UNIVERSIDADE FEDERAL DE PELOTAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOQUÍMICA E BIOPROSPECÇÃO

TESE

1-(7-CLOROQUINOLIN-4-IL)-5-METIL-N-FENIL-1*H*-1,2,3-TRIAZOL-4 CARBOXAMIDA, TEM EFEITO NA MELHORA DA MEMÓRIA E NA EPILEPTOGÊNESE EM CAMUNDONGOS.

ANE GABRIELA VOGT

PELOTAS, 2021

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Tese apresentada por **Ane Gabriela Vogt** como pré-requisito para obtenção do GRAU DE DOUTORA em Bioquímica e Bioprospecção.

Orientadora: Profª. Drª. Cristiane Luchese
Co-Orientadora: Profª. Drª. Ethel Antunes Wilhelm

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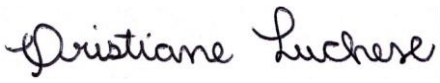

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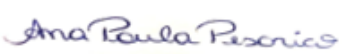

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Resumo

Como o escopo das opções de tratamento para as doenças neurológicas é limitado, faz-se necessário a busca de novos agentes que ajudem a prevenir o agravamento dessas doenças. Nesse sentido, os compostos derivados de quinolina atraem a atenção da comunidade científica, devido as suas atividades farmacológicas. Assim, o objetivo deste estudo foi investigar o possível efeito neuroprotetor do composto 1-(7-cloroquinolin-4-il)-5-metil-N-fenil-1*H*-1,2,3-triazol-4-carboxamida (QTCA-1) frente ao déficit de memória e na epileptogênese. No presente estudo foram utilizados camundongos machos adultos da raça *Swiss* e foram avaliadas (i) a ação anticolinesterásica *in vitro* do QTCA-1, bem como o efeito do tratamento agudo do composto nas diferentes fases da memória (aquisição, consolidação e recuperação) em camundongos amnésicos; (ii) a atividade anti-aminésica do QTCA-1, em um modelo sub-crônico, relacionado a demência em camundongos, bem como avaliar o envolvimento do sistema colinérgico, da atividade da Na^+/K^+-ATPase e do dano oxidativo nas estruturas cerebrais dos camundongos induzidos por escopolamina (ESC); além (iii) do efeito do QTCA-1 na gravidade das crises convulsivas, estresse oxidativo e distúrbio de memória em um modelo de indução de *kindling* por pentilenotetrazol (PTZ) em camundongos, bem como o papel das enzimas Na^+/K^+-ATPase e da acetilcolinesterase (AChE) nas estruturas cerebrais (córtex cerebral e hipocampo) de camundongos. Foi possível verificar que o QTCA-1 (i) modulou o sistema colinérgico, através da inibição da atividade da AChE *in vitro* no córtex cerebral e hipocampo dos camundongos, bem como atenuou as alterações causadas pela ESC nas diferentes fases da memória (aquisição, consolidação e recuperação) no teste da esquiva inibitória, não causando alteração na atividade locomotora e exploratória dos animais; (ii) demonstrou efeito anti-amnésico em um modelo subcrônico, através da normalização das atividades da AChE e Na^+/K^+-ATPase bem como do dano oxidativo em estruturas cerebrais modificados pela ESC; (iii) atenuou a epileptogênese e o déficit de memória, além do dano oxidativo e da atividade da Na^+/K^+-ATPase em estruturas cerebrais, em um modelo de *kindling* induzido por PTZ. Em conclusão, este derivado de quinolina parece exercer ação neuroprotetora na melhora da memória e epileptogênese principalmente por modular o dano oxidativo e a atividade da Na^+/K^+-ATPase. Além disso, o efeito do QTCA-1 também parece estar ligado a atividade anticolinesterásica do composto.

Palavras-Chave: Doenças neurológicas, quinolina, memória, epileptogênese, estresse oxidativo.

Abstract

As the scope of treatment options for neurological diseases is limited, it is necessary to search for new agents that help prevent the worsening of these diseases. In this sense, quinoline-derivative compounds attract the attention of the scientific community, due to their pharmacological activities. Thus, the aim of this study was to investigate the possible neuroprotective effect of the compound 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1*H*-1,2,3-triazole-4-carboxamide (QTCA-1) against memory deficit and epileptogenesis. In the present study, male *Swiss* mice were used and were evaluated (i) the anticholinesterase action of QTCA-1 *in vitro*, as well as the effect of acute treatment of the compound in the different phases of memory (acquisition, consolidation and retrieval) in amnesic mice; (ii) the anti-aminesic activity of QTCA-1, in a sub-chronic model, related to dementia in mice, as well as evaluating the involvement of the cholinergic system, the activity of Na^+/K^+-ATPase and the oxidative damage in brain structures from mice induced by scopolamine (SCO); in addition (iii) the effect of QTCA-1 on the severity of seizures, oxidative stress and memory disorders in a model of kindling induction by pentylenetetrazol (PTZ) in mice, as well as the role of the enzymes Na^+/K^+-ATPase and of acetylcholinesterase (AChE) in mice brain structures (cerebral cortex and hippocampus). It was possible to verify that QTCA-1 (i) modulated the cholinergic system, through the inhibition of AChE activity *in vitro* in the cerebral cortex and hippocampus of mice, as well as attenuated the alterations caused by SCO in the different phases of memory (acquisition, consolidation and retrieval) in the inhibitory avoidance test, not causing changes in the locomotor and exploratory activity of the animals; (ii) demonstrated anti-amnesic effect in a subchronic model, through normalization of AChE and Na^+/K^+-ATPase activities as well as oxidative damage in cerebral structures modified by SCO; (iii) attenuated epileptogenesis and memory deficit, as well as oxidative damage and Na^+/K^+-ATPase activity in brain structures, in a PTZ-induced kindling model. In conclusion, this quinoline derivative seems to exert neuroprotective action in improving memory and epileptogenesis mainly by modulating oxidative damage and Na^+/K^+-ATPase activity. Furthermore, the effect of QTCA-1 also seems to be linked to the anticholinesterase activity of the compound.

Keywords: Neurological diseases, quinoline, memory, epileptogenesis, oxidative stress.

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Fig. 3. Effect of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) at different concentrations (1-500 μM) in the acetylcholinesterase (AChE) activity in cerebral cortex (A) and hippocampus (B) of mice. Data are reported as mean ± standard error of the mean (S.E.M.) of 3 independent experiments, performing in different days. AChE activity was expressed as μmol acetylthiocholine/h/mg protein. (*) denotes $p< 0.05$, (**) denotes $p <0.01$, (***) denotes $p< 0.001$ and (****) denotes $p<0.0001$ as compared with the control group (one-way analysis of variance/ Tukey test).

Fig. 4. Effect of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) on scopolamine (SCO)-induced memory deficit in the step-down inhibitory avoidance task in mice. Data are from the test session for (A) acquisition, (B) consolidation, and (C) retrieval. Each column represents mean±SEM from seven animals per group. (*) denotes $p< 0.05$, (**) denotes $p <0.01$, (***) denotes $p< 0.001$ as compared with the control group. (#) denotes $p< 0.05$, (##) denotes $p <0.01$, (###) denotes $p< 0.001$ as compared with the scopolamine group (one-way analysis of variance/ Tukey test).

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ARTIGO 1

Fig. 1. Chemical structure 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1).

Fig. 2. Scheme of experimental protocol. Thirty minutes before initiating intragastric (i.g.) treatments, mice received scopolamine (SCO) or saline, both intraperitoneally (i.p.). I.g. treatments and i.p. induction were performed every day, until the end of the experimental protocol. From the first day of the experimental protocol, the animals were submitted to the Barnes maze, open-field, object recognition, object location, step-down inhibitory avoidance tasks. On the ninth day, mice were euthanized.

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Fig. 7. Effect of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3- triazole-4-carboxamide (QTCA-1) on acetylcholinesterase (AChE) activity in (7A) hippocampus and (7B) cerebral cortex of mice after induction with scopolamine (SCO). Data are reported as mean ± standard error of the mean (SEM) of seven animals per group. (**) denotes $p < 0.01$, (***) denotes $p < 0.001$ and (****) denotes $p < 0.0001$ as compared to the control group; (##) denotes $p < 0.01$ (###) denotes $p < 0.001$ and (####) denotes $p < 0.0001$ as compared to the SCO group (one-way analysis of variance/Newman-Keuls test).

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Fig. 1. Chemical structure of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1).

Fig. 2. Scheme of experimental protocol. Thirty minutes after initiating intragastric (i.g.) treatments, mice received pentylenetetrazole (PTZ) or saline, both intraperitoneally (i.p.). I.g. and i.p. treatments were performed once every alternate day (1^{th}, 3^{rd} , 5^{th} , 7^{th}, 9^{th} and 11^{th} days) and seizure was evaluated. From the 12^{th} day of the experimental protocol, the animals were submitted to the open-field, object recognition, Y-maze and step-down inhibitory avoidance tasks. On the 16^{th} day, mice were euthanized.

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as mean ± standard error of the mean (SEM) of seven animals per group. (****) denotes $p < 0.0001$ as compared to the control group; (####) denotes $p < 0.0001$ as compared to the PTZ group (two-way ANOVA followed by Fisher's LSD test).

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Fig. 7. Effects of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) and phenobarbital (PHEN) on the latency (s) to fall from the platform in the test phase in the step-down inhibitory avoidance task in mice submitted to pentylenetetrazole (PTZ)-induced kindling. Data are reported

as mean ± standard error of the mean (SEM) of seven animals per group. (****) denotes $p < 0.0001$ as compared to the control group; (####) denotes $p < 0.0001$ as compared to the PTZ group (one-way ANOVA followed by Tukey's test).

Fig. 8. Effects of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) and phenobarbital (PHEN) on thiobarbituric acid reactive species (TBARS) levels in (7A) cerebral cortex and (7B) hippocampus of mice submitted to pentylenetetrazole (PTZ)-induced kindling. Data are reported as mean ± standard error of the mean (SEM) of seven animals per group. (***) denotes $p < 0.001$ and (****) denotes $p < 0.0001$ as compared to the control group; (###) denotes $p< 0.001$ and (####) denotes $p < 0.0001$ as compared to the PTZ group (one-way ANOVA followed by Tukey's test).

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in (10A) cerebral cortex and (10B) hippocampus of mice submitted to pentylenetetrazole (PTZ)-induced kindling. Data are reported as mean ± standard error of the mean (SEM) of seven animals per group. (****) denotes $p < 0.0001$ as compared to the control group; (####) denotes $p < 0.0001$ as compared to the PTZ group (one-way ANOVA followed by Tukey's test).

Fig. 12. Effects of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) and phenobarbital (PHEN) on acetylcholinesterase (AChE) activity in (11A) cerebral cortex and (11B) hippocampus of mice submitted to pentylenetetrazole (PTZ)-induced kindling. Data are reported as mean ± standard error of the mean (SEM) of seven animals per group (one-way ANOVA followed by Tukey's test).

