectro de ação em modelos de DA esporádica.

Por ser uma molécula biodisponível, que atravessa a barreira hematoencefálica (IMAI et al., 2001) o ebselen atua amplamente no SNC, de forma direta. Com relação à sua concentração no plasma, pesquisas mosntram que uma dose aguda de 100 mg/kg de ebselen em ratos apresenta valores séricos de 4 a 5 µM (SALOM et al., 2004), enquanto que 1 mg/kg de ebselen intravenoso atinge 12 µg/ml no plasma de ratos (IMAI et al.,

2001). Ainda, estudos mostram que sua farmacocinética revela que sua porção de selênio na estrutura não está biodisponível e, portanto, não é metabolizado e excretado, explicando sua discreta toxicidade (PARNHAM, M. e SIES, 2000).

Desta forma, considerando os efeitos farmacológicos do ebselen observados no presente estudo com relação à sua farmacocinética, no qual a dose de 10 mg/kg foi efetiva em todos os testes, pode-se considerar que este composto apresenta uma janela terapêutica segura para futuras experiências em humanos para tratar a DA.

De fato, outro ponto importante, é que o uso do modelo da DA esporádica induzida pela ETZ é uma ferramenta para a descoberta do efeito neuroprotetor do ebselen e seu mecanismo de ação, contudo, a busca de outros modelos é interessante pois conseguiríamos verificar se o efeito do ebselen, visto nesta tese, se reproduziria e também, poderíamos ver outros mecanismos envolvidos em sua ação neuroprotetora. Mesmo que, a ETZ seja um modelo metabólico não transgênico de DA esporádica, onde seus efeitos se assemelham às principais características observadas em cérebros de pacientes com DA devemos reconhecer que ainda falta um esclarecimento do mecanismo concreto de ação da ETZ, o que podemos considerar uma limitação deste modelo (GRIEB, 2016).

Por fim, existe uma "crise" no desenvolvimento de medicamentos para a DA, com isso, as indústrias de medicamentos estão deixando de lado a pesquisa e o aumento do índice da DA devido a falta de compreensão mecanicista e de metas validadas (CONN e ROTH, 2008). Isso levou oportunidades para os pesquisadores buscarem estratégias e o uso de fármacos antigos, ou já utilizados para outros fins, fossem reutilizados para assim "resgatar" alguns fármacos (CAVALLA, 2009; CONN e ROTH, 2008). E a grande expectativa dessa estratégia é que elas podem facilitar a rápida comercialização, uma vez que os primeiros obstáculos para o desenvolvimento de fármacos já foram eliminados. Desta forma, o desenvolvimento de uma droga não comercializada como o ebselen para a DA é mais atraente quando comparado à outros compostos que ainda não se sabe a aplicabilidade em humanos.

Ainda, durante a elaboração desta tese, realizamos uma revisão sistemática na literatura a cerca do papel do exercício físico como estilo de vida em indivíduos que desenvolveram a DA na velhice e assim, existem evidências consistentes de que o exercício ajuda a reduzir ou prevenir a deterioração cognitiva e cerebral, entre os idosos e, consequentemente, o aparecimento de doenças neurodegenerativas (CASSILHAS et al., 2007; HOTTING e RODER, 2013; LIU-AMBROSE et al., 2013; MULLEN e HALL, 2015). Ainda, está claramente visto que, o exercício físico tem uma influência

significativa na qualidade de vida e no envelhecimento saudável (MATTSON, 2012; NOUCHI et al., 2014) e na prevenção das doenças neurodegenerativas e mentais, como o Alzheimer, depressão, ansiedade e o estresse ao longo da vida (GOMEZ-PINILLA e HILLMAN, 2013; LIN e KUO, 2013).

Segundo Ratey (RATEY e LOEHR, 2011), onde ele afirma que, se queremos ser mais inteligentes devemos fazer mais exercício, pois *"o exercício é a ferramenta mais poderosa que possuímos para aperfeiçoar a função cerebral... É, simplesmente, um dos melhores tratamentos, de que dispomos, para muitos problemas psiquiátricos"*.

Neste contexto, sabemos que o cérebro está associado ao controle das funções do corpo, entre elas, as funções motoras e cognitivas. Estas funções são dependentes de uma relação complexa entre as estruturas cerebrais (DISHMAN et al., 2006; MATTA MELLO PORTUGAL et al., 2013). No entanto, durante o envelhecimento, vários processos comprometem as estruturas cerebrais e, consequentemente, as funções cerebrais. A disfunção colinérgica (CARRO et al., 2002; REDDY et al., 2010; RENTZ et al., 2010), a redução do fluxo sanguíneo (PARKES et al., 2004) e os déficits cognitivos (HOOGENDAM et al., 2014) são algumas das mudanças ocasionadas pelo envelhecimento. Mais recentemente, a atenção tem sido dada à perda muscular relacionada à idade (HAWKINS et al., 2003; HURLEY et al., 2011) e aos problemas fisiológicos associados, como a perda óssea (STRASSER et al., 2011), diabetes (FLACK et al., 2010), síndrome metabólica (STRASSER et al., 2011), e à mortalidade devido à essas causas (JURCA et al., 2004). Interessantemente, em relação à função cognitiva, uma pesquisa realizada em adultos mais velhos mostrou que a intervenção de um treinamento de força melhora significativamente as habilidades cognitivas (CASSILHAS et al., 2007).

O treinamento físico é uma estimulação positiva de todo o corpo (DESLANDES et al., 2009; MATTA MELLO PORTUGAL et al., 2013) Existem vários mecanismos neurobiológicos que podem explicar os efeitos positivos do exercício sobre a prevenção e o tratamento de doenças mentais, tais como: liberação de neurotransmissores como a serotonina e a dopamina, fatores tróficos, aumento da neuroplasticidade, neurogênese e melhora da cognição (MATTA MELLO PORTUGAL et al., 2013). No entanto, a maioria das evidências sobre o exercício são baseadas em estudos de treinamento aeróbico (MATTA MELLO PORTUGAL et al., 2013)., assim os benefícios mentais e biológicos que o treinamento de força trás não são bem conhecidos, incluindo seus possíveis efeitos positivos na prevenção e tratamento da DA. Além disso, os efeitos do treinamento de

força sobre as funções cognitivas já foram discutidas (WESTCOTT, 2012), mas seus mecanismos ainda são desconhecidos.

Desta forma, o **manuscrito I** visou um tratamento terapêutico não farmacológico com um programa de exercício de força e duração de 4 semanas, sendo a 1º semana apenas familiarização com o aparato e com o programa de exercício, buscando verificar o potencial neuroprotetor do exercício físico de força em modelo de DA induzida por ETZ i.c.v. em camundongos.

Estudos epidemiológicos, também relataram risco reduzido de comprometimento cognitivo leve em idosos que mantêm níveis elevados de atividade física. Além disso, ocorre um aumento na produção de fatores neurogênicos e na formação de sinapses (CASSILHAS et al., 2007), que promovem a afinidade nas ligações aos receptores de membrana acarretando positivamente a duração e a intensidade das cascatas de sinalização. Desta forma, aumenta os fatores neurogênicos, a neurogênese, a memória e o aprendizado (CASSILHAS et al., 2007). No entanto, poucos estudos comparam os efeitos de diferentes modos de exercício na cognição, como por exemplo, o exercício de força.

Mesmo que, o exercício aeróbico seja mais popular na população idosa, cada vez mais o exercício com o uso de carga vem sendo recomendado para essa população, principalmente para a prevenção de distúrbios neuromusculares associados ao envelhecimento, como perda de massa muscular e eficiência neuromuscular (ARENT et al., 2018; SCHIMIDT et al., 2014). Desta forma, alguns estudos começaram a inserir nas atividades com idosos os exercícios multicomponentes que compreendem exercícios que trabalham força, equilíbrio, coordenação, além do condicionamento aeróbico (EGGENBERGER et al., 2015).

Neste contexto, um estudo recente realizado em pacientes com demência mostrou que uma combinação de treinamento de força e o exercício aeróbico é mais efetivos na redução da cognição relacionada à demência do que apenas o exercício aeróbico, sublinhando o potencial terapêutico do treinamento de força (BOSSERS et al., 2015). Em outros 5 estudos, o treinamento de força foi encontrado para melhorar o desempenho cognitivo em idosos e em mulheres idosas com comprometimento cognitivo leve. (CASSILHAS et al., 2007; LIU-AMBROSE et al., 2013). Assim, há evidências recentes para mostrar que o treinamento de força tem efeitos benéficos no funcionamento do cérebro tanto em populações saudáveis quanto clínicas.

Os resultados apresentados no **manuscrito 1** indicam que a integridade da sinalização de *m*BDNF foi diminuída no hipocampo de camundongos injetados com ETZ e o

exercício de força promoveu um aumento desses níveis, *m*BDNF e TrkB, além das proteínas *p*ERK/ERK e *p*CAMK-II/CAMK-II, o que poderia explicar o aumento nos níveis do NeuN no hipocampo dos camundongos. Sabe-se que o CREB é um fator de transcrição que regula diversas respostas celulares, incluindo a proliferação, sobrevivência e diferenciação. Interessantemente, o *p*CREB liga-se a região promotora do BDNF e Bcl2 aumentando a regulação da expressão de ambas as proteínas (FINKBEINER, 2000). Esta poderia ser uma possível explicação para o aumento dos níveis de BDNF e Bcl2 no hipocampo de camundongos induzidos com ETZ i.c.v. e submetidos à um exercício de força. O CREB é um ponto de convergência de muitas vias de sinalização que medeiam o aumento da atividade sináptica, como as proteínas quinases ativadas por mitógeno (MAPK), a proteína quinase dependente de Ca^{+2}-calmodulina tipo II (CaMK-II), que são conhecidas por serem ativadas em resposta ao exercício (MOLTENI et al., 2002). Ainda, o CREB regula a sobrevivência neuronal, a proliferação de precursores, o crescimento e a diferenciação neuronal, bem como as funções de aprendizagem e memória (ALONSO et al., 2002; BENITO e BARCO, 2010).

Ainda, sabemos que o NeuN pode ser usado como um marcador de células neurais recém-geradas no hipocampo. É importante notar que a diminuição da ativação da via apoptótica, demonstrada no **manuscrito I**, também pode estar envolvida com o aumento nos níveis de NeuN. Uma possível explicação seria que novos neurônios podem ser produzidos, pois a sinalização de *m*BDNF estaria envolvida tanto na sobrevivência quanto na neurogênese; ou ainda, um aumento na sobrevivência celular seria uma consequência dos efeitos anti-apoptóticos do exercício de força. No entanto, essa hipótese deve ser interpretada com cautela, pois não determinamos marcadores específicos de neurogênese neste estudo, o que futuramente poderia ser investigado.

Ademais, os resultados apresentados no **manuscrito I** mostram claramente que o exercício de força influência na modulação de proteínas constituintes de vias moleculares reguladas pela DA no hipocampo. De acordo com o nosso conhecimento, este é o primeiro estudo em que o exercício de força foi capaz de aumentar os níveis de marcadores neurogênicos via sinalização BDNF/ERK-CAMK-II/CREB no hipocampo de camundongos, além de suprimir a perda de memória no teste MWM, em um modelo de DA esporádica.