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LISTA DE ABREVIATURAS

Aβ- Beta- amilóide
ACh - Acetilcolina
ACh co-T- Transportador vesicular de acetilcolina
AChE - Acetilcolinesterase
ADP- Adenosina difosfato
ATP- Adenosina trifosfato
BHE- Barreira hematoencefálica
BuChE- Butirilcolinesterase
CAT- Catalase
ChAT - Colina Acetiltranferase
CA -Cornu ammonis
CM- Corpo mamilar
DA- Doença de Alzheimer
DG -Giro dentado
DON- Donepezila
ERs- Espécies reativas
ERNs- Espécies reativas de nitrogênio
EROs- Espécies reativas de oxigênio
ESC- Escopolamina
FDA- Food and Drug Administration
FEN- Fenobarbital
GABA- Ácido gama-aminobutírico
GABA-T - GABA-transaminase
GAD- Enzima glutamato descarboxilase
GPx- Glutationa peroxidase
GSH- Glutationa reduzida
GST- Glutationa-S-transferase
ILAE- Liga Internacional contra epilepsia
LDH- Lactato desidrogenase
mAChR- Receptores muscarínicos
nAChR- Receptores nicotínicos

nat- Núcleo anterior talâmico

NS- Núcleo septal

ntm- Núcleo talâmico médio

OMS- Organização Mundial da Saúde

PTZ- Pentilenotetrazol

SN- Sistema nervoso

SNC- Sistema nervoso central

SOD- Superóxido dismutase

SSADH- Semi-aldeído succínico desidrogenase.

SNAPS- Proteínas sinaptossômicas

SNC – Sistema Nervoso Central

TBARS- Espécies reativas do ácido tiobarbitúrico

Tmm- trato mamilotalâmico

VAMP5- Proteína de membrana associadas à vesículas

SUMÁRIO

1. INTRODUÇÃO

As doenças neurológicas são caracterizadas pela perda progressiva da função e da estrutura neuronal, o que eventualmente leva à morte dos neurônios no sistema nervoso (SN) (KARNATI et al., 2015). Estima-se que centenas de milhões de pessoas em todo o mundo são afetados por distúrbios neurológicos, atingindo cerca de 10,2% da população (ALESSANDRINI et al., 2019). Dentre essas doenças podemos citar a doença de Parkinson, doença de Alzheimer (DA), doença de Huntington, esclerose lateral amiotrófica, epilepsia, dentre outras. Geralmente estas doenças estão acompanhadas por alterações neurodegenerativas que causam modificações celulares e moleculares, com presença de estresse oxidativo, oligomerização e agregação de proteínas, deficiência do transporte axonal nos neurônios, desregulação de cálcio, disfunção mitocondrial e neuroinflamação (KARNATI et al., 2015).

A perda de memória é uma parte particularmente devastadora destas doenças neurológicas (VOGEL e WOOD et al., 2014). A memória pode ser definida como a capacidade de adquirir, guardar e usar uma determinada informação sobre o mundo, sendo um fenômeno comportamental relacionado à plasticidade do sistema nervoso central (SNC) (JANCA et al., 2006). Sendo assim, a disfunção de memória está associada com toda a gama de problemas neurológicos que afetam a função cerebral, principalmente a disfunção colinérgica (MATTHEWS, 2015). Sabe-se que o sistema colinérgico desempenha um papel essencial nos processos de aprendizado e memória, tendo em vista que a diminuição da concentração de acetilcolina (ACh) no cérebro também resulta em uma capacidade diminuída de aprender e formar novas memórias (LIN et al., 2016), Além disso, a hipofunção colinérgica tem sido relacionada com os declínios cognitivos progressivos que levam ao aparecimento da demência (JANCA et al., 2006).

Paralelamente as doenças neurológicas relacionadas a disfunção colinérgica, a epilepsia é uma doença que afeta aproximadamente 50 milhões de pessoas em todo o mundo (WILLEMS et al., 2018). Essa doença é caracterizada como um distúrbio neurológico crônico em que ocorrem crises convulsivas recorrentes e espontâneas, e estão geralmente relacionadas à um desequilíbrio nas redes cerebrais excitatórias e inibitórias (BEGHI, 2019). Além

disso, o prejuízo cognitivo relacionado ao déficit de memória é uma comorbidade grave que geralmente ocorre com mais frequência em pacientes com epilepsia, proveniente da alteração na plasticidade neural, que ocorrem nas crises convulsivas (WANG, X et al., 2018).

Além disso, o estresse oxidativo é outro fator causal bem conhecido na patogênese dos distúrbios neurológicos relacionados à demência (CRUZ et al., 2002) e a epilepsia (WALDBAUM e PATEL, 2010). De fato, estudos mostraram que o estresse oxidativo está relacionado a prejuízos cognitivos como o déficit de memória (TAO et al., 2015), estando também ligado à perda neuronal no cérebro (AZMAN et al., 2015). Acredita-se que o estresse oxidativo perturbe a homeostase do cálcio intracelular gradualmente, levando a um aumento da excitabilidade neuronal, suscetibilidade a ataques e vulnerabilidade a estresse adicional e perda de células neuronais (CHANG e YU, 2010).

Como essas doenças neurológicas citadas anteriormente englobam uma série de distúrbios do SN, o escopo das opções de tratamento para as mesmas é limitado e as taxas de aprovação de medicamentos para tratamentos aprimorados permanecem baixas, quando comparadas com outras áreas terapêuticas (ALESSANDRINI et al, 2019). Desta forma, faz-se necessário o desenvolvimento de estratégias eficazes que ajudem a prevenir o agravamento da estrutura e da função cerebral relacionadas com essas doenças podendo, assim, proporcionar benefícios significativos para a sociedade e para os sistemas de cuidados de saúde.

Desta forma, nosso grupo de pesquisa tem dedicado atenção ao efeito de derivados da quinolina em distúrbios que afetam o SNC (PINZ et al., 2018; BARTH et al., 2019). Entre os derivados da quinolina, pode-se citar a 1-(7-cloroquinolin-4-il)-5-metil-N-fenil-1H-1,2,3-triazol-4-carboxamida (QTCA-1) que apresentou atividade anticonvulsivante, devido a sua natureza lipofílica, sendo o tecido cerebral o alvo deste composto (WILHELM et al., 2014).

Neste contexto, considerando que o QTCA-1 apresenta propriedades farmacológicas importantes, podendo vir a ser uma alternativa no tratamento dos distúrbios neurológicos que geram muitos prejuízos na qualidade de vida dos indivíduos, além dos elevados custos sócios-econômicos, o presente estudo tem por objetivo, investigar o efeito neuroprotetor do QTCA-1 frente ao déficit de memória relacionado a disfunção colinérgica e na epileptogênese.

2. OBJETIVOS

2.1. Objetivo geral

Investigar o possível efeito neuroprotetor do QTCA-1, um derivado de quinolina, no déficit de memória e na epileptogênese em camundongos.

2.2. Objetivos específicos

- Avaliar se o QTCA-1 modula o sistema colinégico em estruturas cerebrais (córtex cerebral e hipocampo) de camundongos *in vitro*;

- Verificar o efeito do QCTA-1 nas diferentes fases da memória em camundongos, bem como investigar a atividade locomotora e explotatória dos animais;

- Averiguar o envolvimento do sistema colinérgico nas estruturas cerebrais dos camundongos tratados com o QTCA-1 nas diferentes fases da memória;

- Investigar a atividade anti-aminésica do QTCA-1 em um modelo de amnésia em camundongos, bem como avaliar o envolvimento do sistema colinérgico, da atividade da Na^+/K^+-ATPase e do dano oxidativo nas estruturas cerebrais dos camundongos;

- Determinar o efeito terapêutico do QTCA-1 na gravidade das crises convulsivas, no estresse oxidativo e no distúrbio de memória em um modelo de kindling em camundongos, bem como investigar o possível papel da atividade da Na^+/K^+-ATPase e do sistema colinérgico nas estruturas cerebrais de camundongos.

3. REVISÃO BIBLIOGRÁFICA

3.1. Doenças neurológicas e a demência

Distúrbios do SNC e periférico são heterogêneos e diversos. Estima-se que centenas de milhões de pessoas em todo o mundo são afetados por alguma doença neurológica (ALESSANDRINI et al., 2019). As doenças neurológicas ficam atrás apenas das doenças cardiovasculares em relação às causas de morte (GROUP GBDNDC, 2017). Além disso, foi relatado anteriormente que o custo anual de gestão as doenças neurológicas, apenas nos Estados Unidos, chegam a 800 bilhões de dólares. Apesar destas doenças afetarem muitas pessoas no mundo, o cenário de tratamento é limitado, com poucas opções disponíveis e baixas taxas de aprovação para novos medicamentos em potencial (PANKEVICH et al., 2014).

Dentre as desordens neurológicas mais comuns pode-se destacar a doença de Parkinson, DA, doença de Huntington, epilepsia, dentre outras (KARNATI et al., 2015). A maioria destas doenças neurológicas são caracterizadas pela perda progressiva da função neuronal e estrutural, que eventualmente levam à morte dos neurônios no SN (KARNATI et al., 2015). Sendo assim, nas desordens neurológicas, ocorrem alterações celulares e moleculares, que são acompanhadas de uma aceleração na alteração neuronal fisiológica, e quando abrange funções superiores, tem-se o processo de demência (IZQUIERDO, 2002).

A demência é uma síndrome neurológica que esta associada a várias doenças, além de ser considerada uma das principais causas de morbimortalidade entre idosos (PURI et al., 2014). Segundo dados da OMS, estima-se que 50 milhões de pessoas sofram de algum tipo de demência, no mundo (OMS, 2017).

As síndromes demenciais podem ser classificadas de duas formas: degenerativas e não-degenerativas. As demências não-degenerativas decorrem de acidentes vasculares, crises convulsivas, processos infecciosos, traumatismos, contaminações tóxicas, tumores, entre outros. Já a demência do tipo degenerativa, está presente em doenças como a DA ou doença de Huntington (KURUPPU e MATTHEWS, 2013).

Dessa forma, a demência é caracterizada como uma síndrome neurológica, que leva ao aparecimento de diversos sintomas, dentre eles a amnésia, na qual ocorre uma perda da memória total ou parcial, e o indivíduo é incapaz de manter ou recuperar informações anteriormente armazenadas (JANCA et al., 2006). Apesar da demência acometer muitos aspectos da cognição dos pacientes, a perda da memória tem um destaque especial nesse processo (JANCA et al., 2006).

3.2. Aspectos gerais da memória

A memória é definida como a capacidade de adquirir, guardar e usar uma determinada informação sobre o mundo (JANCA et al., 2006). Este processo é um fenômeno comportamental relacionado à plasticidade do SNC. A memória pode ser definida como a capacidade de armazenar e evocar experiências aprendidas, não apenas a capacidade de repetir, mas sim de variar a resposta frente a uma nova aprendizagem (IZQUIERDO, 2002). Desta forma, a memória é um processo que requer a atividade integrada de diferentes regiões do encéfalo e sistemas de neurotransmissão (IZQUIERDO et al., 2006).

Pelo fato das memórias serem provenientes das experiências, é mais adequado relaciona-las à processos de memória ou “memórias”, do que simplesmente “memória”, uma vez que podem existir tantas memórias quanto o número de experiências possíveis (IZQUIERDO, 2002). Como a natureza das experiências vivenciadas é a mais variada possível, é de se esperar que existam diferentes tipos de memórias e que estas estejam relacionadas a diferentes áreas cerebrais e, consequentemente, mecanismos celulares distintos (IZQUIERDO, 2002).

Sendo assim, a memória não é um processo único, e também não é adquirida imediatamente na sua forma final. É necessário um tempo no qual a memória vai sendo preparada para se tornar permanente. Desta maneira, a memória é o resultado de pelo menos três tipos de processamento distintos e pode então ser dividida em aquisição, consolidação e recuperação (IZQUIERDO et al., 2006).

A aquisição ou aprendizagem refere-se aos processos pelos quais novas informações aprendidas são tratadas e processadas por sistemas neurais específicos, quando encontradas pela primeira vez (IZQUIERDO et al., 2006). Logo após a aquisição, tem-se a consolidação, ou seja, a fixação e o armazenamento de uma informação recém adquirida ou aprendida. Esta fase, refere-se àqueles processos que alteram a informação recém retida e ainda lábil, de modo a torná-la mais estável para a retenção em longo prazo. Por sua vez, a consolidação envolve a expressão de genes e a síntese de novas proteínas, dando origem a alterações estruturais que mantêm a estabilidade da memória ao longo do tempo (DUDAI, 2004).

À última etapa dá-se o nome de recuperação, que se refere àqueles processos que permitem a lembrança e o uso das informações retidas. A recuperação é definida como o processo de avaliação da memória e o uso da informação consolidada. Em humanos, ela pode ser vista na mudança de um comportamento, adequação a um determinado padrão ou no reconhecimento de pessoas, lugares, palavras, etc (IZQUIERDO et al., 2006).

Sendo assim, em relação ao aprendizado, o mesmo pode ser modificado, de forma que se pode manipular uma ou mais das etapas de formação da memória. Esta interferência pode ocorrer nas diferentes fases da memória: aquisição, consolidação e evocação. Assim, a interferência pode ocorrer antes da exposição a uma nova experiência, ou nos momentos iniciais da aquisição. Em relação a consolidação, a interferência pode ocorrer após o treino (após a aquisição). Já o processo de recuperação, pode ser interferido com tratamento aplicado antes do teste (IZQUIERDO et al., 1997). Desta forma, a esquiva inibitória é um teste que permite a avaliação dos mecanismos envolvidos nas memórias de curta e longa duração. Por ser adquirido em uma única sessão de treino, o aprendizado da esquiva inibitória permite isolar cada uma das fases do processamento da memória (IZQUIERDO et al., 1997).

Além disso, a memória pode ser classificada de acordo com a sua duração. A memória de curto prazo é uma forma de memória de capacidade limitada e de curta duração, que faz referência a experiências recém-vividas e conhecimentos adquiridos há pouco tempo, com o seu armazenamento por um período limitado. Um exemplo seria a recordação de uma sequência pequena de letras ou números (VESELIS, 2015). Já a memória de longo prazo é mais

estável e de longa duração, onde há lembranças que tem relação com conhecimentos adquiridos há muito tempo, consolidadas ao longo do tempo depois do processo de aprendizado. A memória de longo prazo esta diretamente relacionada com o armazenamento de grande quantidade de informações, por um período de tempo indefinido (VESELIS, 2015).

A memória de longo prazo é dividida em declarativa e não-declarativa. A memória declarativa está relacionada com a capacidade de coleta consciente de informações sobre fatos e eventos e a capacidade de descrevê-los. Dessa forma, a memória do tipo declarativa é representacional, provendo um modelo do mundo externo, que pode ser verdadeiro ou fictício, sendo que suas funções estão comprometidas em indivíduos com amnésia e demências (SQUIRE, 2004).

A memória do tipo declarativa pode ser dividida em semântica e episódica. A memória semântica está relacionada a fatos concretos sobre o mundo a partir de conhecimentos simbolicamente representáveis, como, por exemplo, o fato de o céu de fim de tarde ser alaranjado (AMEEN-ALI et al., 2015). Já a memória episódica é definida como a capacidade de reviver um evento no contexto original em que ocorreu e está diretamente relacionada com a lembrança pessoal de um indivíduo. Portanto, esta é a lembrança do que aconteceu, como, onde e quando (AMEEN-ALI et al., 2015).

A memória não-declarativa, por sua vez, é expressa via alterações no padrão comportamental do indivíduo. Ela é composta de modificações em áreas cerebrais responsáveis pelo desempenho em uma determinada tarefa (GASBARRI et al., 2014). Dessa forma, as memórias adquiridas ocorrem pela reativação de áreas em que o aprendizado já foi consolidado. Isso é possível pela aquisição, consolidação e evocação de habilidades motoras e cognitivas. Um exemplo a este fato seria o ato de dirigir. Mesmo que ele possa ter surgido de eventos inerentes ao indivíduo, depois de um tempo a ação torna-se quase automática (SQUIRE, 2004).

Acredita-se que a memória seja uma função cerebral que envolve diversas regiões, tais como o córtex cerebral e várias estruturas do sistema límbico. A busca pela localização espacial da memória existe há mais de um século (IZQUIERDO, 2002). No princípio, os pesquisadores afirmavam que os processos cognitivos estavam centrados no neocórtex (IZQUIERDO, 2002).

Hoje em dia, sabe-se que diversas estruturas encefálicas estão envolvidas com as diferentes etapas (aquisição, consolidação e recuperação) da memória, com destaque para o hipocampo, a amígdala, o septo medial, o córtex temporal, o córtex pré-frontal, o estriado e o cerebelo (IZQUIERDO, 2002).

Estas descobertas em relação a memória destacam a anatomia complexa das várias regiões envolvidas com seu processamento, como os lóbulos temporal medial que incluem tanto o hipocampo (subcampos CA1 a CA3, giro dentado e subículo) e estruturas extra-hipocampal (entorrinal, perirrinal e córtices parahipocampais) (Figura 1). Sabe-se que o hipocampo é considerado importante para o processo de consolidação da memória (MATTHEWS, 2015). Além disso, danos às regiões do cérebro conectadas com os lobos temporais mediais também resultam em graus variáveis no comprometimento da memória episódica. Lesões no giro cingulado posterior que está funcionalmente conectado ao hipocampo, também pode prejudicar memória. Os lóbulos frontais também desempenham um papel importante na memória episódica, impactando principalmente nas funções de codificação e recuperação. Adicionalmente, a conexão entre o tálamo pré-frontal e tálamo retrosplenial contribuem significativamente para a rede de memória episódica e pode fazer contribuições diferenciais para a recordação e familiaridade (MATTHEWS, 2015).

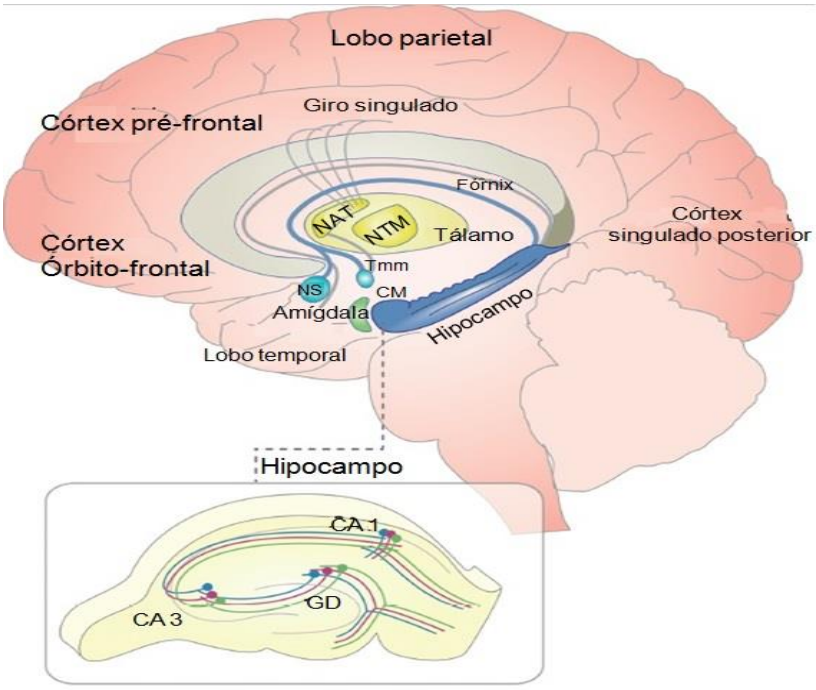

Figura 1. Estruturas encefálicas envolvidas nos processos de aprendizagem e memória. A inserção mostra a via de alimentação trissináptica envolvida no processamento de informações dentro do hipocampo, conhecida por ser crítica para o estágio de codificação da memória. nat: núcleo anterior talâmico; ntm: núcleo talâmico médio, Tmm: trato mamilotalâmico, NS: núcleo septal, CM: corpo mamilar; CA = cornu ammonis; DG = Giro dentado. Adaptado de MATTHEWS, 2015.

3.3. Relação da via colinérgica com a memória

O sistema colinérgico desempenha um papel essencial nos processos de aprendizado e memória. A transmissão colinérgica (Figura 2) é baseada em proteínas que estão envolvidas na síntese, estocagem, transporte e degradação do neurotransmissor ACh (FERREIRA-VIEIRA et al., 2016). A ACh é considerada um neurotransmissor muito importante, envolvida principalmente na regulação das funções cognitivas (AGRAWAL et al., 2009; ISHRAT et al., 2009). Isso se dá pelo fato de que, durante o processo de envelhecimento, os neurônios colinérgicos sofrem degeneração, levando a uma hipofunção colinérgica, sendo relacionados com os declínios cognitivos progressivos (SCHLIEBS e ARENDT, 2011).

A síntese de ACh ocorre nos terminais nervosos dos neurônios colinérgicos a partir de dois precursores, a colina e a acetil-coenzima A. A colina acetiltranferase (ChAT) catalisa a síntese de ACh que, por sua vez, pode interagir com receptores colinérgicos pré- e pós-sinápticos (DEIANA et al., 2011). A ChaT é ativada quando há despolarização, influxo de cálcio e fosforilação por diversas proteínas quinases. À medida que o neurônio colinérgico é despolarizado, a ACh é liberada na fenda sináptica por exocitose, e assim ativa seus receptores. Posteriormente, o neurotransmissor é rapidamente inativado pela AChE, uma enzima que está geralmente presente nos neurônios da fenda sináptica (DEIANA et al., 2011).

O neurotransmissor ACh liga-se a duas classes de receptores: os receptores colinérgicos nicotínicos, de ação ionotrópica, e os receptores colinérgicos muscarínicos, de ação metabotrópica (FERREIRA-VIEIRA et al., 2016). Os primeiros, ao serem ativados, permitem o influxo de íons como Na^+,

K^+ e Ca^{2+}, e após sua ativação há uma despolarização e excitação celular, resultando em uma atividade modulatória do SNC. Já os receptores muscarínicos, que são os mais abundantes, pertencem à classe dos receptores acoplados à proteína G (FERREIRA-VIEIRA et al., 2016). Centralmente, a estimulação desses receptores facilita a liberação de diversos neurotransmissores, como o glutamato, o ácido gama-aminobutírico (GABA), a dopamina e a ACh. Por sua vez, a alta concentração de ACh está envolvida nos processos de memória e aprendizado (FERREIRA-VIEIRA et al., 2016).

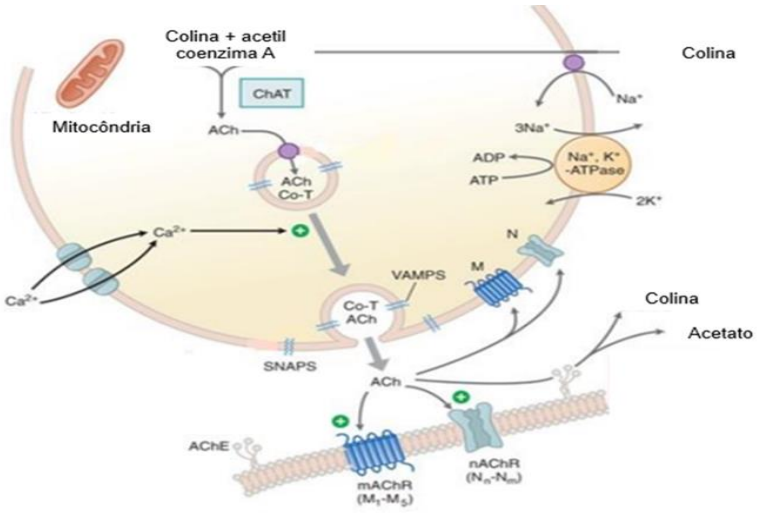

Figura 2. Representação da uma sinapse da via colinérgica com síntese, armazenamento, transporte e degradação da acetilcolina (ACh). Siglas: Acetilcolina (ACh); AChE (Acetilcolinesterase); Colina acetiltranferase (ChAT); Adenosina difosfato (ADP); Adenosina trifosfato (ATP); Proteína de membrana associadas à vesículas (VAMP5); Proteínas sinaptossômicas (SNAPS); Receptores nicotínicos (nAChR); Receptores muscarínicos (mAChR); Transportador vesicular de acetilcolina (ACh co-T). Adaptado de BRUNTON et al., (2011).