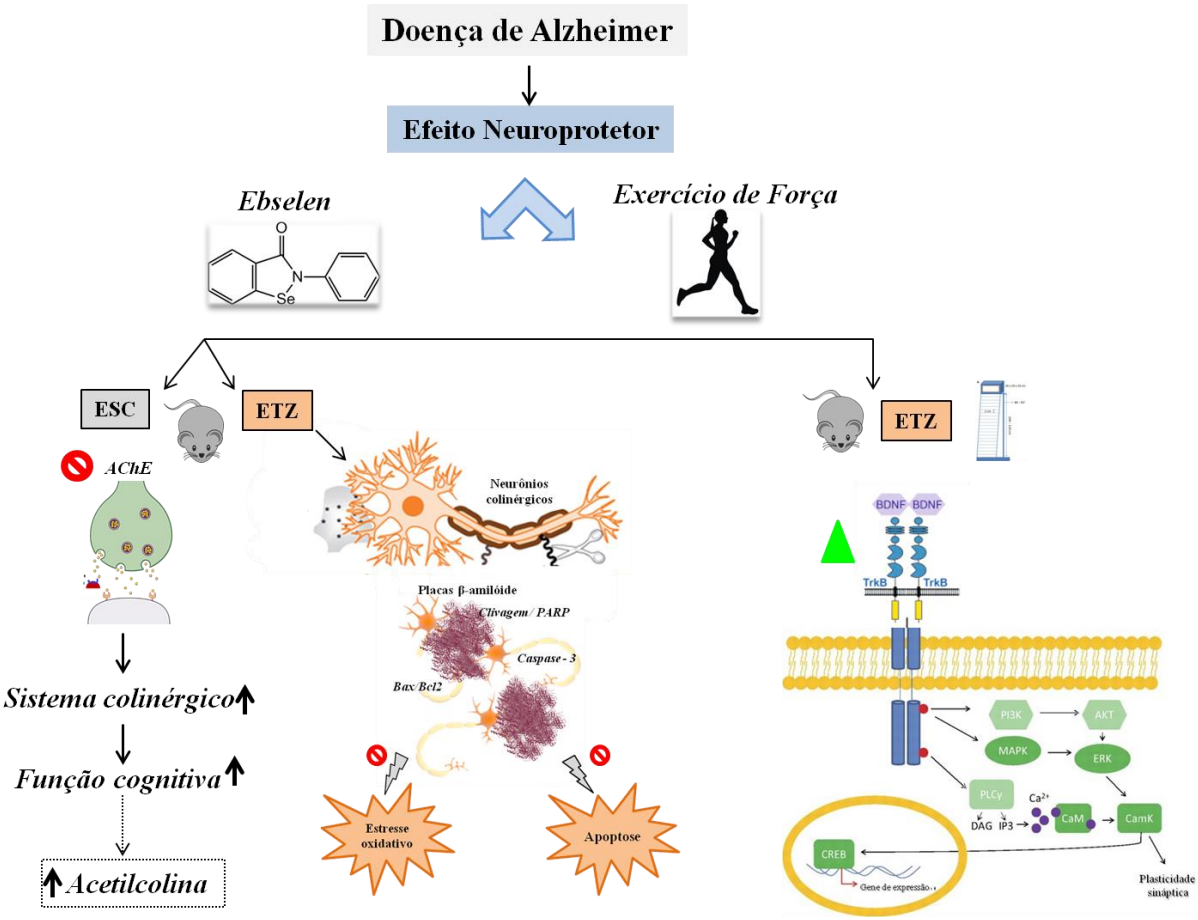
Cabe ressaltar, que o **manuscrito I** tinha como proposta inicial, verificar o efeito sinérgico do exercício de força + ebselen, em uma dose subefetiva (0.1 mg/kg), no entanto não houve aumento conforme o esperado, ou seja o efeito sinérgico não foi observado.

Frente à isso, nossa hipótese com relação à este achado, é que o efeito do exercício atingiu um “teto” e, consequentemente, o efeito do ebselen não foi evidenciado (**Anexo E**).

Além disso, dentre os mecanismos desencadeadores de apoptose, o CREB estimula a expressão de fatores pró-sobrevivência incluindo membros da família Bcl2 (WILSON et al., 1996). Com base nesses mecanismos propostos, há um aparente aumento da sinalização anti- apoptótica pelo CREB em hipocampo de animais induzidos por ETZ i.c.v. e isto corrobora com os efeitos neurogênicos da via de sinalização BDNF/ERK-CAMK-II/CREB. Ainda, foi relatado que a DA provoca redução significativa na expressão do gene BDNF (GREENBERG et al., 2009). Este impacto negativo sobre o BDNF tem importamtes implicações para uma série de condições patológicas. Por exemplo, comprometimento da memória dependente do hipocampo (DAVIS, S. et al., 2000) e aumento do apoptose neuronal (DAVIS, S. et al., 2000), características que estão envolvidas em vários estados patológicos associados às doenças neurodegenerativas, em especial à DA. Os resultados presentes no **manuscrito I** mostram uma redução dos níveis de BDNF maduro (mBDNF) bem como a fosforilação do TrkB e da *p*ERK no hipocampo nos camundongos induzidos por ETZ e uma reversão destes níveis pelo exercício de força, no qual os animais foram submetidos por 4 semanas. O mBDNF desencadeia a ativação da proteína quinase regulada por sinal extracelular (ERK) (CHEN et al., 2013) e assim podemos sugerir que isso possa contribuir com aumento da fosforilação da ERK e a melhora da memória no MWM nos animais que praticaram exercício de força. De fato o CREB por ser um fator de transcrição que regula diversas respostas celulares, incluindo a proliferação, sobrevivência e diferenciação, interessantemente, o *p*CREB liga-se a região promotora do BDNF e Bcl2 aumentando a regulação da expressão de ambas as proteínas (FINKBEINER, 2000). Esta poderia ser uma possível explicação para o aumento do BDNF e Bcl2 no hipocampo de camundongos induzidos com ETZ submetido ao exercício de força.

Estes resultados permitiram a proposta de uma possível hipótese para ter-se um melhor entendimento dos efeitos do ebselen e do exercício de força sobre as alterações induzidas pelos modelos animais de DA esporádica (Figura 10), estimulando novos estudos para sua possível confirmação.

Figura 10. Esquema geral dos mecanismos envolvidos nos efeitos farmacológicos do ebselen e do exercício físico de força sobre as alterações induzidas pelos modelos animais de DA esporádica.


Doença de Alzheimer
Efeito Neuroprotetor
Ebselen
Exercício de Força
ESC
ETZ
ETZ
AChE
Neurônios colinérgicos
Placas β-amilóide
Clivagem/ PARP
Caspase - 3
Bax/Bcl2
Sistema colinérgico
Função cognitiva
Acetilcolina
Estresse oxidativo
Apoptose
BDNF
BDNF
TrkB
TrkB
PI3K
AKT
MAPK
ERK
PLCγ
DAG
IP3
CaM
CamK
CREB
Gene de expressão
Plasticidade sináptica

5. CONCLUSÃO

Os resultados apresentados nesta tese indicam que o composto orgânico de selênio ebselen protegeu o comprometimento da memória induzida pela escopolamina, em ambos os testes utilizados, Y-maze e TRO. Inibiu a atividade da AChE no hipocampo dos camundongos, além de inibir a atividade da isoforma AChE/G4 no córtex frontal e no hipocampo *in vitro*

Ainda, a presente tese demonstrou que o composto multifuncional, ebselen, reverteu o comprometimento da memória nos testes de TRO, LO e Y- maze, modulou o estresse oxidativo e os níveis de proteínas relacionadas a apoptose no modelo de DA esporádica induzida por ETZ i.c.v., em camundongos.

Com isso, observamos que, os principais mecanismos envolvidos no efeito neuroprotetor do ebselen são, antioxidante; modulador da atividade da AChE e supressor da morte neuronal.

Com relação à terapia não farmacológica, o exercício de força foi eficaz em reverter o dano de memória no MWM nesta modalidade de exercício físico de força em um modelo de DA esporádica induzida por ETZ i.c.v., no qual seu principal mecanismo foi a supressão do comprometimento da memória espacial através da modulação da via de sinalização BDNF/ERK – CAMK – II/ CREB, no hipocampo dos camundongos.

Finalmente, esta tese contribui para o esclarecimento dos mecanismos neuroprotetores envolvidos na ação do ebselen e reforça a hipótese de que composto pode ser uma interessante alternativa terapêutica para o tratamento da DA. Além disso, a importância do exercício de força para retardar a progressão em pacientes com DA, uma modalidade de exercício que seus benefícios ainda não são completamente elucidados.

6. PERSPECTIVAS

- Investigar se o ebselen, em doses mais baixas, exerce efeito diretamente nos níveis do neurotransmissor ACh e em seus receptores muscarínicos, por exemplo, o M1;
- Avaliar se o ebselen diminui os emaranhados nerofibrilares e as placas senis, fisiopatologias importantes da DA;
- Avaliar o efeito sinérgico em diferentes doses do ebselen e aplicar em outras modalidades de exercício físico, como terapia para o tratamento de doenças neurodegenerativas;

7. REFERÊNCIAS

AGRAWAL, R. et al. Effect of curcumin on brain insulin receptors and memory functions in STZ (ICV) induced dementia model of rat. Pharmacol Res, v. 61, n. 3, p. 247-52, Mar 2010.

AGRAWAL, R. et al. Insulin receptor signaling in rat hippocampus: a study in STZ (ICV) induced memory deficit model. Eur Neuropsychopharmacol, v. 21, n. 3, p. 261-73, Mar 2011.

AHLSKOG, J. E. et al. Physical exercise as a preventive or disease-modifying treatment of dementia and brain aging. Mayo Clin Proc, v. 86, n. 9, p. 876-84, Sep 2011.

ALBERT, M. S. et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement, v. 7, n. 3, p. 270-9, May 2011.

ALONSO, M. et al. Signaling mechanisms mediating BDNF modulation of memory formation in vivo in the hippocampus. Cell Mol Neurobiol, v. 22, n. 5-6, p. 663-74, Dec 2002.

ANTOR, M. A. et al. The effect of transdermal scopolamine for the prevention of postoperative nausea and vomiting. Front Pharmacol, v. 5, p. 55, 2014.

ARENT, S. M. et al. The Combined Effects of Exercise, Diet, and a Multi-Ingredient Dietary Supplement on Body Composition and Adipokine Changes in Overweight Adults. J Am Coll Nutr, v. 37, n. 2, p. 111-120, Feb 2018.

ATTA UR, R. et al. Acetyl and butyrylcholinesterase-inhibiting triterpenoid alkaloids from Buxus papillosa. Phytochemistry, v. 58, n. 6, p. 963-8, Nov 2001.

BALLARD, C. et al. Quetiapine and rivastigmine and cognitive decline in Alzheimer's disease: randomised double blind placebo controlled trial. BMJ, v. 330, n. 7496, p. 874, Apr 16 2005a.

BALLARD, C. et al. The k variant of the butyrylcholinesterase gene is associated with reduced phosphorylation of tau in dementia patients. Dement Geriatr Cogn Disord, v. 19, n. 5-6, p. 357-60, 2005b.