Sabe-se que o sistema colinérgico está relacionado com os processos de memória. Além disso, o papel da ACh nos processos de memória e aprendizado está sendo esclarecido. Sugere-se que a mesma tem um papel importante no hipocampo, onde colabora com a aquisição e a recuperação das informações. Nesse modelo, chamado de hipótese colinérgica, a alta concentração de ACh facilitaria a aquisição de novas memórias, enquanto sua

baixa concentração seria responsável pela recuperação de memórias anteriores (FERREIRA-VIEIRA et al., 2016). Essa hipótese parece ser confirmada, pois Klinkenberg e Blokland (2010) demonstraram que na doença de DA ocorre a degeneração dos neurônios colinérgicos do núcleo basalis de Meinert, causando uma diminuição significativa da liberação de ACh na amígdala, hipocampo e neocórtex, e uma consequente diminuição da modulação dos receptores sob as funções neuronais nessas estruturas. Desta forma, o sistema colinérgico tem importante papel nos processos de formação da memória e há evidências de que o aprendizado e a memória podem ser modificados por fármacos que afetam a função colinérgica central (DEIANA et al., 2011).

3.4. Fármacos que afetam a função colinérgica central

3.4.1. Escopolamina

Na antiguidade já se sabia que a ingestão de extratos de algumas plantas como *Atropa belladona L*., *Datura stramponium L*. e *Hyosyamus niger L.* poderia influenciar o estado mental dos indivíduos. Mais tarde, descobriu-se que essas plantas eram ricas em alcalóides tropânicos que eram responsáveis por tais efeitos, dentre eles, a escopolamina (ESC) de estrutura química $C_{17}H_{21}NO_4$ (Figura 3) (BALMUS e CIOBICA, 2017).

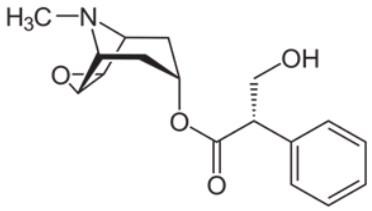

Figura 3. Estrutura da ESC.

A ESC é um fármaco antagonista colinérgico não seletivo dos receptores muscarínicos, definida como uma substância anticolinérgica (MATTSON, 2004). Sendo assim, o bloqueio de receptores colinérgicos muscarínicos interfere no armazenamento de novas informações, consequentemente afeta a memória, sendo que o mecanismo envolvido neste processo se pronuncia pela supressão da atividade neural excitatória. Desta forma, este fármaco atua impedindo a passagem de determinados impulsos

nervosos no SNC pela redução dos efeitos da ACh ao se ligar a receptores muscarínicos, sem gerar a despolarização (Figura 4) (ZHANG et al., 2017). Cabe salientar que a ESC quando administrada em altas doses, também pode antagonizar receptores nicotínicos (Figura 4) (ZHANG et al., 2017).

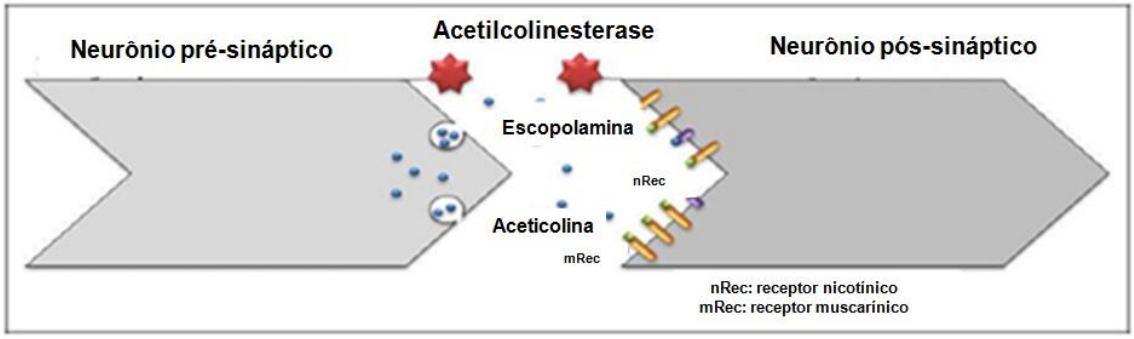

Figura 4. Ação Molecular da ESC na fenda sináptica. Adaptado de BALMUS e CIOBICA (2017).

Atualmente a ESC é utilizada no tratamento de disfunções do SNC, como náusea, doença de Parkinson e dependência por opióides, podendo apresentar-se nas formas farmacêuticas como comprimido, solução injetável, adesivos transdérmicos, entre outros. Como a ESC penetra facilmente a barreira sangue- cérebro e apresenta uma ação prejudicial em relação à cognição, formação e armazenamento da memória em roedores, ela vem sendo amplamente utilizada na área da neuropsicofarmacologia (BALMUS e CIOBICA, 2017). Desde 1974, a ESC é utilizada como um modelo animal de indução de amnésia, déficits cognitivos e perda de memória, simulando um modelo de demência a até mesmo mimetizando a DA (DEIANA et al., 2011).

A ESC é amplamente utilizada em modelos de estudo de memória em que se objetiva a procura de compostos que tenham a capacidade de reverter os danos causados pela hipofunção colinérgica provocada pela sua administração, uma vez que os efeitos causados podem ser revertidos por determinadas drogas (MORE et al., 2016). Além disso, cabe salientar que muitos estudos avaliaram parâmetros comportamentais relacionados ao aprendizado e à memória em roedores, como esquiva inibitória, labirinto em T, labirinto em Y, entre outros, e todos demonstraram uma redução no desempenho cognitivo nos animais induzidos por ESC (SOUZA et al., 2013; MORE et al., 2016; ZHANG et al., 2017).

Desta forma, cabe salientar que os antagonistas muscarínicos como a ESC produzem déficits na memória de curto prazo (BALMUS e CIOBICA, 2017), sendo que ela também pode vir a ser usada para causar comprometimento nas diferentes fases da memória (aquisição, consolidação e recuperação) e na avaliação da memória de longo prazo (SOUZA et. al., 2013). Além disso, a ESC prejudica tarefas relacionadas a procedimentos com intervalos de retenção, o que sugere que esse fármaco acomete a memória operacional espacial (KLINKERBERG e BLOKLAND, 2010; MORE et al., 2016).

Tem sido demonstrado que a administração de ESC a partir da dose de 0,03 mg/kg provoca prejuízo na memória de reconhecimento de objetos, e dependendo dos parâmetros utilizados, o seu efeito é dose-dependente (JONES e HIGGINS, 1995). Além disso, a ESC pode ser usada como um modelo em testes que permitem avaliar a memória declarativa do tipo episódica (OLIVEIRA, 2017). Desta forma, o modelo da ESC permite avaliar as propriedades farmacológicas de novos compostos, principalmente aqueles que possam vir a ser promissores para o tratamento de demência relacionada com a perda de memória.

3.4.2. Inibidores da AChE

Alguns fármacos que afetam a função colinérgica central são os inibidores da AChE. Atualmente os inibidores da AChE, ou também chamados anticolinesterásicos são utilizados na terapêutica, principalmente para o tratamento da demência na DA. Os efeitos farmacológicos dos inibidores da AChE, caracterizam-se pelo impedimento da hidrólise da ACh pela AChE, por inibição da enzima, que é realizada na presença destes fármacos que interagem com a enzima, bloqueando-a, permitindo assim a manutenção do gradiente de concentração do neurotransmissor durante os processos de condução de sinal para outros neurônios (transmissão sináptica) (TAYLOR, 2006).

Dentre estes fármacos anticolinesterásicos podem ser citados galantamina, donepezila (DON), tacrina e rivastigmina (Figura 5). Estes quatro inibidores da AChE são aprovados pela agência *Food and Drug Administration*

(FDA) para o tratamento da DA, e agem alterando o tônus colinérgico central, aumentando a possibilidade da ACh de estimular os receptores nicotínicos e muscarínicos cerebrais (PORCEL e MONTALBAN, 2006).

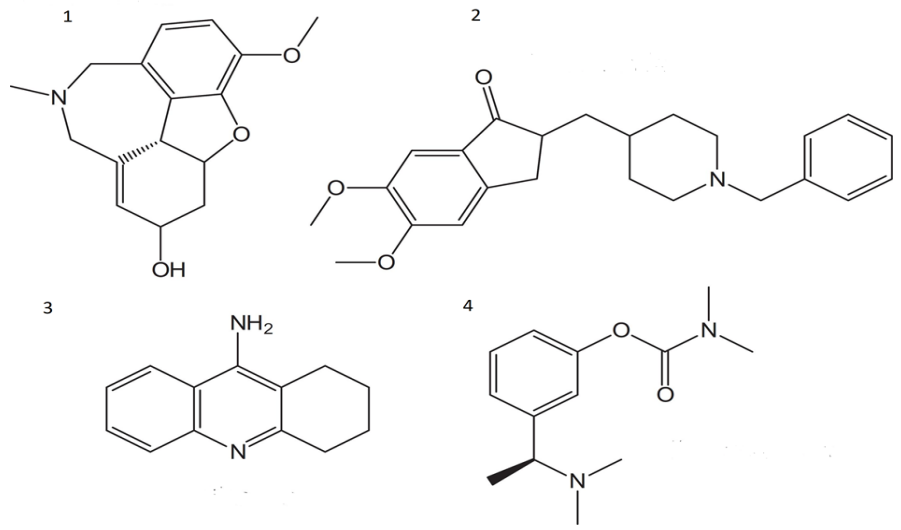

Figura 5. Estruturas dos principais inibidores da AChE aprovados para o tratamento da DA. 1) galantamina; 2) donepezila; 3) tacrina; 4) rivastigmina.

Os anticolinesterásicos podem ser classificados de acordo com a possibilidade de reversão de sua inibição e também pela duração de sua ação sobre a AChE (BENTLEY et al., 2011). A galantamina e a DON são inibidores reversíveis da AChE, apresentando duração intermediária e longa, respectivamente. A rivastigmina tem ação de duração intermediária e é lentamente reversível (BENTLEY et al., 2011). A tacrina foi o primeiro inibidor reversível da AChE a ser utilizado no tratamento de doenças neurodegenerativas (LEVIN 2000).

O uso de anticolinesterásicos é ainda a forma mais eficiente de controle da evolução das doenças neurodegenerativas. Infelizmente, a utilização destes é limitada, pois apresentam muitas vezes, baixa biodisponibilidade e diversos efeitos adversos, como a hepatotoxicidade e distúrbios gastrintestinais, além de apresentarem apenas um alívio sintomático para os prejuízos causados na demência (PORCEL e MONTALBAN, 2006). Desta forma, faz-se necessário a busca por novos compostos principalmente aqueles que possam vir a ser promissores para o tratamento de demência relacionada com a perda de memória.

3.5. Epilepsia

A epilepsia é um distúrbio neurológico crônico com quadro clínico caracterizado por crises epilépticas recorrentes, na ausência de doenças tóxico- metabólicas ou febris e que podem ocorrer em qualquer faixa etária (ILAE, 1989; FALKO- WALTER et al., 2018; WHO 2019). As crises epilépticas são resultantes de uma atividade neuronal excessiva, devido a descargas elétricas anormais e recorrentes, associadas a uma via complexa de neurotransmissores, envolvendo os sistemas glutamatérgico, colinérgico e GABAérgico (ROBEL et al., 2015). O termo convulsão pode ser definido como uma ocorrência transitória e isolada de sinais e/ou sintomas devido à atividade hipersincrônica e repetitiva de um grupamento neuronal do córtex cerebral, cuja distribuição anatômica e duração da sua atividade determinam a natureza da crise (ILAE, 1989). A Organização Mundial da Saúde descreve que em torno de 10% da população mundial apresenta alguma crise convulsiva durante a vida (OMS, 2019).

A epilepsia é considerada uma doença do cérebro, sendo uma das enfermidades neurológicas mais prevalentes, afetendo cerca de 70 milhões de pessoas em todo o mundo (LÖSCHER, 2017). Sua incidência tem uma distribuição bimodal com maior risco em crianças e grupos de idade mais avançada. Além disso, alguns pacientes com epilepsia apresentam crises refratárias ao tratamento medicamentoso disponível no mercado (LÖSCHER, 2017).

A epilepsia é uma doença que apresenta diversos sintomas, com múltiplos fatores de risco e uma forte predisposição genética, em vez de uma condição com uma única expressão e causa. No entanto, a causa da epilepsia em muitos pacientes é desconhecida, as convulsões podem ser o resultado de quase qualquer insulto que perturba a função cerebral. Esses insultos incluem causas adquiridas, como após acidente vascular cerebral ou lesão cerebral traumática, doenças autoimunes e infecciosas (DEVINSKY et al., 2018).

Devido a fatores como alta prevalência, gravidade, morbidade e impacto socioeconômico, as pesquisas científicas no campo da epileptologia têm adquirido caráter prioritário nas políticas em saúde pública. Diversos modelos experimentais usando roedores estão sendo utilizados, tendo em vista

que reproduzem as alterações comportamentais e eletroencefalográficas que são semelhantes às que ocorrem nas crises epilépticas em humanos. Esses modelos são utilizados com a finalidade de estudar o envolvimento dos sistemas de neurotransmissores com os moduladores da epileptogênese (LÖSCHER, 2017).

3.5.1. Comorbidades cognitivas na epilepsia

As comorbidades são cada vez mais reconhecidas como importantes marcadores etiológicos e de prognósticos da epilepsia (FISHER et al., 2017). Várias doenças, incluindo depressão, ansiedade, demência, enxaqueca, doenças cardíacas, úlceras pépticas e artrite são até oito vezes mais comuns em pessoas com epilepsia do que na população em geral. Vários mecanismos explicam como a epilepsia e as comorbidades estão associadas, incluindo fatores de risco compartilhados e relações bidirecionais (KEEZER et al., 2016).

A associação mais significativa é representada por uma relação causal. Assim, pessoas com DA e/ou ou outras demências que afetam a memória tendem a ter convulsões e epilepsia durante o curso da doença a uma taxa de 1,3-6,1% (BEGHI et al., 2020). Sendo assim, quando comparado com a população em geral, pacientes com DA apresentam maior risco de desenvolverem convulsões e epilepsia (BEGHI et al., 2020).

Ainda o comprometimento cognitivo é característico em pacientes epilépticos de várias idades (HALÁSZ et al., 2019). Fatores relacionados à convulsão epiléptica, incluindo idade precoce de início, maior duração da história epiléptica e maior frequência nas crises convulsivas foram significativamente associados com o déficit de memória (WANG et al., 2018). Sendo que os pacientes com algum tipo de epilepsia classificam os problemas de memória como um de suas três principais preocupações, e os problemas de memória são uma preocupação para 42% dos pacientes (WANG et al., 2018).

Ainda não é totalmente compreendido como a epilepsia causa déficit de memória, embora existam muitos fatores contribuintes conhecidos. Notavelmente, quando ocorrem as crises convulsivas na epilepsia, uma variedade de mudanças celulares podem ocorrer no hipocampo, dentre estas, a perda neuronal, gliose reativa, inflamação e neurogênese alterada

(PITKANEN e SUTUL, 2002). Desta forma, déficits cognitivos frequentemente se manifestam em pacientes que apresentem crises convulsivas, uma vez que a neurogênese hipocampal é afetada pela mesma (MING e SONG, 2005).

Estudos demonstram que a consolidação da memória pode ser prejudicada por epilepsias do lobo temporal ou por epilepsias idiopáticas generalizadas que envolvem diretamente estruturas anatômicas temporais mesiais importantes para o processamento da memória (REALMUTO et al., 2015; YANG et al., 2016). Da mesma forma, Wang et al. (2018) demonstraram que pacientes com epilepsia do lobo temporal exibiam baixo desempenho no processamento da memória, sugerindo que o lobo temporal pode desempenhar um papel mais dominante na função cognitiva.

Foi descrito que além de dificuldades com trabalho e memória imediata, os pacientes com epilepsia muitas vezes sofrem de esquecimento acelerado de longo prazo, no qual algum defeito do processo de consolidação lento causa degradação anormalmente rápida das memórias ao longo do tempo (BLAKE et al., 2000). Além disso, a atividade epileptiforme subclínica foi detectada em 42,4% dos pacientes com DA, sendo que a atividade epileptiforme subclínica também foi observada em pacientes com amnesia, comprometimento cognitivo leve ou demência precoce (KANNER et al., 2016).

Ainda o próprio uso de medicamentos anticonvulsivantes pode contribuir para o comprometimento da memória (HORAK et al., 2017). No entanto, ainda faltam intervenções terapêuticas para tratar déficits cognitivos relacionados à epilepsia (HORAK et al., 2017). Além disso, a expectativa de vida está aumentando progressivamente, sendo assim a epilepsia, demência e, consequentemente, comorbidade epilepsia-demência também deverá aumentar. Desta forma, na ausência de tratamentos eficazes para a DA e outros tipos de demência relacionados ao déficit de memória, ainda são necessários maiores avanços na investigação das comorbidades da epilepsia e a busca de novos tratamentos para estas comorbidades.

3.5.2. *Kindling* (abrasamento) induzido por Pentilenotetrazol

Os modelos animais para epilepsia e convulsão são de grande importância para aumentar a nossa compreensão dos mecanismos básicos subjacentes à epileptogênese e para descoberta de novos fármacos

anticonvulsivantes (LÖSCHER, 2017). *Kindling* elétricos ou químicos são modelos epileptogênicos usados para a compreensão do processo epileptogênico e para o estudo de novas moléculas para o tratamento da epilepsia (PAVLOVA et al., 2004).

O *kindling* é um fenômeno que resulta em uma atividade convulsiva de intensidade progressiva devido à administração repetitiva de estimuladores sub-convulsivos elétricos ou químicos (PAVLOVA et al., 2004). Os *kindlings* químicos induzidos por PTZ reproduzem diversos tipos de crises convulsivas que ocorrem na epilepsia humana, como crises de ausência, crises mioclônicas, tônico-clônicas generalizadas, dentre outros modelos de convulsão (WU et al., 2006).

A administração de PTZ é uma abordagem comumente utilizada para estudar a excitabilidade cerebral (KLIOUEVA et al., 2001) e para desenvolver novos anticonvulsivantes (LÖSCHER et al., 2002). O modelo de *kindling* por PTZ, induz a alterações cerebrais, emocionais (PAVLOVA et al., 2006) e comportamentais em roedores, como comprometimento da memória, revelando que este modelo animal também imita comorbidades de epilepsia (KAUR et al., 2016; ABDEL-ZAHER et al., 2017).

O PTZ é caracterizado por ser um agente antagonista do receptor $GABA_A$, que ligando-se aos seus sítios de reconhecimento, inibe as correntes de íons cloreto associadas a esse canal. Quando isso ocorre, há uma redução dos efeitos endógenos de GABA, o que resulta em um estado de hiperexcitabilidade do SNC (KANDRATAVICIUS, 2014). Desta forma, o PTZ suprime a função das sinapses inibitórias, levando ao aumento da atividade neuronal, sendo que essa regulamentação causa convulsões generalizadas em animais (SHIMADA e YAMAGATA, 2018). Além disso, o PTZ também está relacionado com canais iônicos de Na^+ e Ca^+, aumentando as correntes de membrana e consequentemente a excitabilidade neuronal, segundo dados eletroencefalográficos (LOSCHER, 1998).

Ainda, o PTZ também causa uma alteração na densidade e sensibilidade dos diferentes subtipos de receptores de glutamato (SCHROEDER et al., 1998) em muitas partes do cérebro e um aumento na densidade do neurotransmissor glutamato na região do hipocampo. É relatado que mudanças na expressão molecular nos transportadores de glutamato

durante o processo de *kindling* podem desencadear o desenvolvimento de epileptogênese (LI et al., 2004). É sugerido que o N-metil-D-aspartato (NMDA) desempenha um papel na epileptogênese do *kindling*, sendo que alterações relacionadas à subunidade e região de receptores NMDA durante a síntese no desenvolvimento de convulsão induzida por PTZ em ratos sugere que essas alterações podem ser responsáveis pela disseminação da hiperatividade neuronal induzida por PTZ. Desta forma, a plasticidade neocortical de longo prazo despertada por *kindling* pode ser gerada por uma alteração no delicado equilíbrio entre inibição e excitação neuronal (uma diminuição relativa na inibição e um aumento relativo na excitação ou uma combinação de ambos) (GETOVA et al., 1998).

Desta forma, o modelo de *kindling* induzido por PTZ pode ser apresentado como um método mais preferível para o tempo, esforço e aspectos éticos, na busca de novas moléculas, pois gera crises convulsivas e alterações neurocognitivas que ocorrem na epilepsia com menor dose e número de induções de PTZ em comparação com outros modelos (ÖZLEM et al., 2015).

3.6. Fármacos anticonvulsivantes

Os fármacos anticonvulsivantes atuam em diversos alvos moleculares para modificar seletivamente a excitabilidade dos neurônios, de modo que o disparo relacionado com as crises convulsivas possa ser bloqueado, sem alterar a atividade dos neurônios não epilépticos que atendem aos sinais normais no cérebro (STEFAN e FEUERSTEIN, 2007). Estes fármacos, atingem um ou mais alvos no cérebro, como, enzimas metabólicas, canais iônicos, e transportadores de neurotransmissores. Desta forma, quando estes alvos são ativados vão promover o sincronismo e a diminuição da excitabilidade dos neurônios, inibindo assim o comportamento convulsivo (ROGAWSKI e LOSCHER, 2004).

Os primeiros fármacos anticonvulsivantes foram detectados no século XX, sendo desenvolvidos e introduzidos entre 1910 e 1970, como por exemplo, fenobarbital, primidona, benzodiazepínicos, etossuximida, e valproato de sódio, sendo posteriormente chamados de fármacos anticonvulsivantes de primeira

geração. Após um hiato de mais de 20 anos, vários fármacos anticonvulsivantes foram introduzidos na prática clínica, como a vigabatrina, gabapentina, felbamato, lamotrigina, oxcarbazepina, tiagabina e topiramato, referidos como “novos medicamentos” ou fármacos anticonvulsivantes de segunda geração. Além disso, nas últimas décadas mais de 15 fármacos anticonvulsivantes de terceira geração foram introduzidos, proporcionando aos médicos e pacientes mais opções para o tratamento de muitos tipos de epilepsias (STEFAN e FEUERSTEIN, 2007).

Desta forma, como pode-se observar na figura 6 os fármacos anticonvulsivantes disponíveis atualmente no mercado agem somente suprimindo as crises epilépticas. Os principais mecanismos de ação desses FA são: (1) modulação de canais de íons dependentes de voltagem, (2) aumento da inibição sináptica mediada por GABA, (3) inibição da excitação sináptica mediada pelo glutamato. (LÖSCHER et al., 2013).