BEHNE, D. et al. Subcellular distribution of selenoproteins in the liver of the rat. Biochim Biophys Acta, v. 1033, n. 3, p. 219-25, Mar 26 1990.

BENITO, E.; BARCO, A. CREB's control of intrinsic and synaptic plasticity: implications for CREB-dependent memory models. Trends Neurosci, v. 33, n. 5, p. 230-40, May 2010.

BENNETT, D. A. et al. Neurofibrillary tangles mediate the association of amyloid load with clinical Alzheimer disease and level of cognitive function. Arch Neurol, v. 61, n. 3, p. 378-84, Mar 2004.

BIRKS, J.; CRAIG, D. Galantamine for vascular cognitive impairment. Cochrane Database Syst Rev, n. 4, p. CD004746, Jan 25 2006.

BIRKS, J. S. et al. Rivastigmine for Alzheimer's disease. Cochrane Database Syst Rev, v. 9, p. CD001191, Sep 22 2015.

BISHOP, N. A. et al. Neural mechanisms of ageing and cognitive decline. Nature, v. 464, n. 7288, p. 529-35, Mar 25 2010.

BLENNOW, K. et al. Alzheimer's disease. Lancet, v. 368, n. 9533, p. 387-403, Jul 29 2006.

BLOKLAND, A. Acetylcholine: a neurotransmitter for learning and memory? Brain Res Brain Res Rev, v. 21, n. 3, p. 285-300, Nov 1995.

BORGES, V. C. et al. Effect of diphenyl diselenide, diphenyl ditelluride and ebselen on cerebral Na(+), K(+)-ATPase activity in rats. Toxicology, v. 215, n. 3, p. 191-7, Nov 15 2005.

BOSSERS, S. M. et al. Experience in Prehospital Endotracheal Intubation Significantly Influences Mortality of Patients with Severe Traumatic Brain Injury: A Systematic Review and Meta-Analysis. PLoS One, v. 10, n. 10, p. e0141034, 2015.

BRADLEY, M. A. et al. Elevated 4-hydroxyhexenal in Alzheimer's disease (AD) progression. Neurobiol Aging, v. 33, n. 6, p. 1034-44, Jun 2012.

BRILEY, D. et al. Preserved neurogenesis in non-demented individuals with AD neuropathology. Sci Rep, v. 6, p. 27812, Jun 14 2016.

BRODATY, H. et al. Dementia time to death: a systematic literature review on survival time and years of life lost in people with dementia. Int Psychogeriatr, v. 24, n. 7, p. 1034-45, Jul 2012.

BROOKMEYER, R. et al. Forecasting the global burden of Alzheimer's disease. Alzheimers Dement, v. 3, n. 3, p. 186-91, Jul 2007.

BUTTERFIELD, D. A. et al. Redox proteomics in selected neurodegenerative disorders: from its infancy to future applications. Antioxid Redox Signal, v. 17, n. 11, p. 1610-55, Dec 1 2012.

CARRO, E. et al. Serum insulin-like growth factor I regulates brain amyloid-beta levels. Nat Med, v. 8, n. 12, p. 1390-7, Dec 2002.

CARUSO, A. et al. Stress as risk factor for Alzheimer's disease. Pharmacol Res, Apr 21 2018.

CASSILHAS, R. C. et al. Spatial memory is improved by aerobic and resistance exercise through divergent molecular mechanisms. Neuroscience, v. 202, p. 309-17, Jan 27 2012a.

CASSILHAS, R. C. et al. Resistance exercise improves hippocampus-dependent memory. Braz J Med Biol Res, v. 45, n. 12, p. 1215-20, Dec 2012b.

CASSILHAS, R. C. et al. The impact of resistance exercise on the cognitive function of the elderly. Med Sci Sports Exerc, v. 39, n. 8, p. 1401-7, Aug 2007.

CAVALLA, D. APT drug R&D: the right active ingredient in the right presentation for the right therapeutic use. Nat Rev Drug Discov, v. 8, n. 11, p. 849-53, Nov 2009.

CHAKRABARTI, S. et al. Metabolic Risk Factors of Sporadic Alzheimer's Disease: Implications in the Pathology, Pathogenesis and Treatment. Aging Dis, v. 6, n. 4, p. 282-99, Aug 2015.

CHEN, C. et al. 7,8-dihydroxyflavone ameliorates scopolamine-induced Alzheimer-like pathologic dysfunction. Rejuvenation Res, v. 17, n. 3, p. 249-54, Jun 2014.

CHI, S. et al. Depression in Alzheimer's disease: epidemiology, mechanisms, and management. J Alzheimers Dis, v. 42, n. 3, p. 739-55, 2014.

CHIAM, J. T. et al. No Evidence to Suggest that the Use of Acetylcholinesterase Inhibitors Confounds the Results of Two Blood-Based Biomarker Studies in Alzheimer's Disease. J Alzheimers Dis, v. 47, n. 3, p. 741-50, 2015.

CONN, P. J.; ROTH, B. L. Opportunities and challenges of psychiatric drug discovery: roles for scientists in academic, industry, and government settings. Neuropsychopharmacology, v. 33, n. 9, p. 2048-60, Aug 2008.

COTMAN, C. W.; BERCHTOLD, N. C. Physical activity and the maintenance of cognition: learning from animal models. Alzheimers Dement, v. 3, n. 2 Suppl, p. S30-7, Apr 2007.

COYLE, J. T.; PUTTFARCKEN, P. Oxidative stress, glutamate, and neurodegenerative disorders. Science, v. 262, n. 5134, p. 689-95, Oct 29 1993.

CRISMON, M. L. Tacrine: first drug approved for Alzheimer's disease. Ann Pharmacother, v. 28, n. 6, p. 744-51, Jun 1994.

CUMMINGS, J. L. Cognitive and behavioral heterogeneity in Alzheimer's disease: seeking the neurobiological basis. Neurobiol Aging, v. 21, n. 6, p. 845-61, Nov-Dec 2000.

_____. Alzheimer's disease. N Engl J Med, v. 351, n. 1, p. 56-67, Jul 1 2004.

CUMMINGS, J. L. et al. Alzheimer's disease drug-development pipeline: few candidates, frequent failures. Alzheimers Res Ther, v. 6, n. 4, p. 37, 2014.

DAS, A. et al. Role of molecular isoforms of acetylcholinesterase in learning and memory functions. Pharmacol Biochem Behav, v. 81, n. 1, p. 89-99, May 2005.

DAVIES, K. J. Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. IUBMB Life, v. 50, n. 4-5, p. 279-89, Oct-Nov 2000.

DAVIES, P.; MALONEY, A. J. Selective loss of central cholinergic neurons in Alzheimer's disease. Lancet, v. 2, n. 8000, p. 1403, Dec 25 1976.

DAVIS, K. L.; YAMAMURA, H. I. Cholinergic underactivity in human memory disorders. Life Sci, v. 23, n. 17-18, p. 1729-33, Oct 30 1978.

DAVIS, S. et al. The MAPK/ERK cascade targets both Elk-1 and cAMP response element-binding protein to control long-term potentiation-dependent gene expression in the dentate gyrus in vivo. J Neurosci, v. 20, n. 12, p. 4563-72, Jun 15 2000.

DE BRUIN, N.; POUZET, B. Beneficial effects of galantamine on performance in the object recognition task in Swiss mice: deficits induced by scopolamine and by prolonging the retention interval. Pharmacol Biochem Behav, v. 85, n. 1, p. 253-60, Sep 2006.

DEARDORFF, W. J. et al. The Use of Cholinesterase Inhibitors Across All Stages of Alzheimer's Disease. Drugs Aging, v. 32, n. 7, p. 537-47, Jul 2015.

DEL BO, R. et al. VEGF genetic variability is associated with increased risk of developing Alzheimer's disease. J Neurol Sci, v. 283, n. 1-2, p. 66-8, Aug 15 2009.

DESHMUKH, R. et al. Amelioration of intracerebroventricular streptozotocin induced cognitive dysfunction and oxidative stress by vinpocetine -- a PDE1 inhibitor. Eur J Pharmacol, v. 620, n. 1-3, p. 49-56, Oct 12 2009.

DESLANDES, A. et al. Exercise and mental health: many reasons to move. Neuropsychobiology, v. 59, n. 4, p. 191-8, 2009.

DISHMAN, R. K. et al. Neurobiology of exercise. Obesity (Silver Spring), v. 14, n. 3, p. 345-56, Mar 2006.

DORAISWAMY, P. M. Non-cholinergic strategies for treating and preventing Alzheimer's disease. CNS Drugs, v. 16, n. 12, p. 811-24, 2002.

DRACHMAN, D. A.; LEAVITT, J. Human memory and the cholinergic system. A relationship to aging? Arch Neurol, v. 30, n. 2, p. 113-21, Feb 1974.

DREVER, B. D. et al. The cholinergic system and hippocampal plasticity. Behav Brain Res, v. 221, n. 2, p. 505-14, Aug 10 2011.

DURACKOVA, Z. Some current insights into oxidative stress. Physiol Res, v. 59, n. 4, p. 459-69, 2010.

DUZEL, E. et al. Can physical exercise in old age improve memory and hippocampal function? Brain, v. 139, n. Pt 3, p. 662-73, Mar 2016.

EGGENBERGER, P. et al. Multicomponent physical exercise with simultaneous cognitive training to enhance dual-task walking of older adults: a secondary analysis of a 6-month randomized controlled trial with 1-year follow-up. Clin Interv Aging, v. 10, p. 1711-32, 2015.

EHNINGER, D.; KEMPERMANN, G. Regional effects of wheel running and environmental enrichment on cell genesis and microglia proliferation in the adult murine neocortex. Cereb Cortex, v. 13, n. 8, p. 845-51, Aug 2003.

ELLIS, J. M. Cholinesterase inhibitors in the treatment of dementia. J Am Osteopath Assoc, v. 105, n. 3, p. 145-58, Mar 2005.

ERICKSON, K. I. et al. Exercise training increases size of hippocampus and improves memory. Proc Natl Acad Sci U S A, v. 108, n. 7, p. 3017-22, Feb 15 2011.

FARFEL, J. M. et al. Very low levels of education and cognitive reserve: a clinicopathologic study. Neurology, v. 81, n. 7, p. 650-7, Aug 13 2013.

FINKBEINER, S. CREB couples neurotrophin signals to survival messages. Neuron, v. 25, n. 1, p. 11-4, Jan 2000.

FISHMAN, E. B. et al. Distribution of the molecular forms of acetylcholinesterase in human brain: alterations in dementia of the Alzheimer type. Ann Neurol, v. 19, n. 3, p. 246-52, Mar 1986.

FLACK, K. D. et al. Aging, resistance training, and diabetes prevention. J Aging Res, v. 2011, p. 127315, Dec 15 2010.

FLOOD, J. F.; CHERKIN, A. Scopolamine effects on memory retention in mice: a model of dementia? Behav Neural Biol, v. 45, n. 2, p. 169-84, Mar 1986.

FRATIGLIONI, L. et al. Incidence of dementia and major subtypes in Europe: A collaborative study of population-based cohorts. Neurologic Diseases in the Elderly Research Group. Neurology, v. 54, n. 11 Suppl 5, p. S10-5, 2000.