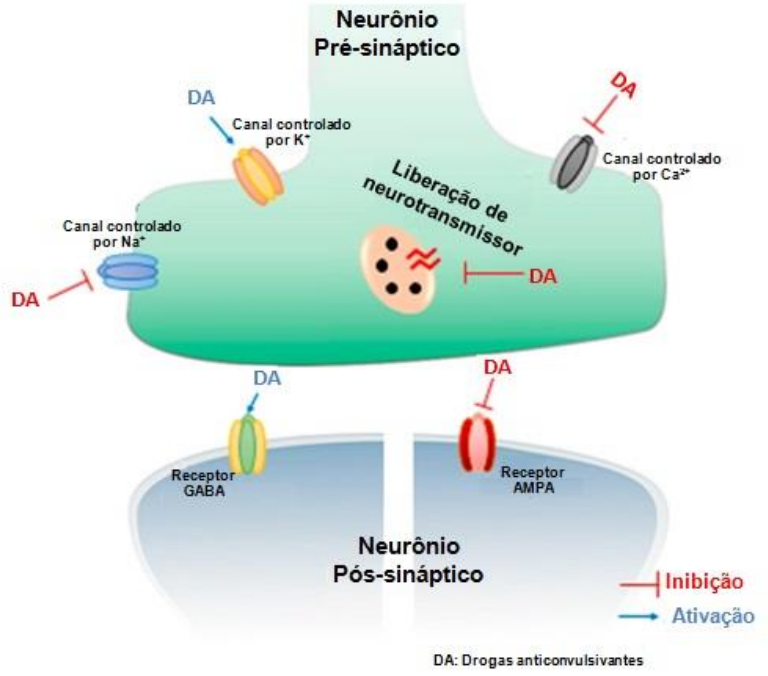

Figura 6. Principais mecanismos de ação dos fármacos anticonvulsivantes. Adaptado de da Fonsêca et al., (2019).

O tratamento com fármacos anticonvulsivantes apresenta um número considerável de reações adversas, sendo que cerca de 30% dos pacientes com epilepsia não têm tratamento adequado e 40% apresentam farmacorresistência aos medicamentos atualmente disponíveis (LÖSCHER e

SCHMIDT, 2006). Além disso, estudos têm demonstrado que o uso de fármacos anticonvulsivantes não trata as comorbidades relacionadas a epilepsia, estando associados ao aparecimento de vários efeitos adversos relacionados a cognição, ou seja, acentuando o déficit cognitivo (EDDY et al., 2011). Os fármacos anticonvulsivantes podem afetar adversamente as funções cognitivas através da excitabilidade neuronal ou aumentando a neurotransmissão inibitória, sendo que os principais efeitos cognitivos destes fármacos são o comprometimento da memória, atenção, vigilância e velocidade psicomotora (EDDY et al., 2011).

Nesse contexto, os fármacos anticonvulsivantes presentes na clínica atualmente não apresentam o efeito esperado, que seria capaz de neutralizar os efeitos da epileptogênese, incluindo a prevenção, a modificação da doença e a remissão total das crises convulsivas e suas comorbidades associadas. Desta forma, a busca por novos fármacos, está em andamento para melhor atender as necessidades dos pacientes com epilepsia e suas comorbidades (LÖSCHER et al., 2013).

3.7. Estresse oxidativo, memória e epilepsia

As espécies reativas (ERs) são moléculas que englobam uma série de espécies radicalares ou não radicalares, podendo ser derivadas tanto do oxigênio (EROs), como do nitrogênio (ERNs). As ERs radicalares incluem moléculas com um elétron desemparelhado em sua órbita externa denominadas radicais livres (exemplos: superóxido; óxido nítrico; peroxinitrito), enquanto as ER não radicalares incluem moléculas sem elétrons desemparelhados (exemplos: ozônio, ácido hipocloroso, peróxido de hidrogênio), mas que também possuem grande reatividade com moléculas orgânicas (HALLIWEL e GUTTERIDGE, 2015). As ERs são produzidas de forma endógena ou exógena, podendo ser originadas no curso de diversas reações fisiológicas, tais como na respiração celular, nos macrófagos durante a fagocitose, entre outras. No entanto, um desequilíbrio entre a geração de ERs e a atuação dos sistemas de defesa antioxidante, podem causar vários danos a todas as estruturas celulares, incluindo o DNA, os lipídios e as proteínas, levando ao aparecimento de diversos distúrbios, tais como os neurológicos (LI

et al., 2013; HALLIWELL, GUTTERIDGE, 2015). Essa situação é denominada estresse oxidativo e pode prejudicar o funcionamento de organelas, tais como a mitocôndria, e danificar a membrana das células, levando à morte celular (HALLIWEL e GUTTERIDGE, 2015).

Para minimizar o processo de estresse oxidativo o organismo possui um sistema de defesa antioxidante que tem a função de inibir e/ou reduzir os danos causados pela ação deletéria das ERs (HALLIWEL e GUTTERIDGE, 2015). O sistema de defesa antioxidante pode ter diferentes origens: endógena ou exógena.Os antioxidantes de origem endógena são produzidos pelo organismo, e dentre eles pode-se destacar as defesas antioxidantes enzimáticas, tais como a glutationa peroxidase (GPx), a superóxido dismutase (SOD), a catalase (CAT), a glutationa-S-transferase (GST), entre outras; e as defesas antioxidantes não-enzimáticas, tais como a glutationa reduzida (GSH), os peptídeos de histidina, a ubiquinona, a bilirrubina, entre outros (HALLIWEL e GUTTERIDGE, 2015). Além disso, os antioxidantes podem ser de origem exógena tais como as vitaminas C e E, os β-carotenos, os polifenóis, os taninos, o selênio, entre outros (HALLIWEL e GUTTERIDGE, 2015).

O cérebro é particularmente suscetível ao estresse oxidativo, uma vez que usa maiores quantidades de oxigênio do que outros órgãos. O cérebro também contém altas concentrações de ácidos graxos poli-insaturados que são propensos a peroxidação lipídica, é rico em ferro, que pode catalisar a formação do radical hidroxila e tem baixa atividade das enzimas antioxidantes (DRINGEN et al., 2005). Nesse sentido, o estresse oxidativo está envolvido na patogênese de algumas condições neurológicas e distúrbios neurodegenerativos, como a DA, doença de Parkinson e esclerose lateral amiotrófica, epilepsia, dentre outras (MIGLIORE et al., 2005).

Assim, o estresse oxidativo esta diretamente envolvido com o déficit de memória e com a epilepsia (SULTANA et al., 2006, ARMENTA et al., 2014), sendo que os aumentos prolongados de ERs induzem um risco inerente de aumento no déficit de memória (SULTANA et al., 2006) e neurodegeneração observada na epilepsia (ARMENTA et al., 2014; SHIN et al., 2011). Apesar de ainda não ser bem conhecido se o estresse oxidativo é uma causa ou consequência dessas patologias, tem sido amplamente descrito que o aumento da geração de ERs pode levar a convulsões prolongadas podendo resultar em

disfunção mitocondrial no hipocampo precedendo morte de células neuronal e causando subsequente epileptogênese e déficit de memória (JODKO e LITWINIENKO, 2010; ARMENTA et al., 2014).

Desta forma, muitas vezes a atividade das defesas antioxidantes enzimáticas e os níveis de peroxidação lipídica decorrentes da ação das ERs tem sido utilizadas como um meio de avaliação do papel do estresse oxidativo na atividade de novas moléculas que atenuem o déficit de memória e a epileptogênese (EL-SHERBINY et al., 2003, WALDBAUM e PATEL, 2010, HUANG et al., 2012).

3.8. Na^+/K^+-ATPase, memória e epilepsia

A Na^+/K^+-ATPase é uma enzima ligada à membrana conhecida por desempenhar um papel fundamental na manutenção do gradiente iônico celular. Esta enzima é abundante no cérebro e desempenha um papel importante na atividade neuronal, modulando também indiretamente a concentração intracelular de outros íons como Ca^{2+}, Cl e H^+, bem como o movimento transmembrana de água, glicose e diversos mediadores químicos (GULLEDGE et al., 2013).

No cérebro, a enzima Na^+/K^+-ATPase é um importante regulador da excitabilidade dos neurônios, sendo um importante contribuidor para manutenção do gradiente eletroquímico subjacente pós-hiperpolarização neuronal e potencial de repouso (APERIA, 2012). Além disso, muitos distúrbios neurológicos têm sido associados com mudanças na atividade das Na^+/K^+-ATPase (HOLM et al., 2016).

Nesse sentido como esta enzima é responsável por manter o gradiente eletroquímico celular e é crucial no ciclo celular normal e na diferenciação do sistema nervoso, mudanças nesta atividade podem ter consequências extensas na função neuronal (HOLM et al., 2016). Estudos recentes relacionaram que a inibição da atividade da enzima Na^+/K^+-ATPase apresenta consequências deletérias na memória. Na verdade, foi demonstrado que a inibição da atividade da enzima Na^+/K^+-ATPase pode levar a déficits de aprendizagem e memória e também podem induzir apoptose (APERIA et al 2007).

Além disso, a atividade reduzida da enzima Na^+/K^+-ATPase contribui também para o início e/ ou disseminação de convulsões em pacientes com epilepsia (HOLM et al., 2016). Além disso, diminuição da atividade da Na^+/K^+-ATPase foi encontrada em o córtex cerebral humano epiléptico e no hipocampo de camundongos (FUNCK et al., 2014).

3.9. Quinolinas e Compostos Quinolínicos

As quinolinas são compostos heterocíclicos que estão presentes na natureza e em uma variedade de produtos naturais, sendo que os mesmos apresentam farmacóforos úteis que desempenham um papel fundamental no desenvolvimento de novos medicamentos (CHU et al., 2019). Como pode ser observado na figura 7, os compostos quinolínicos apresentam um anel benzênico fundido na posição 2 e 3 de um anel piridínico, e um átomo de nitrogênio, com fórmula molecular C_9H_7N (ROTH et al., 2000).

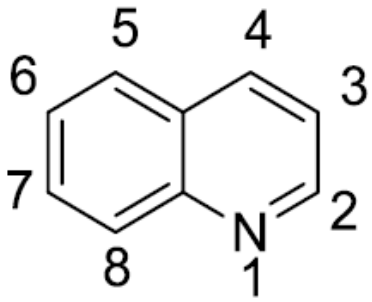

Figura 7. Estrutura química da quinolina

A quinolina na sua forma impura foi obtida pela primeira vez a partir do alcatrão de carvão por Runge no ano de 1834, e em 1842 Gerhardt obteve a mesma como um produto de degradação da quinina e cinchonina, sendo mais tarde chamada de quinolina. O alcatrão de carvão também contém isoquinolina, bem como várias alquil quinolinas e alquil isoquinolinas, que são difíceis de separar, sendo assim esse processo aconteceu durante alguns anos antes que a quinolina estivesse disponível na sua forma mais pura para investigação de suas propriedades (MATADA et al., 2021).

As quinolinas e os compostos derivados das mesmas apresentam diversas propriedades farmacológicas como antibacteriana (ZHANG et al.,

2018), anti-inflamatória (UPADHYAY et al., 2018), anticancerígena (UPADHYAY et al., 2018), anti-tubercular (FAN et al., 2018), atividades anti-malária (HU et al., 2017) e anti-Alzheimer (WANG, Z. R. et al., 2018). Os compostos quinolínicos representam uma classe importante de compostos heterocíclicos bioativos no campo dos produtos farmacêuticos (CHU et al., 2019).