FU, H. et al. Tau Pathology Induces Excitatory Neuron Loss, Grid Cell Dysfunction, and Spatial Memory Deficits Reminiscent of Early Alzheimer's Disease. Neuron, v. 93, n. 3, p. 533-541 e5, Feb 8 2017.

FURSTENAU, C. R. et al. The effect of ebselen on adenine nucleotide hydrolysis by platelets from adult rats. Chem Biol Interact, v. 148, n. 1-2, p. 93-9, Jun 30 2004.

GALLUZZI, S. et al. Supporting evidence for using biomarkers in the diagnosis of MCI due to AD. J Neurol, v. 260, n. 2, p. 640-50, Feb 2013.

GIACOBINI, E. Cholinesterase inhibitors: new roles and therapeutic alternatives. Pharmacol Res, v. 50, n. 4, p. 433-40, Oct 2004.

GOEDERT, M.; SPILLANTINI, M. G. A century of Alzheimer's disease. Science, v. 314, n. 5800, p. 777-81, Nov 3 2006.

GOLDMAN, D. P. et al. Accelerating Alzheimer's disease drug innovations from the research pipeline to patients. Alzheimers Dement, v. 14, n. 6, p. 833-836, Jun 2018.

GOMEZ-PINILLA, F.; HILLMAN, C. The influence of exercise on cognitive abilities. Compr Physiol, v. 3, n. 1, p. 403-28, Jan 2013.

GOTZ, J.; ITTNER, L. M. Animal models of Alzheimer's disease and frontotemporal dementia. Nat Rev Neurosci, v. 9, n. 7, p. 532-44, Jul 2008.

GOVERDHAN, P. et al. Neuroprotective effects of meloxicam and selegiline in scopolamine-induced cognitive impairment and oxidative stress. Int J Alzheimers Dis, v. 2012, p. 974013, 2012.

GRAHAM, J. H. et al. Functional central nicotinic acetylcholine receptor antagonism by systemic administration of Tinuvin 770 (BTMPS). Curr Alzheimer Res, v. 2, n. 2, p. 141-7, Apr 2005.

GREENBERG, M. E. et al. New insights in the biology of BDNF synthesis and release: implications in CNS function. J Neurosci, v. 29, n. 41, p. 12764-7, Oct 14 2009.

GREIG, N. H. et al. A new therapeutic target in Alzheimer's disease treatment: attention to butyrylcholinesterase. Curr Med Res Opin, v. 17, n. 3, p. 159-65, 2001.

GRIEB, P. Intracerebroventricular Streptozotocin Injections as a Model of Alzheimer's Disease: in Search of a Relevant Mechanism. Mol Neurobiol, v. 53, n. 3, p. 1741-1752, Apr 2016.

GUILLOZET, A. L. et al. Neurofibrillary tangles, amyloid, and memory in aging and mild cognitive impairment. Arch Neurol, v. 60, n. 5, p. 729-36, May 2003.

GUTTERIDGE, J. M. C.; HALLIWELL, B. Mini-Review: Oxidative stress, redox stress or redox success? Biochem Biophys Res Commun, v. 502, n. 2, p. 183-186, Jul 12 2018.

HALLIWELL, B. Oxidative stress and neurodegeneration: where are we now? J Neurochem, v. 97, n. 6, p. 1634-58, Jun 2006.

HAMER, M. et al. Walking speed and subclinical atherosclerosis in healthy older adults: the Whitehall II study. Heart, v. 96, n. 5, p. 380-4, Mar 2010.

HARDY, J.; SELKOE, D. J. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science, v. 297, n. 5580, p. 353-6, Jul 19 2002.

HAWKINS, S. A. et al. Exercise and the master athlete--a model of successful aging? J Gerontol A Biol Sci Med Sci, v. 58, n. 11, p. 1009-11, Nov 2003.

HELMES, E.; OSTBYE, T. Beyond memory impairment: cognitive changes in Alzheimer's disease. Arch Clin Neuropsychol, v. 17, n. 2, p. 179-93, Feb 2002.

HELZNER, E. P. et al. Survival in Alzheimer disease: a multiethnic, population-based study of incident cases. Neurology, v. 71, n. 19, p. 1489-95, Nov 4 2008.

HILLMAN, C. H. et al. Be smart, exercise your heart: exercise effects on brain and cognition. Nat Rev Neurosci, v. 9, n. 1, p. 58-65, Jan 2008.

HOOGENDAM, Y. Y. et al. The role of cerebellar volume in cognition in the general elderly population. Alzheimer Dis Assoc Disord, v. 28, n. 4, p. 352-7, Oct-Dec 2014.

HOTTING, K.; RODER, B. Beneficial effects of physical exercise on neuroplasticity and cognition. Neurosci Biobehav Rev, v. 37, n. 9 Pt B, p. 2243-57, Nov 2013.

HOYER, S.; LANNERT, H. Long-term abnormalities in brain glucose/energy metabolism after inhibition of the neuronal insulin receptor: implication of tau-protein. J Neural Transm Suppl, n. 72, p. 195-202, 2007.

_____. Long-term effects of corticosterone on behavior, oxidative and energy metabolism of parietotemporal cerebral cortex and hippocampus of rats: comparison to intracerebroventricular streptozotocin. J Neural Transm, v. 115, n. 9, p. 1241-9, Sep 2008.

HURLEY, B. F. et al. Strength training as a countermeasure to aging muscle and chronic disease. Sports Med, v. 41, n. 4, p. 289-306, Apr 1 2011.

IMAI, H. et al. Ebselen protects both gray and white matter in a rodent model of focal cerebral ischemia. Stroke, v. 32, n. 9, p. 2149-54, Sep 2001.

IQBAL, K. et al. Stratification of patients is the way to go to develop neuroprotective/disease-modifying drugs for Alzheimer's disease. J Alzheimers Dis, v. 15, n. 2, p. 339-45, Oct 2008.

ISHRAT, T. et al. Selenium prevents cognitive decline and oxidative damage in rat model of streptozotocin-induced experimental dementia of Alzheimer's type. Brain Research, v. 1281, p. 117-27, Jul 24 2009.

IWATA, H. et al. Subcellular compartment and molecular subdomain of beta-amyloid precursor protein relevant to the Abeta 42-promoting effects of Alzheimer mutant presenilin 2. J Biol Chem, v. 276, n. 24, p. 21678-85, Jun 15 2001.

JACKLIN, D. L. et al. Evidence for a specific role for muscarinic receptors in crossmodal object recognition in rats. Neurobiol Learn Mem, v. 118, p. 125-32, Feb 2015.

JAVED, H. et al. Rutin prevents cognitive impairments by ameliorating oxidative stress and neuroinflammation in rat model of sporadic dementia of Alzheimer type. Neuroscience, v. 210, p. 340-52, May 17 2012.

JOHNSON, J. W.; KOTERMANSKI, S. E. Mechanism of action of memantine. Curr Opin Pharmacol, v. 6, n. 1, p. 61-7, Feb 2006.

JURCA, R. et al. Associations of muscle strength and fitness with metabolic syndrome in men. Med Sci Sports Exerc, v. 36, n. 8, p. 1301-7, Aug 2004.

KAMAT, P. K. Streptozotocin induced Alzheimer's disease like changes and the underlying neural degeneration and regeneration mechanism. Neural Regen Res, v. 10, n. 7, p. 1050-2, Jul 2015.

KARRAN, E. et al. The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. Nat Rev Drug Discov, v. 10, n. 9, p. 698-712, Aug 19 2011.

KLEEMEYER, M. M. et al. Changes in fitness are associated with changes in hippocampal microstructure and hippocampal volume among older adults. Neuroimage, v. 131, p. 155-61, May 1 2016.

KLINKENBERG, I.; BLOKLAND, A. The validity of scopolamine as a pharmacological model for cognitive impairment: a review of animal behavioral studies. Neurosci Biobehav Rev, v. 34, n. 8, p. 1307-50, Jul 2010.

KRYUKOV, G. V. et al. Characterization of mammalian selenoproteomes. Science, v. 300, n. 5624, p. 1439-43, May 30 2003.

KUMAR, R. et al. Effects of erythropoietin on memory deficits and brain oxidative stress in the mouse models of dementia. Korean J Physiol Pharmacol, v. 14, n. 5, p. 345-52, Oct 2010.

LANNERT, H.; HOYER, S. Intracerebroventricular administration of streptozotocin causes long-term diminutions in learning and memory abilities and in cerebral energy metabolism in adult rats. Behav Neurosci, v. 112, n. 5, p. 1199-208, Oct 1998.

LAW, A. et al. Say NO to Alzheimer's disease: the putative links between nitric oxide and dementia of the Alzheimer's type. Brain Res Brain Res Rev, v. 35, n. 1, p. 73-96, Mar 2001.

LESTER-COLL, N. et al. Intracerebral streptozotocin model of type 3 diabetes: relevance to sporadic Alzheimer's disease. J Alzheimers Dis, v. 9, n. 1, p. 13-33, Mar 2006.

LIN, T. W.; KUO, Y. M. Exercise benefits brain function: the monoamine connection. Brain Sci, v. 3, n. 1, p. 39-53, Jan 11 2013.

LIU-AMBROSE, T. et al. Emerging concept: 'central benefit model' of exercise in falls prevention. Br J Sports Med, v. 47, n. 2, p. 115-7, Jan 2013.

LUO, Z. et al. Synthesis and evaluation of multi-target-directed ligands against Alzheimer's disease based on the fusion of donepezil and ebselen. J Med Chem, v. 56, n. 22, p. 9089-99, Nov 27 2013.

MAHADEVAN, J. et al. Ebselen treatment prevents islet apoptosis, maintains intranuclear Pdx-1 and MafA levels, and preserves beta-cell mass and function in ZDF rats. Diabetes, v. 62, n. 10, p. 3582-8, Oct 2013.

MARKESBERY, W. R. Oxidative stress hypothesis in Alzheimer's disease. Free Radic Biol Med, v. 23, n. 1, p. 134-47, 1997.

MARTINI, F. et al. Inhibitory effect of ebselen on cerebral acetylcholinesterase activity in vitro: kinetics and reversibility of inhibition. Curr Pharm Des, v. 21, n. 7, p. 920-4, 2015.

MARTINI, F. et al. Ebselen inhibits the activity of acetylcholinesterase globular isoform G4 in vitro and attenuates scopolamine-induced amnesia in mice. J Cell Biochem, Feb 5 2018.

MASAKI, C. et al. Effects of the potential lithium-mimetic, ebselen, on brain neurochemistry: a magnetic resonance spectroscopy study at 7 tesla. Psychopharmacology (Berl), v. 233, n. 6, p. 1097-104, Mar 2016.

MATTA MELLO PORTUGAL, E. et al. Neuroscience of exercise: from neurobiology mechanisms to mental health. Neuropsychobiology, v. 68, n. 1, p. 1-14, 2013.

MATTSON, M. P. Energy intake and exercise as determinants of brain health and vulnerability to injury and disease. Cell Metab, v. 16, n. 6, p. 706-22, Dec 5 2012.

MCKINNEY, W. T. Overview of the past contributions of animal models and their changing place in psychiatry. Semin Clin Neuropsychiatry, v. 6, n. 1, p. 68-78, Jan 2001.

MEDEIROS, R. et al. The role of tau in Alzheimer's disease and related disorders. CNS Neurosci Ther, v. 17, n. 5, p. 514-24, Oct 2011.

MISHRA, S. K. et al. Intracerebroventricular streptozotocin impairs adult neurogenesis and cognitive functions via regulating neuroinflammation and insulin signaling in adult rats. Neurochem Int, v. 113, p. 56-68, Feb 2018.

MOLTENI, R. et al. Differential effects of acute and chronic exercise on plasticity-related genes in the rat hippocampus revealed by microarray. Eur J Neurosci, v. 16, n. 6, p. 1107-16, Sep 2002.

MOORE, A. H.; O'BANION, M. K. Neuroinflammation and anti-inflammatory therapy for Alzheimer's disease. Adv Drug Deliv Rev, v. 54, n. 12, p. 1627-56, Dec 7 2002.

MORRIS, E. et al. Diagnostic accuracy of (18)F amyloid PET tracers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. Eur J Nucl Med Mol Imaging, v. 43, n. 2, p. 374-85, Feb 2016.

MU, Y.; GAGE, F. H. Adult hippocampal neurogenesis and its role in Alzheimer's disease. Mol Neurodegener, v. 6, p. 85, Dec 22 2011.

MUELLER, S. G. et al. Ways toward an early diagnosis in Alzheimer's disease: the Alzheimer's Disease Neuroimaging Initiative (ADNI). Alzheimers Dement, v. 1, n. 1, p. 55-66, Jul 2005.

MUGESH, G. et al. Chemistry of biologically important synthetic organoselenium compounds. Chem Rev, v. 101, n. 7, p. 2125-79, Jul 2001.

MULLEN, S. P.; HALL, P. A. Editorial: Physical activity, self-regulation, and executive control across the lifespan. Front Hum Neurosci, v. 9, p. 614, 2015.

MULLER, U. C.; ZHENG, H. Physiological functions of APP family proteins. Cold Spring Harb Perspect Med, v. 2, n. 2, p. a006288, Feb 2012.

NICOLAS, G. et al. Somatic variants in autosomal dominant genes are a rare cause of sporadic Alzheimer's disease. Alzheimers Dement, Aug 13 2018.

NOGUCHI, N. Ebselen, a useful tool for understanding cellular redox biology and a promising drug candidate for use in human diseases. Arch Biochem Biophys, v. 595, p. 109-12, Apr 1 2016.

NOGUEIRA, C. W.; ROCHA, J. B. Toxicology and pharmacology of selenium: emphasis on synthetic organoselenium compounds. Arch Toxicol, v. 85, n. 11, p. 1313-59, Nov 2011.

NOGUEIRA, C. W. et al. Exposure to ebselen changes glutamate uptake and release by rat brain synaptosomes. Neurochem Res, v. 27, n. 4, p. 283-8, Apr 2002.

NOGUEIRA, C. W. et al. Organoselenium and organotellurium compounds: toxicology and pharmacology. Chem Rev, v. 104, n. 12, p. 6255-85, Dec 2004.

NORTON, S. et al. Potential for primary prevention of Alzheimer's disease: an analysis of population-based data. Lancet Neurol, v. 13, n. 8, p. 788-94, Aug 2014.

NOSENGO, N. Can you teach old drugs new tricks? Nature, v. 534, n. 7607, p. 314-6, Jun 16 2016.

NOUCHI, R. et al. Four weeks of combination exercise training improved executive functions, episodic memory, and processing speed in healthy elderly people: evidence from a randomized controlled trial. Age (Dordr), v. 36, n. 2, p. 787-99, Apr 2014.

NOVAES, G. S. et al. Chronic effects of strength training vs. Hydro aerobics on functional and cardiorespiratory ability in postmenopausal women. J Hum Kinet, v. 43, p. 57-66, Sep 29 2014.

PAILLARD, T. et al. Protective Effects of Physical Exercise in Alzheimer's Disease and Parkinson's Disease: A Narrative Review. J Clin Neurol, v. 11, n. 3, p. 212-9, Jul 2015.

PAPP, L. V. et al. From selenium to selenoproteins: synthesis, identity, and their role in human health. Antioxid Redox Signal, v. 9, n. 7, p. 775-806, Jul 2007.

PARIHAR, M. S.; BREWER, G. J. Amyloid-beta as a modulator of synaptic plasticity. J Alzheimers Dis, v. 22, n. 3, p. 741-63, 2010.

PARK, M. H.; HAN, C. Is there an MCI reversion to cognitively normal? Analysis of Alzheimer's disease biomarkers profiles. Int Psychogeriatr, v. 27, n. 3, p. 429-37, Mar 2015.

PARKES, J. P. et al. Antibody responses to rabbit haemorrhagic disease virus in predators, scavengers, and hares in New Zealand during epidemics in sympatric rabbit populations. N Z Vet J, v. 52, n. 2, p. 85-9, Apr 2004.

PARNHAM, M.; SIES, H. Ebselen: prospective therapy for cerebral ischaemia. Expert Opin Investig Drugs, v. 9, n. 3, p. 607-19, Mar 2000.

PARNHAM, M. J.; KINDT, S. A novel biologically active seleno-organic compound--III. Effects of PZ 51 (Ebselen) on glutathione peroxidase and secretory activities of mouse macrophages. Biochem Pharmacol, v. 33, n. 20, p. 3247-50, Oct 15 1984.

PARNHAM, M. J.; SIES, H. The early research and development of ebselen. Biochem Pharmacol, v. 86, n. 9, p. 1248-53, Nov 1 2013.

PARSONS, C. G. et al. Memantine and cholinesterase inhibitors: complementary mechanisms in the treatment of Alzheimer's disease. Neurotox Res, v. 24, n. 3, p. 358-69, Oct 2013.

PEIXINHO-PENA, L. F. et al. A strength exercise program in rats with epilepsy is protective against seizures. Epilepsy Behav, v. 25, n. 3, p. 323-8, Nov 2012.

PERRY, R. H. et al. Extensive loss of choline acetyltransferase activity is not reflected by neuronal loss in the nucleus of Meynert in Alzheimer's disease. Neurosci Lett, v. 33, n. 3, p. 311-5, Dec 13 1982.

PERSSON, T. et al. Oxidative stress in Alzheimer's disease: why did antioxidant therapy fail? Oxid Med Cell Longev, v. 2014, p. 427318, 2014.

PETERSEN, R. C. et al. Mild cognitive impairment: a concept in evolution. J Intern Med, v. 275, n. 3, p. 214-28, Mar 2014.

PETERSEN, R. C.; MORRIS, J. C. Mild cognitive impairment as a clinical entity and treatment target. Arch Neurol, v. 62, n. 7, p. 1160-3; discussion 1167, Jul 2005.

PLASCHKE, K.; HOYER, S. Action of the diabetogenic drug streptozotocin on glycolytic and glycogenolytic metabolism in adult rat brain cortex and hippocampus. Int J Dev Neurosci, v. 11, n. 4, p. 477-83, Aug 1993.

PLASCHKE, K. et al. Insulin-resistant brain state after intracerebroventricular streptozotocin injection exacerbates Alzheimer-like changes in Tg2576 AbetaPP-overexpressing mice. J Alzheimers Dis, v. 19, n. 2, p. 691-704, 2010.

PLASSMAN, B. L. et al. Prevalence of dementia in the United States: the aging, demographics, and memory study. Neuroepidemiology, v. 29, n. 1-2, p. 125-32, 2007.

POLLACK, M. et al. The role of apoptosis in the normal aging brain, skeletal muscle, and heart. Ann N Y Acad Sci, v. 959, p. 93-107, Apr 2002.

PORCEL, J.; MONTALBAN, X. Anticholinesterasics in the treatment of cognitive impairment in multiple sclerosis. J Neurol Sci, v. 245, n. 1-2, p. 177-81, Jun 15 2006.

PORCIUNCULA, L. O. et al. Ebselen prevents excitotoxicity provoked by glutamate in rat cerebellar granule neurons. Neurosci Lett, v. 299, n. 3, p. 217-20, Feb 23 2001.

PORCIUNCULA, L. O. et al. Neuroprotective effect of ebselen on rat hippocampal slices submitted to oxygen-glucose deprivation: correlation with immunocontent of inducible nitric oxide synthase. Neurosci Lett, v. 346, n. 1-2, p. 101-4, Jul 31 2003.

PRAKASH, A. K.; KUMAR, A. Effect of chronic treatment of carvedilol on oxidative stress in an intracerebroventricular streptozotocin induced model of dementia in rats. J Pharm Pharmacol, v. 61, n. 12, p. 1665-72, Dec 2009.

PRINCE, M. et al. Recent global trends in the prevalence and incidence of dementia, and survival with dementia. Alzheimers Res Ther, v. 8, n. 1, p. 23, Jul 30 2016.

PRINCE, M. et al. The global prevalence of dementia: a systematic review and metaanalysis. Alzheimers Dement, v. 9, n. 1, p. 63-75 e2, Jan 2013.

QIU, C. Preventing Alzheimer's disease by targeting vascular risk factors: hope and gap. J Alzheimers Dis, v. 32, n. 3, p. 721-31, 2012.

QIU, W. Q. et al. Insulin-degrading enzyme regulates extracellular levels of amyloid beta-protein by degradation. J Biol Chem, v. 273, n. 49, p. 32730-8, Dec 4 1998.

RATEY, J. J.; LOEHR, J. E. The positive impact of physical activity on cognition during adulthood: a review of underlying mechanisms, evidence and recommendations. Rev Neurosci, v. 22, n. 2, p. 171-85, 2011.

REDDY, P. H. et al. Amyloid-beta and mitochondria in aging and Alzheimer's disease: implications for synaptic damage and cognitive decline. J Alzheimers Dis, v. 20 Suppl 2, p. S499-512, 2010.

REITZ, C. et al. Epidemiology of Alzheimer disease. Nat Rev Neurol, v. 7, n. 3, p. 137-52, Mar 2011.

RENTZ, D. M. et al. Cognition, reserve, and amyloid deposition in normal aging. Ann Neurol, v. 67, n. 3, p. 353-64, Mar 2010.

ROCCA, W. A. et al. Trends in the incidence and prevalence of Alzheimer's disease, dementia, and cognitive impairment in the United States. Alzheimers Dement, v. 7, n. 1, p. 80-93, Jan 2011.

ROSSATO, J. I. et al. Antioxidant properties of new chalcogenides against lipid peroxidation in rat brain. Neurochem Res, v. 27, n. 4, p. 297-303, Apr 2002.

SAITO, Y. et al. Heparin-selenocystamine conjugate with selenol groups. Biol Pharm Bull, v. 21, n. 8, p. 805-8, Aug 1998.

SALKOVIC-PETRISIC, M. et al. Cerebral amyloid angiopathy in streptozotocin rat model of sporadic Alzheimer's disease: a long-term follow up study. J Neural Transm, v. 118, n. 5, p. 765-72, May 2011.

SALOM, J. B. et al. Single-dose ebselen does not afford sustained neuroprotection to rats subjected to severe focal cerebral ischemia. Eur J Pharmacol, v. 495, n. 1, p. 55-62, Jul 8 2004.

SCANDALIOS, J. G. Oxygen Stress and Superoxide Dismutases. Plant Physiol, v. 101, n. 1, p. 7-12, Jan 1993.