Muitos dos derivados quinolínicos são usados hoje em dia como produtos agrícolas e medicamentos. Os medicamentos mais usados dessa classe são: antimaláricos (cloroquina, quinino, quinidina), anti-helmíntico (Oxamniquina), antiviral (Saquinavir), antibacteriano (fluoroquinolonas, como ciprofloxacina, esparfloxacina, etc.), anestésico local (Dibucaína), antiasmático (montelucaste), anticâncer (camptotecina, irinotecano, topotecano, gefitinibazol), antifúngico-antiprozoal (clioquinol), antipsicótico (Brexpiprazol, etc.) (TIWARY et al., 2015).

3.9.1. Compostos quinolínicos x efeito neuroprotetor

Tendo em vista a importância e as atividades biológicas significativas dos compostos derivados de quinolina, além da grande prevalência de doenças neurológicas atualmente, diversos estudos tem sido realizados com o objetivo de investigar o efeito neuroprotetor das quinolinas (PINZ et al., 2018; de FREITAS COUTO et al., 2019; MAHDAVI et al., 2019).

Em um estudo realizado Grychowska e colaboradores (2019), foi demonstrado que o composto (S) -1 -[(3-clorofenil) sulfonil] -4- (pirrolidina-3-il-amino)-1H-pirrolo[3,2-c]quinolina (CPPQ) (Figura 8A), bloqueou os receptores de serotonina 5-HT6 e dopamina D3, melhorando o declínio cognitivo induzido por fenciclidina. Neste estudo também foi desmonstrado que este composto apresentou propriedades neuroprotetoras contra o dano astrocitário induzido pela doxorrubicina, avaliando a atividade metabólica celular e liberação de lactato desidrogenase (LDH) como um índice de ruptura da membrana celular.

Em estudo realizado por Mahdavi e colaboradores (2019) várias benzocromenoquinolinas foram sintetizadas e avaliadas quanto às suas atividades inibidoras de colinesterase, bem como propriedades neuroprotetoras. Entre os compostos sintetizados, o 14-amino-13- (3-nitrofenil) -2,3,4,13- tetra-hidro-1H-benzo [6,7]cromeno[2,3-b]quinolina-7,12-diona (Figura 8B) representou a melhor atividade inibitória para AChE e BUChE com IC50 de 0,86 e 6,03 μm, respectivamente. Além disso, o composto poderia inibir a β-secretase 1 com IC50 = 19,60 μm. Além disso, o estudo de docking demonstrou interações desejáveis do composto 6m com resíduos de aminoácidos caracterizando AChE, BChE e a β-secretase 1.

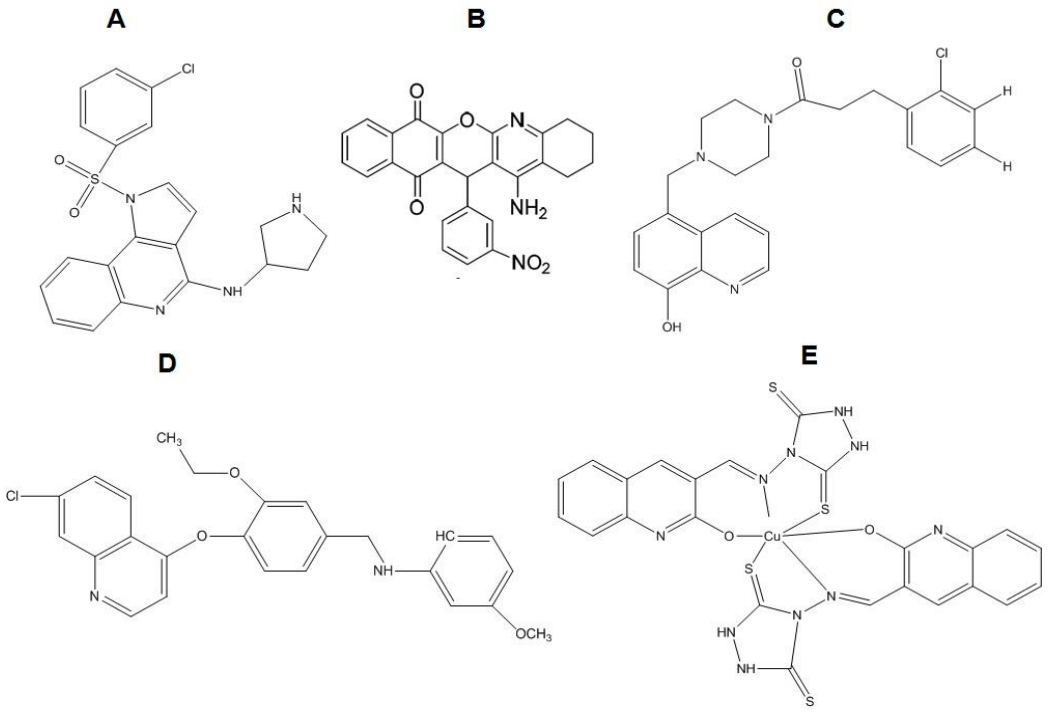

Figura 8. Estruturas química de compostos quinolínicos com efeito neuroprotetor.

Yang e colaboradores (2018) demonstraram que uma série de derivados de 8-hidroxiquinolina sintetizados para o tratamento da DA apresentaram efeitos inibitórios significativos *in vitro* contra a agregação do peptídeos beta-amiloide (Aβ) especialmente o composto 5b (denominado no estudo) (Figura 8C) (IC50 = 5,64 μM para agregação Aβ auto-induzida). Além disso, foi demonstrado que o composto 5b pode penetrar a barreira hematoencefálica (BHE) *in vitro*, sendo um composto promissor na terapia com DA.

Em estudo realizado por Umar e colaboradores (2018), uma série de derivados quinolínicos, inibidores de agregação Aβ foram sintetizados (Figura 8D). Os compostos 5g e 5a (denominados no estudo) apresentaram o maior potencial inibitório, 53,73% e 53,63% a 50 μM, respectivamente; esse potencial foi maior que o agente desagregante padrão de Aβ, a curcumina. A molécula 5g desagregou a agregação de Aβ induzida por AChE (58,26 %), duas vezes mais do que o medicamento padrão DON (23,66%) e inibiu a agregação de Aβ induzida por Cu^{2+}, demonstrando que os mesmos podem vir a ser promissores para o tratamento da perda de memória relacionado com a DA.

Em um estudo realizado por Kulkarni e colaboradores (2010) um novo complexo de Cu^{2+} e Zn^{2+} de derivados de triazol-quinolina foram sintetizados e suas propriedades farmacológicas foram avaliadas. O composto C3 (denominado no estudo) (Figura 8E), um derivado de quinolina, apresentou atividade anticonvulsivante promissora frente às convulsões induzidas por eletrochoque em ratos *Wistar*, demonstrando também baixa toxicidade e alto perfil de segurança.

Desta forma, pode-se afirmar que os compostos derivados de quinolinas apresentam diversas propriedades biológicas interessantes, principalmente a neuroprotetoras, como pode ser observado nos trabalhos citados anteriormente (GRYCHOWSKA et al., 2019; MAHDAVI et al., 2019; UMAR et al., 2018; YANG et al., 2018; KULKARNI et al., 2010). Sendo assim, os mesmos podem vir a ser uma alternativa terapêutica para o tratamento de doenças neurológicas, relacionadas ao déficit de memória e também a epilepsia. Nesse sentido, vários estudos estão sendo realizados com o intuito de esclarecer as ações farmacológicas destes compostos.

3.9.2. 1-(7-cloroquinolin-4-il)-5-metil-N-fenil-1H-1,2,3-triazol-4 carboxamida (QTCA-1)

Os compostos quinolínicos com nitrogênio, 1,2,3-triazóis são uma classe interessante de heterociclos baseados em nitrogênio amplamente usados na descoberta e modulação de candidatos a novos fármacos. Desta forma, a síntese de heterociclos complexos, tais como quinolinas e derivados de 1,2,3-triazol, tem se mostrado muito promissora (SARAIVA et al., 2016). Além disso,

os derivados de quinolina são entidades biologicamente ativas e apresentam uma ampla gama de atividades farmacológicas (PINZ et al., 2018; VOGT et al., 2018, BARTH et al., 2019). Sendo assim, devido à sua importância como sub-estrutura em uma ampla variedade de produtos sintéticos e naturais, esforços consideráveis têm sido direcionados a descoberta de novas moléculas baseadas em derivados quinolínicos.

Desta forma, tendo em vista as diversas propriedades farmacológicas dos derivados quinolínicos, o nosso grupo de pesquisa tem buscado elucidar as propriedades farmacológicas do QTCA-1 (Figura 18). Em um estudo realizado por Wilhelm e colaboradores (2014) avaliou o potencial antinociceptivo, anti-inflamatório em camundongos, além do efeito anticonvulsivante em ratos jovens do QTCA-1. Foi observado o efeito deste composto no teste de formalina, que é um modelo experimental clássico para avaliar a atividade antinociceptiva, demonstrando que o tratamento com o QTCA-1 (doses 25, 50 e 100 mg/kg) causou uma inibição significativa no tempo de lambida da pata dos camundongos induzidos por formalina, tanto na fase inicial (0-5 min) quanto na fase tardia (15 a 30 min). Também foi demonstrado neste estudo que o tratamento com QTCA- 1, nas doses de 50 e 100 mg/kg, inibiu as constrições abdominais induzidas pelo ácido acético.

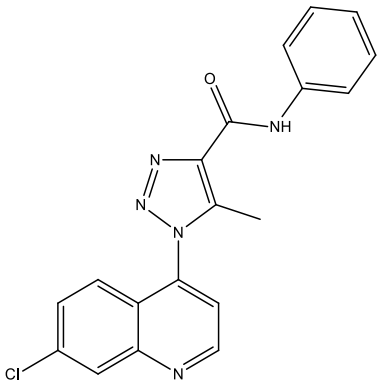

Figura 9. Estrutura química do 1-(7-cloroquinolin-4-il)-5-metil-N-fenil-1H-1,2,3-triazol-4 carboxamida (QTCA-1).

Além disso, o pré-tratamento de ratos jovens com uma dose de 100 mg/kg do composto QTCA-1 foi estatisticamente eficaz em diminuir a aparecimento de convulsões induzidas pela pilocarpina e aumentou a latência do início do primeiro episódio de crise tônico-clônica. Neste mesmo estudo,

também foi observado uma abolição das convulsões e da morte induzida por PTZ em ratos jovens tratados com este derivado quinolínico. Esse efeito pode se dar principalmente sua natureza lipofílica, sendo o tecido cerebral o alvo deste composto. Devido a estas propriedades, o QTCA-1 pode ser considerado promissor no desenvolvimento de fármacos para o tratamento das alterações fisiológicas na memória, bem como de doenças neurológicas, relacionada a demência, e também como alternativa terapêutica nas crises convulsivas resultantes da epilepsia.

CAPÍTULO 1

MANUSCRITO 1

A quinoline derivative improves acquisition, consolidation and retrieval in amnesic mice through anticholinesterase action.

A quinoline derivative improves acquisition, consolidation and retrieval in amnesic mice through anticholinesterase action

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Abstract

The present study evaluated the anticholinesterase action of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1), as well as whether compound has an impact on different phases of memory (acquisition, consolidation, and retrieval) in amnesic mice. Male mice Swiss were treated with QTCA-1 (10 mg/kg, i intragastrically (i.g.)) or donepezil (DON) (10 mg/kg; (i.g.)) thirty minutes before the injection of scopolamine (SCO) (1 mg/kg, intraperitoneally (i.p.)). SCO was administered 30 min before training (acquisition), test (retrieval), or immediately after training (consolidation) in the step-down inhibitory avoidance task. After behavioral tests mice were euthanized, and cerebral cortices and hippocampus of mice were removed to determine the acetylcholinesterase (AChE) activities. *In vitro* experiments demonstrated that the QTCA-1 inhibited the AChE activity in cerebral cortex (at concentration 200 and 500 μM) and hippocampus (at concentrations equal to or greater 10, 100, 200 500 μM) of mice. The compound QTCA-1 increased step-down latency in memory impaired mice, improving memory deficits during the acquisition, consolidation and retrieval phases induced by SCO. Treatment with QCTA-1 normalized the AChE activity in the cerebral structures (cerebral cortices and hippocampus) induced by SCO. QTCA-1 improved memory in mice, might offer a useful therapeutic choice to alleviate memory impairments, especially those related to cholinergic dysfunction.