SCHABLE, S. et al. The NK3 receptor agonist senktide ameliorates scopolamine-induced deficits in memory for object, place and temporal order. Neurobiol Learn Mem, v. 97, n. 2, p. 235-40, Feb 2012.

SCHETINGER, M. R. et al. New benzodiazepines alter acetylcholinesterase and ATPDase activities. Neurochem Res, v. 25, n. 7, p. 949-55, Jul 2000.

SCHEWE, T. Molecular actions of ebselen--an antiinflammatory antioxidant. Gen Pharmacol, v. 26, n. 6, p. 1153-69, Oct 1995.

SCHIMIDT, H. L. et al. Memory deficits and oxidative stress in cerebral ischemia-reperfusion: neuroprotective role of physical exercise and green tea supplementation. Neurobiol Learn Mem, v. 114, p. 242-50, Oct 2014.

SCOTT, T. J. et al. Economic analysis of opportunities to accelerate Alzheimer's disease research and development. Ann N Y Acad Sci, v. 1313, p. 17-34, Apr 2014.

SELKOE, D. J.; HARDY, J. The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Mol Med, v. 8, n. 6, p. 595-608, Jun 2016.

SHARMA, V. et al. Neuroprotective effect of RO-20-1724-a phosphodiesterase4 inhibitor against intracerebroventricular streptozotocin induced cognitive deficit and oxidative stress in rats. Pharmacol Biochem Behav, v. 101, n. 2, p. 239-45, Apr 2012.

SHIMIZU, S. et al. Differential effects of acetylcholinesterase inhibitors on clinical responses and cerebral blood flow changes in patients with Alzheimer's disease: a 12-month, randomized, and open-label trial. Dement Geriatr Cogn Dis Extra, v. 5, n. 1, p. 135-46, Jan-Apr 2015.

SINGH, N. et al. A safe lithium mimetic for bipolar disorder. Nat Commun, v. 4, p. 1332, 2013.

SINGH, N. et al. Effect of the Putative Lithium Mimetic Ebselen on Brain Myo-Inositol, Sleep, and Emotional Processing in Humans. Neuropsychopharmacology, v. 41, n. 7, p. 1768-78, Jun 2016.

SINHA, M. et al. Antioxidant role of amyloid beta protein in cell-free and biological systems: implication for the pathogenesis of Alzheimer disease. Free Radic Biol Med, v. 56, p. 184-92, Mar 2013.

SOUCHAY, C. Metamemory in Alzheimer's disease. Cortex, v. 43, n. 7, p. 987-1003, Oct 2007.

SPERLING, R. Potential of functional MRI as a biomarker in early Alzheimer's disease. Neurobiol Aging, v. 32 Suppl 1, p. S37-43, Dec 2011.

SPIERS, H. J.; BENDOR, D. Enhance, delete, incept: manipulating hippocampus-dependent memories. Brain Res Bull, v. 105, p. 2-7, Jun 2014.

SPUCH, C. et al. LRP-1 and LRP-2 receptors function in the membrane neuron. Trafficking mechanisms and proteolytic processing in Alzheimer's disease. Front Physiol, v. 3, p. 269, 2012.

STIX, G. Alzheimer's: forestalling the darkness. Sci Am, v. 302, n. 6, p. 50-7, Jun 2010.

STRASSER, B. et al. The effects of strength and endurance training in patients with rheumatoid arthritis. Clin Rheumatol, v. 30, n. 5, p. 623-32, May 2011.

TARASOFF-CONWAY, J. M. et al. Clearance systems in the brain-implications for Alzheimer disease. Nat Rev Neurol, v. 11, n. 8, p. 457-70, Aug 2015.

TERRY, A. V., JR.; BUCCAFUSCO, J. J. The cholinergic hypothesis of age and Alzheimer's disease-related cognitive deficits: recent challenges and their implications for novel drug development. J Pharmacol Exp Ther, v. 306, n. 3, p. 821-7, Sep 2003.

TILLEMENT, L. et al. Alzheimer's disease: effects of beta-amyloid on mitochondria. Mitochondrion, v. 11, n. 1, p. 13-21, Jan 2011.

TIRABOSCHI, P. et al. The importance of neuritic plaques and tangles to the development and evolution of AD. Neurology, v. 62, n. 11, p. 1984-9, Jun 8 2004.

TURON-ESTRADA, A. et al. [Tolerance and adverse events of treatment with acetylcholinesterase inhibitors in a clinical sample of patients with very slight and mild Alzheimer s disease over a six-month period]. Rev Neurol, v. 36, n. 5, p. 421-4, Mar 1-15 2003.

UBHI, K. et al. Fluoxetine ameliorates behavioral and neuropathological deficits in a transgenic model mouse of alpha-synucleinopathy. Exp Neurol, v. 234, n. 2, p. 405-16, Apr 2012.

UPADHYAY, D. et al. Particulate matter induces alveolar epithelial cell DNA damage and apoptosis: role of free radicals and the mitochondria. Am J Respir Cell Mol Biol, v. 29, n. 2, p. 180-7, Aug 2003.

VAN DAM, D.; DE DEYN, P. P. Drug discovery in dementia: the role of rodent models. Nat Rev Drug Discov, v. 5, n. 11, p. 956-70, Nov 2006.

VAN MAURIK, I. S. et al. Interpreting Biomarker Results in Individual Patients With Mild Cognitive Impairment in the Alzheimer's Biomarkers in Daily Practice (ABIDE) Project. JAMA Neurol, v. 74, n. 12, p. 1481-1491, Dec 1 2017.

VAN PRAAG, H. Neurogenesis and exercise: past and future directions. Neuromolecular Med, v. 10, n. 2, p. 128-40, 2008.

VAN PRAAG, H. et al. Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc Natl Acad Sci U S A, v. 96, n. 23, p. 13427-31, Nov 9 1999.

VAN PRAAG, H. et al. Exercise enhances learning and hippocampal neurogenesis in aged mice. J Neurosci, v. 25, n. 38, p. 8680-5, Sep 21 2005.

VAVAKOVA, M. et al. Markers of Oxidative Stress and Neuroprogression in Depression Disorder. Oxid Med Cell Longev, v. 2015, p. 898393, 2015.

VAYNMAN, S. et al. Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. Eur J Neurosci, v. 20, n. 10, p. 2580-90, Nov 2004.

WANG, C. et al. The role of pro-inflammatory S100A9 in Alzheimer's disease amyloid-neuroinflammatory cascade. Acta Neuropathol, v. 127, n. 4, p. 507-22, Apr 2014a.

WANG, X. et al. Glutathione peroxidase mimic ebselen improves glucose-stimulated insulin secretion in murine islets. Antioxid Redox Signal, v. 20, n. 2, p. 191-203, Jan 10 2014b.

WATKINS, P. B. et al. Hepatotoxic effects of tacrine administration in patients with Alzheimer's disease. JAMA, v. 271, n. 13, p. 992-8, Apr 6 1994.

WEINER, M. W. et al. Military risk factors for Alzheimer's disease. Alzheimers Dement, v. 9, n. 4, p. 445-51, Jul 2013.

WENDEL, A. et al. A novel biologically active seleno-organic compound--II. Activity of PZ 51 in relation to glutathione peroxidase. Biochem Pharmacol, v. 33, n. 20, p. 3241-5, Oct 15 1984.

WESTCOTT, W. L. Resistance training is medicine: effects of strength training on health. Curr Sports Med Rep, v. 11, n. 4, p. 209-16, Jul-Aug 2012.

WILLNER, P. Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. Psychopharmacology (Berl), v. 134, n. 4, p. 319-29, Dec 1997.

WILSON, B. E. et al. Induction of bcl-2 expression by phosphorylated CREB proteins during B-cell activation and rescue from apoptosis. Mol Cell Biol, v. 16, n. 10, p. 5546-56, Oct 1996.

WIMO, A. et al. The worldwide costs of dementia 2015 and comparisons with 2010. Alzheimers Dement, v. 13, n. 1, p. 1-7, Jan 2017.

XIE, Y. et al. Ebselen ameliorates beta-amyloid pathology, tau pathology, and cognitive impairment in triple-transgenic Alzheimer's disease mice. J Biol Inorg Chem, v. 22, n. 6, p. 851-865, Aug 2017.

YAMADA, N. et al. Improvement of scopolamine-induced memory impairment by Z-ajoene in the water maze in mice. Pharmacol Biochem Behav, v. 78, n. 4, p. 787-91, Aug 2004.

YAMAGUCHI, T. et al. Ebselen in acute ischemic stroke: a placebo-controlled, double-blind clinical trial. Ebselen Study Group. Stroke, v. 29, n. 1, p. 12-7, Jan 1998.

YANG, J. et al. Induced pluripotent stem cells in Alzheimer's disease: applications for disease modeling and cell-replacement therapy. Mol Neurodegener, v. 11, n. 1, p. 39, May 17 2016.

YOUNKIN, S. G. et al. Molecular forms of acetylcholinesterases in Alzheimer's disease. Fed Proc, v. 45, n. 13, p. 2982-8, Dec 1986.

ZHAO, R.; HOLMGREN, A. A novel antioxidant mechanism of ebselen involving ebselen diselenide, a substrate of mammalian thioredoxin and thioredoxin reductase. J Biol Chem, v. 277, n. 42, p. 39456-62, Oct 18 2002.

ZUCCHELLA, C. et al. The Multidisciplinary Approach to Alzheimer's Disease and Dementia. A Narrative Review of Non-Pharmacological Treatment. Front Neurol, v. 9, p. 1058, 2018.

ANEXOS

ANEXO A- CARTAS DE APROVAÇÃO DO PROJETO PELA COMISSÃO DE ÉTICA NO USO DE ANIMAIS DA UNIVERSIDADE FEDERAL DE SANTA MARIA

Comissão de Ética no Uso de Animais
da
Universidade Federal de Santa Maria

CERTIFICADO

Certificamos que a proposta intitulada "AVALIAÇÃO DO POSSÍVEL EFEITO TERAPÊUTICO DO COMPOSTO EBSELEN FRENTE A PREJUÍZOS COGNITIVOS INDUZIDOS POR DIFERENTES MODELOS DE DOENÇA DE ALZHEIMER EM CAMUNDONGOS SWISS", protocolada sob o CEUA nº 7372110915, sob a responsabilidade de **Cristina W. Nogueira** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria (CEUA/UFSM) na reunião de 23/06/2016.

We certify that the proposal "EVALUATION OF POSSIBLE THERAPEUTIC EFFECT OF COMPOUND EBSELEN IN FRONT OF COGNITIVE DEFICIT INDUCED BY DIFFERENT ALZHEIMER'S DISEASE MODELS IN SWISS MICE", utilizing 310 Heterogenics mice (310 males), protocol number CEUA 7372110915, under the responsibility of **Cristina W. Nogueira** - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Federal University of Santa Maria (CEUA/UFSM) in the meeting of 06/23/2016.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 10/2016 a 10/2017 Área: Bioquímica E Biologia Molecular

Origem:	Biotério Central UFSM						
Espécie:	Camundongos heterogênicos	sexo:	Machos	idade:	42 a 42 dias	N:	310
Linhagem:	Swiss			Peso:	25 a 35 g		

Resumo: Os compostos orgânicos de selênio apresentam importantes propriedades farmacológicas já reportadas, destacando-se entre elas os efeitos protetores sobre o déficit cognitivo em diferentes modelos experimentais. O ebselen, um composto orgânico sintético de selênio, é considerado um potente agente farmacológico em função de seus diversos efeitos já demonstrados, entre eles antioxidante, anti-inflamatório e neuroprotetor, e também pela baixa toxicidade atribuída à molécula. Sabe-se que o ebselen atravessa a barreira hematoencefálica e é seguro com base na toxicidade celular e ensaios clínicos de Fase I-III. Estudos demonstram que o ebselen inibe a atividade da Acetilcolinesterase (AChE) ex vivo (Mazzanti et al., 2006) e também inibe a atividade da enzima AChE cerebral in vitro (Martini et al., 2014). A acetilcolina (ACh) é um mediador químico de sinapses no sistema nervoso central (SNC), e sabe-se que há uma relação entre o déficit da transmissão colinérgica e doenças relacionadas com o prejuízo da memória (Cummings et al., 1998). AChE é um dos catalisadores biológicos mais eficientes e desempenha um papel chave na neurotransmissão colinérgica através da hidrólise da ACh (Soreq e Seidman, 2001; Mesulam et al., 2002). Dessa forma, os inibidores da AChE, conhecidos como IAChE, foram inicialmente desenvolvidos para tratar a disfunção cognitiva na doença de Alzheimer (DA) através do aumento dos níveis da ACh na fenda sináptica. Para este projeto, avaliaremos os possíveis efeitos protetores do composto ebselen sobre modelos que mimetizam a DA, para isso serão utilizados os modelos da injeção escopolamina e peptídeo ?-amilóide em camundongos Swiss. Os animais serão avaliados comportalmente nos seguintes testes: reconhecimento do objeto, localização do objeto, esquiva passiva e labirinto aquático de Morris. A atividade locomotora dos animais também será avaliada através da caixa de atividades, onde serão monitorados número de elevações, a distância percorrida e a contagem do tempo de permanência no quadrante central. Além disso, amostras de tecido cerebral serão analisadas em diferentes técnicas experimentais (western blot, imunohistoquímica e ELISA) com a finalidade de investigar um possível mecanismo de ação do composto orgânico de selênio estudado.

Local do experimento: Sala 2424 - Prédio 18 Sala 3209 - Prédio 19

Santa Maria, 01 de outubro de 2018

Avenida Roraima, 1000, Reitoria, 2º andar - CEP 97105-900 Santa Maria, RS - tel: 55 (55) 3220-9362 / fax:
Horário de atendimento: das 8:30 ás 12h e 14h ás 17hs : e-mail: ceua.ufsm@gmail.com
CEUA N 7372110915

Comissão de Ética no Uso de Animais
da
Universidade Federal de Santa Maria

Prof. Dr. Denis Broock Rosemberg
Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Prof. Dr. Saulo Tadeu Lemos Pinto Filho
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Comissão de Ética no Uso de Animais
da
Universidade Federal de Santa Maria

CERTIFICADO

Certificamos que a proposta intitulada "AVALIAÇÃO DA AÇÃO NEUROPROTETORA E ANTI-INFLAMATÓRIA DO EBSELEN E DO EXERCÍCIO DE NATAÇÃO EM MODELO DE DANO COGNITIVO INDUZIDO POR ESTREPTOZOTOCINA", protocolada sob o CEUA nº 6145050717, sob a responsabilidade de **Cristina W. Nogueira** *e equipe; Franciele Martini; Luiza Souza Marques* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria (CEUA/UFSM) na reunião de 24/08/2017.

We certify that the proposal "EVALUATION OF NEUROPROTETARY AND ANTI-INFLAMMATORY ACTION OF EBSELEN AND SWIMMING EXERCISE IN MODEL OF COGNITIVE DAMAGE INDUCED BY ESTREPTOZOTOCIN", utilizing 240 Heterogenics mice (240 males), protocol number CEUA 6145050717, under the responsibility of **Cristina W. Nogueira** *and team; Franciele Martini; Luiza Souza Marques* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Federal University of Santa Maria (CEUA/UFSM) in the meeting of 08/24/2017.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 09/2017 a 09/2018 Área: Bioquímica E Biologia Molecular

Origem:	Biotério Central UFSM						
Espécie:	Camundongos heterogênicos	sexo:	Machos	idade:	50 a 60 dias	N:	240
Linhagem:	Swiss			Peso:	25 a 35 g		

Resumo: Os compostos orgânicos de selênio apresentam importantes propriedades farmacológicas já reportadas, destacando-se entre elas os efeitos protetores sobre o déficit cognitivo em diferentes modelos experimentais. O ebselen, um composto orgânico sintético de selênio, é considerado um potente agente farmacológico em função de seus diversos efeitos já demonstrados, entre eles antioxidante, anti-inflamatório e neuroprotetor, e também pela baixa toxicidade atribuída à molécula (NOGUEIRA e ROCHA 2011). Sabe-se que o ebselen atravessa a barreira hematoencefálica e é seguro com base na toxicidade celular e ensaios clínicos de Fase I-III. A doença de Alzheimer (DA) é uma disfunção neurodegenerativa progressiva e multifatorial, caracterizada principalmente por uma diminuição da sinalização colinérgica e perda da memória (SCHLIEBS e ARENDT, 2011). A DA é evidenciada pelo depósito de fragmentos ?-amilóides, emaranhados neurofibrilares e neuroinflamação. Estudos mostram que, o exercício físico regular juntamente com terapia farmacológica, é um dos tratamentos para a prevenção da progressão da DA por possuir efeitos sobre múltiplos sistemas neurotransmissores e promovendo desta forma um aumento da expectativa de vida (UBHI e MASLIAH, 2013). Assim, mais pesquisas são necessárias para investigar a capacidade do exercício em modular, em parte, muitos marcadores da DA. Para este projeto, avaliaremos os possíveis efeitos protetores do composto ebselen juntamente com o exercício de natação sobre um modelo que mimetiza a DA. Para isso a estreptozotocina será utilizada como modelo indução de déficit cognitivo em camundongos Swiss (SHARMA, 2012). Os animais serão submetidos à adaptação do exercício de natação 20 min /dia durante 5 dias. Após a fase de adaptação, os animais serão submetidos ao exercício de natação 30 min/ dia, 5 vezes por semana, durante 4 semanas. Ainda, os animais receberão o composto ebselen nas doses de 5, 10 ou 20 mg/kg durante 14 dias. Na última semana, os camundongos serão avaliados comportamentalmete no teste do labirinto aquático de Morris. Além disso, amostras de córtex e hipocampo serão analisadas nas técnicas experimentais de western blot e imunohistoquímica, com a finalidade de investigar o possível mecanismo de ação do composto orgânico de selênio juntamente com o exercício físico de natação.

Local do experimento: Laboratório- 2424 Durante todo o curto período em que os animais estarão em nossa sala de experimentação laboratorial, a limpeza e a troca das palhas de cada uma das caixas serão efetuadas por um funcionário, sendo que, o mesmo está devidamente treinado para realizar o procedimento, causando o mínimo possível de desconforto para os animais experimentais.

Santa Maria, 01 de outubro de 2018

Comissão de Ética no Uso de Animais
da
Universidade Federal de Santa Maria

Prof. Dr. Denis Broock Rosemberg
Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Prof. Dr. Saulo Tadeu Lemos Pinto Filho
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

ANEXO B- Autorização para reprodução do artigo científico "Ebselen inhibits the activity of acetylcholinesterase globular isoform G4 in vitro and attenuates scopolamine-induced amnesia in mice" publicado na revista *Journal of Cell Biochemistry 119(7):5598-5608.*

JOHN WILEY AND SONS LICENSE TERMS AND CONDITIONS

Jan 11, 2019

This Agreement between Ms. Suzan Rosa ("You") and John Wiley and Sons ("John Wiley and Sons") consists of your license details and the terms and conditions provided by John Wiley and Sons and Copyright Clearance Center.

License Number	4505870390526
License date	Jan 11, 2019
Licensed Content Publisher	John Wiley and Sons
Licensed Content Publication	Journal of Cellular Biochemistry
Licensed Content Title	Ebselen inhibits the activity of acetylcholinesterase globular isoform G4 in vitro and attenuates scopolamine-induced amnesia in mice
Licensed Content Author	Franciele Martini, Ana P. Pesarico, César A. Brüning, et al
Licensed Content Date	Mar 12, 2018
Licensed Content Volume	119
Licensed Content Issue	7
Licensed Content Pages	11
Type of use	Dissertation/Thesis
Requestor type	Author of this Wiley article
Format	Print and electronic
Portion	Full article
Will you be translating?	No
Title of your thesis / dissertation	Ação neuroprotetora do composto ebselen e do exercício físico na neuroproteção em modelos de doença de Alzheimer esporádica em camundongos
Expected completion date	Feb 2019
Expected size (number of pages)	100
Requestor Location	Rua coronel niederauer 947 Santa Maria, Rio Grande do Sul 97015-122 Brazil Attn: Franciele Martini
Publisher Tax ID	EU826007151
Total	0.00 USD
Terms and Conditions	

TERMS AND CONDITIONS

This copyrighted material is owned by or exclusively licensed to John Wiley & Sons, Inc. or one of its group companies (each a"Wiley Company") or handled on behalf of a society with which a Wiley Company has exclusive publishing rights in relation to a particular work (collectively "WILEY"). By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the billing and payment terms and conditions established by the Copyright Clearance Center Inc., ("CCC's Billing and Payment terms and conditions"), at the time that you opened your RightsLink account (these are available at any time at http://myaccount.copyright.com).

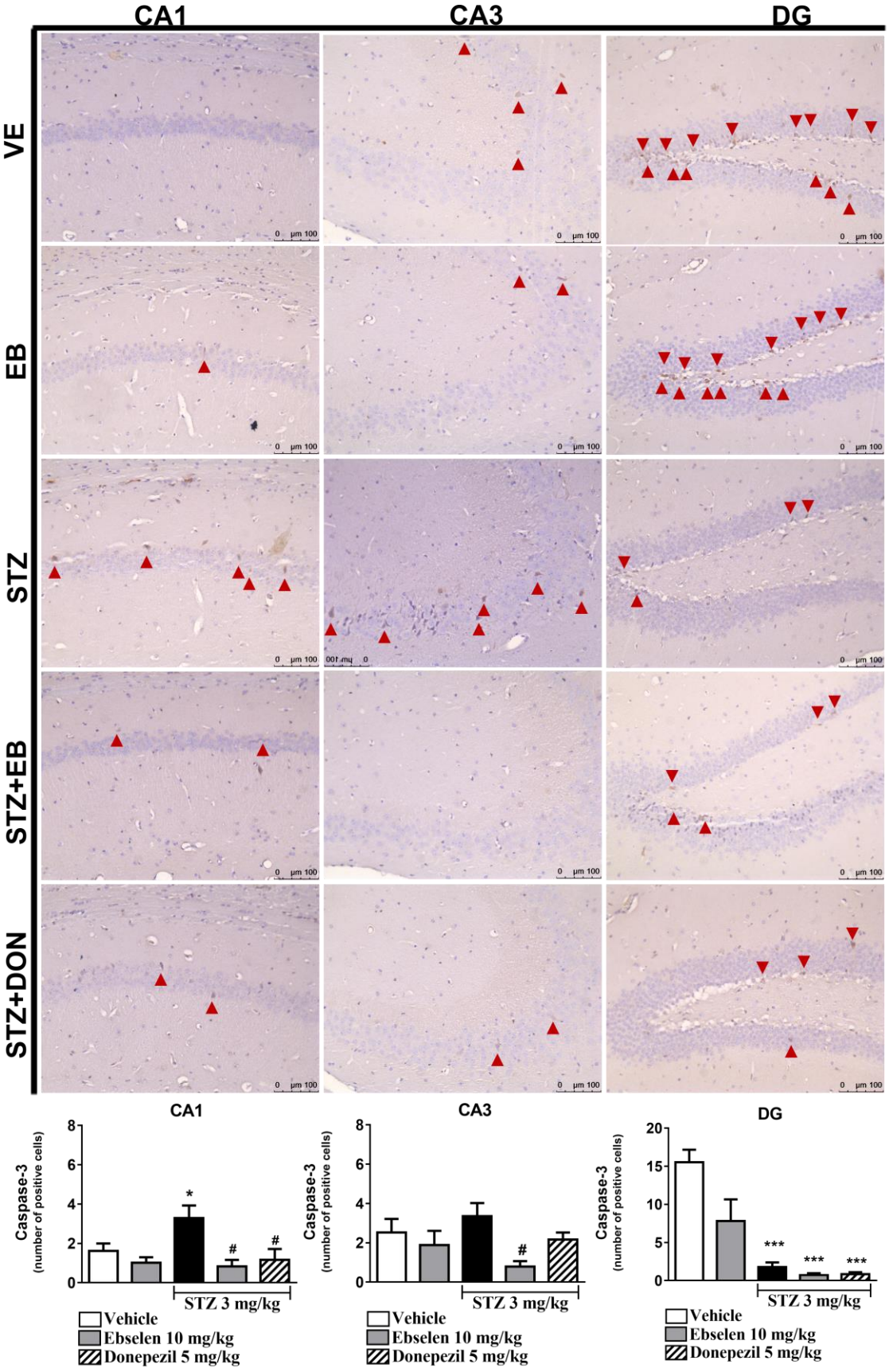
ANEXO C- Autorização para reprodução do artigo científico "A multifunctional compund ebselen reverses memory impairment, apoptosis and oxidative stress in a mouse of sporadic Alzheimer's disease" publicado na revista *Journal of PsychiatricResearch 109(11):107-117.*

ELSEVIER ORDER DETAILS

Jan 11, 2019

Order Number	501455823
Order date	Jan 11, 2019
Licensed Content Publisher	Elsevier
Licensed Content Publication	Journal of Psychiatric Research
Licensed Content Title	A multifunctional compound ebselen reverses memory impairment, apoptosis and oxidative stress in a mouse model of sporadic Alzheimer's disease
Licensed Content Author	Franciele Martini,Suzan Gonçalves Rosa,Isabella Pregardier Klann,Bruna Cruz Weber Fulco,Fabiano Barbosa Carvalho,Francine Luciano Rahmeier,Marilda Cruz Fernandes,Cristina Wayne Nogueira
Licensed Content Date	February 2019
Licensed Content Volume	109
Licensed Content Issue	n/a
Licensed Content Pages	11
Start Page	107
End Page	117
Type of Use	reuse in a thesis/dissertation
Portion	full article
Format	both print and electronic
Are you the author of this Elsevier article?	Yes
Will you be translating?	No
Title of your thesis/dissertation	Ação neuroprotetora do composto ebselen e do exercício físico na neuroproteção em modelos de doença de Alzheimer esporádica em camundongos
Author of new work	Cristina Nogueira
Expected completion date	Feb 2019
Estimated size (number of pages)	100
Requestor Location	Rua coronel niederauer 947 Santa Maria, Rio Grande do Sul 97015-122 Brazil Attn: Franciele Martini
Publisher Tax ID	GB 494 6272 12
Total	Not Available

ANEXO D- Resultado suplementar à tese, referente ao artigo 2



ANEXO E- Resultado suplementar à tese, referente ao manuscrito I

Figure 1. Experimental design of this study.

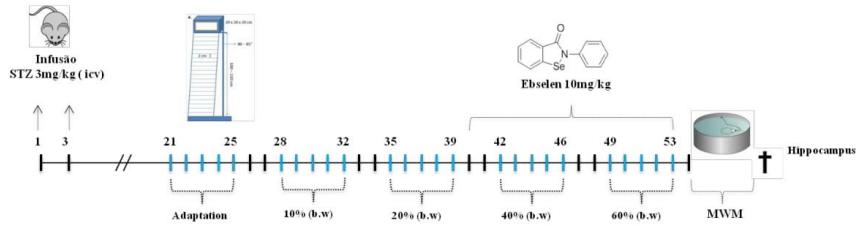


Figure 2. Strength exercise plus ebselen and strength exercise perse revert the learning and memory impairment induced by ICV-STZ in mice.

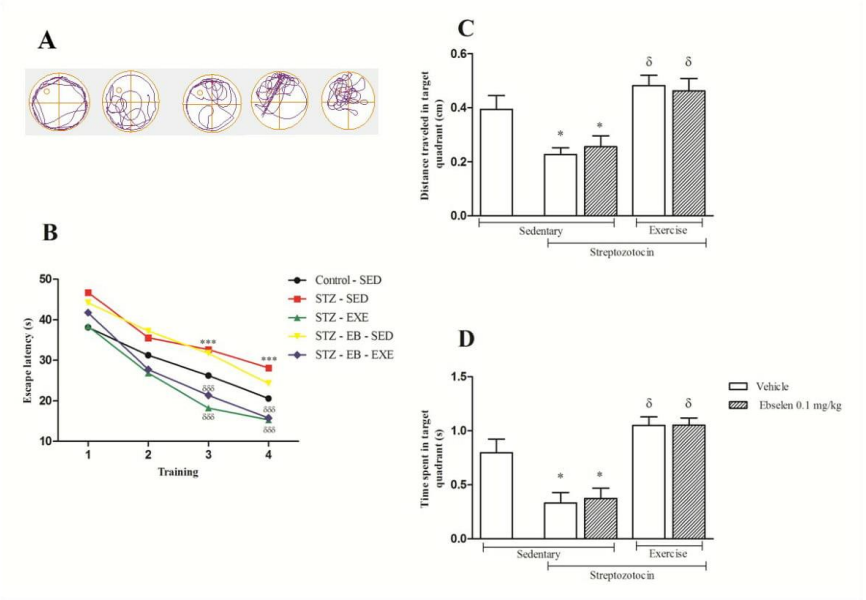


Figure 3. Strength exercise plus strength and exercise exercises revert the levels of protein involved in neuroprotection and proteins involved in apoptotic processes.

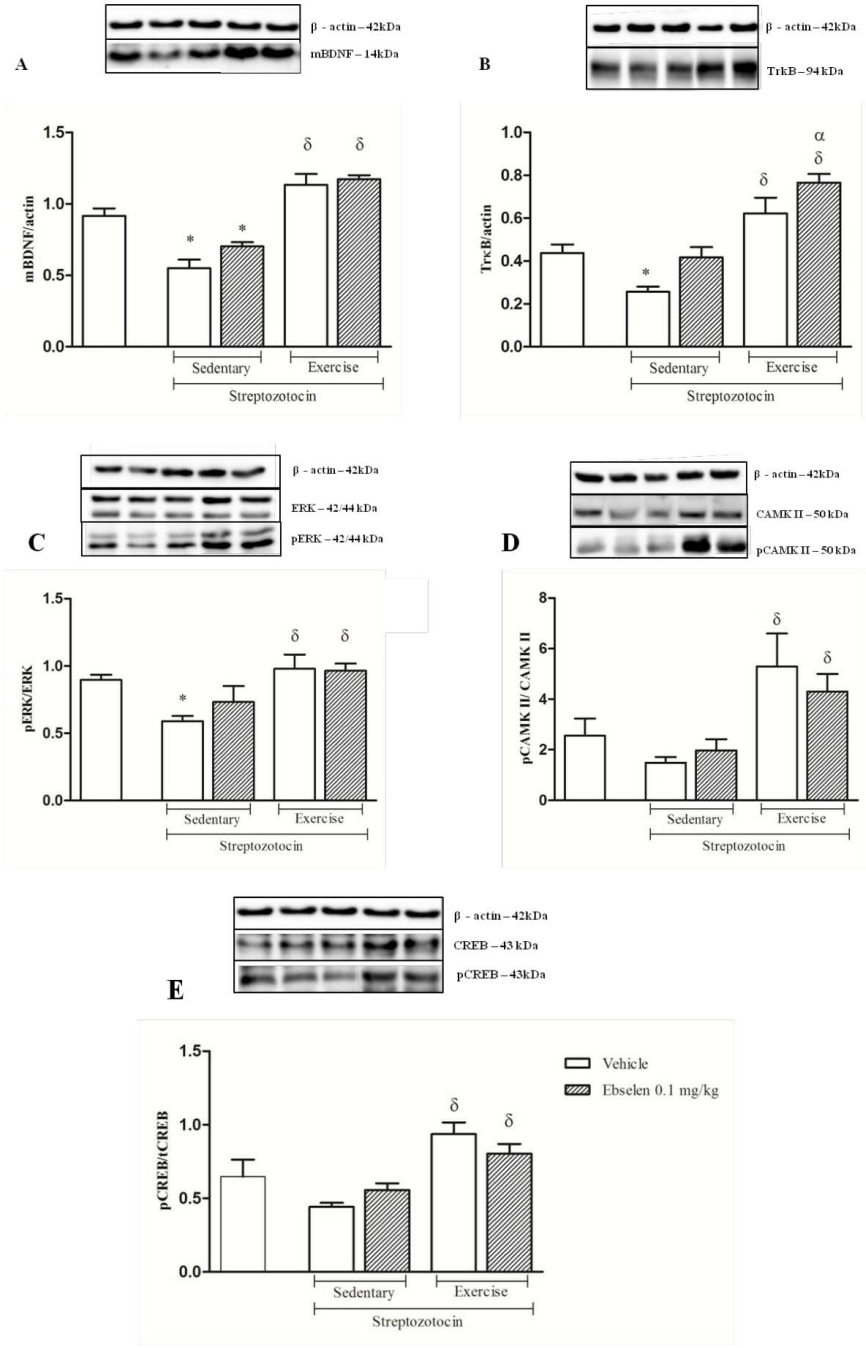

A
β - actin – 42kDa
mBDNF – 14kDa
mBDNF/actin
B
β - actin – 42kDa
TrkB – 94 kDa
TrcB/actin
C
β - actin – 42kDa
ERK – 42/44 kDa
pERK – 42/44 kDa
pERK/ERK
D
β - actin – 42kDa
CAMK II – 50 kDa
pCAMK II – 50 kDa
pCAMK II/ CAMK II
E
β - actin – 42kDa
CREB – 43 kDa
pCREB – 43kDa
pCREB/tCREB
Vehicle
Ebselen 0.1 mg/kg
Sedentary
Exercise
Streptozotocin

Figure 4. Strength exercise plus strength and exercise exercises revert the levels of protein involved in neuroprotection and proteins involved in apoptotic processes.