Keywords: Phases of memory, Memory Impairments, Quinoline, Acetylcholinesterase.

1. Introduction

Memory is ability to recall life experiences, and it defines a person's identity (Vogel-Ciernia and Wood, 2014). Accurate memory performance is essential for a variety of tasks, from academic/professional performance to the simplest daily activities. In this context, disturbances of memory can affect cognitive capabilities of individuals and thus their life quality at all stages of life.

For the good proper function of memory is fundamental to maintain the individual physical and mental integrity, besides being crucial for the display of survival strategies (Méndez-Díaz et al., 2005). The loss of memory is a particularly devastating part of a variety of cognitive disorders, diseases, and injuries. In this way, memory is affected when the synapses responsible for making or evoking it are inhibited or altered. This occurs gradually in adulthood, being a physiological process that hardly generates a functional deficit before 80-85 years of age (Vogel-Ciernia and Wood, 2014).

Indeed, memory is a highly complex cognitive function. Memory formation requires a sequence of complementary processes to register, maintain, and retrieve events. Declarative memory, a type of memory classified according to its nature, provides the basis for conscious recollection of acquired knowledge or experienced facts and events, people, places, and objects. Moreover, it is common to segregate memory processes into an acquisition, consolidation, and retrieval phase (Kandel et al., 2014). Major aspects of declarative memory require the hippocampus and cerebral cortex.

Cholinergic system is one of the main neurotransmission pathways in the brain involved in memory and cognitive mechanisms. During aging occurs a moderate degeneration of cholinergic neurons, inducing cholinergic hypofunction, and this event has been related to progressive cognitive decline (Janca et al., 2006). Moreover, the

significant reduction in cholinergic activity was the first pathophysiological component identified in Alzheimer's disease (AD) (Hampel et al., 2018).

In addition, acetylcholine (ACh) is a neurotransmitter present in the hippocampus and cerebral cortex and it is essential for regulation of brain functions, such as learning and memory processes (Orta-Salazar et al., 2014). Choline acetyltransferase (ChAT) catalyzes the synthesis of ACh from choline acetyl-CoA and choline, which is decomposed by acetylcholinesterase (AChE) (Nalivaeva and Turner, 2016). Indeed, neurodegeneration of the cholinergic neurons is accompanied by a reduction in ChAT, in the cholinergic neuron numbers, in the ACh receptors levels, and also by an alteration in the synthesis of ACh or its presynaptic recapture (Graef et al., 2011).

Moreover, a deficiency of ACh can be caused by a disorder in the AChE activity (Mokrani et al., 2019). Some evidence suggests that the increase in the AChE activity is one of the important causes of AD (Lahiri et al., 2002; Racchi et al., 2001). In this context, clinically available AChE inhibitors for AD, such as donepezil (DON), galantamine, and rivastigmine, can ameliorate the cognitive symptoms and enhance the living quality for AD patients (Lleo et al., 2006). However, these drugs have demonstrated modest benefits with some limitations, including side effects (Ali et al., 2015). In this sense, the search for new compounds with better therapeutic efficacies and fewer side effects has been the subject of research (Duarte et al., 2017; Pinz et al., 2018; Pinz et al., 2021).

Quinoline derivatives are an important class of compounds found in various bioactive natural products and pharmaceuticals (Jiang et al., 2011; Oz et al., 2009). Our research group has dedicated attention to study the effect of quinoline derivatives in disorders affecting the central nervous system. Among the quinoline derivatives studied by us, the compound 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-

carboxamide (QTCA-1) stands out. QTCA-1 showed anticonvulsant action (Wilhelm et al., 2014) and anti-amnesic activity after a subchronic treatment (Luchese et al., 2020), being cerebral tissue the target of this compound.

In order to search new compounds against memory impairment, the present study is aimed at evaluating the anticholinesterase action of QTCA-1, as well as whether compound has an impact on different phases of memory (acquisition, consolidation, and retrieval) in amnesic mice.

2. Materials and methods

2.1. Chemicals

QTCA-1 (Fig. 1) was prepared according to the literature method (Wilhelm et al., 2014). Analysis of the ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of QTCA-1 (99.9%) was determined by gas chromatography–mass spectrometry (GC/MS). (−)Scopolamine hydro bromide (SCO), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) and thiobarbituric acid (TBA) were purchased from Sigma Chemical Co. (St Louis, Missouri, USA). DON was purchased from Eurofarma (standard commercial). All other chemicals were of analytical grade and obtained from standard commercial suppliers. For *in vitro* assays, QCTA-1 was dissolved in dimethylsulfoxide (DMSO) and used at different concentrations (μM). For *in vivo* experiments, QTCA-1 and DON were dissolved in canola oil, and SCO was dissolved in saline 0.9%.

2.2. Animals

The experiments were conducted using 60-day old male Swiss mice (25–35 g), obtained from a local breeding colony. Animals were kept in a separate animal room, in controlled conditions with a constant temperature (22 ± 1ºC) and a 12 h dark/light cycle (with light on at 6:00 a.m.). Animals had free access to food and water. The experiments were approved by the Committee on Care and Use of Experimental Animal Resources, Federal University of Pelotas, Brazil (CEEA 1974-2016).

2.3. *In vitro* experiments

In vitro experiments were carried out to investigate the effect of compound QTCA-1 in inhibiting AChE activity. Mice were euthanized with isoflurane and cerebral cortex and hippocampus were removed and dissected. Cerebral tissues were homogenized in a 0.25 M sucrose buffer (1/10, w/v). The homogenates were centrifuged at 900×g at 4°C for 10 min and the supernatants were used for determination of AChE activity.

2.3.1. AChE activity assay

Enzymatic assay was determined according to the method by (Ellman et al., 1961), with some modifications, using acetylthiocholine as substrate. An aliquot of 100 µl of supernatant (protein of 2.8 mg/ml) was pre-incubated for 2 min at 25ºC in the presence of QCTA-1 at different concentrations (1-500 µM), in a medium containing 100 mM potassium phosphate buffer, pH 7.5. Enzymatic reaction was initiated by adding DTNB (final concentration of 0.5 mM) and acetylthiocholine (final concentration of 0.8 mM). The rate of hydrolysis of acetylthiocholine iodide was measured at 412 nm. Results were expressed as µmol acetylthiocholine/h/mg protein. The experiments were performed in different days within 3 independent assays.

2.4. *In vivo* experiments

Mice were divided into five groups of seven nimals each: Control (saline 0.9% and canola oil), QTCA-1 (saline and QTCA-1), SCO (SCO and canola oil), QTCA-1 + SCO and DON + SCO. Experimental protocol is presented in Fig. 2. SCO (1 mg/kg, intraperitoneally (i.p.)) or saline 0.9% (5 ml/kg, i.p.) were administered 30 min before training (acquisition), test (retrieval), or immediately after training (consolidation) (Souza et al., 2013). These memory phases were evaluated in the step-down inhibitory avoidance task. QTCA-1 (10 mg/kg, intragastric (i.g.)) or DON (10 mg/kg, i.g) or canola oil (10 ml/kg, i.g.) were administered 30 min before SCO or saline (Fig. 2). After test session in the step-down inhibitory avoidance task, locomotor and exploratory activities were evaluated in the open-field test.

2.5.1. Behavioral tests

2.5.1.1. Open-field test

Spontaneous locomotor and exploratory activities were determined in the open-field test (Walsh and Cummins, 1976). The open-field was made of plywood and surrounded by walls 30 cm in height. The floor of the open field, 45 cm in length and 45 cm in width, was divided by masking tape markers into nine squares (three rows of three). Each animal was placed individually at the center of the apparatus and observed for 4 min to record locomotion (number of segments crossed with the four paws) and exploration (expressed by the number of time rearing on the hind limbs).

2.5.1.2. Step-down inhibitory avoidance

Nonspatial long-term memory was investigated using a step-down inhibitory avoidance task according to the method of (Sakaguchi et al., 2006), with some

modifications. Each mouse was placed on the platform and the latency to step down with four paws on the grid was automatically recorded in training and test sessions. In the training session, upon stepping down, mice received a 0.5 mA scrambled footshock for 2 s. Test sessions were performed 24 h later with the same procedure, except that no shock was administered after stepping down. An upper cut-off time of 300 s was set.

2.6. *Ex vivo* assays

Immediately after behavioral tests, mice were euthanized with isoflurane and cerebral structures (cerebral cortex and hippocampus) were removed and dissected. Cerebral cortex and hippocampus of mice were homogenized in 0.25 M sucrose buffer (1/10, w/v) and centrifuged at 900×g at 4°C for 10 minutes. The supernatants were used for determination of AChE activity.

2.6.1. AChE activity

The activity of AChE was determined by a modified method by (Ellman et al., 1961), using acetylthiocholine as substrate. An aliquot of 100 µl of supernatant (protein of 2.8 mg/ml) was pre-incubated for 2 min at 25ºC in a medium containing 100 mM potassium phosphate buffer, pH 7.5. Enzymatic reaction was initiated by adding DTNB (final concentration of 0.5 mM) and acetylthiocholine (final concentration of 0.8 mM). The method is based on the formation of the yellow anion, 5,50- dithio-bis-acid-nitrobenzoic, measured spectrophotometrically at 412 nm. Results were expressed as µmol acetylthiocholine/h/mg protein.

2.6.2. Protein determination

The protein was measured by the Comassie blue method by (Bradford, 1976), using bovine serum albumin as standard.

2.7. Statistical analysis

Data are expressed as means ± standard error of the mean (SEM). The normality of data was evaluated by the D'Agostino and Pearson omnibus normality test. For *in vitro* assays the data were analyzed using a unpaired *t*-test. Maximal inhibition (I_{max} - maximum percentage that an inhibitor reduced a response) was calculated at the most effective concentration used. For in vivo e ex vivo experiments, statistical analysis was performed using one-way ANOVA followed by the *Tukey* multiple comparisons test. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. *In vitro* experiments

3.1.1. AChE activity

In the cerebral cortex, QTCA-1 inhibited the AChE activity at concentration of 200 μM and 500 μM (around 57 and 61%, respectively) (Fig. 3A) (ANOVA: $F_{5,12} = 3.974$, $p < 0.05$). In hippocampus, QTCA-1 inhibited the enzyme activity at concentrations equal to and greater than 10 μM (37, 45, 59 and 76 % for 10, 100, 200 and 500 μM, respectively) (Fig. 3B) (ANOVA: $F_{5, 12} = 19.58$, $p < 0.0001$).

The I_{max} was 61% for cerebral cortices and ~ 74% for hippocampus (Table 1).

3.2. *In vivo* experiments

3.2.1. Open-field test

Table 2 demonstrates the effects of treatments on the number of crossing and rearing of mice in the open-field test in different memory phases. No changes was observed in the number of crossing (ANOVA: $F_{4,30} = 0.3425$, $p > 0.05$ for acquisition; ANOVA: $F_{4,30} = 0.7976$, $p > 0.05$ for consolidation; ANOVA: $F_{4,30} = 2.928$, $p > 0.05$ for retrieval) and rearing (ANOVA: $F_{4,30} = 0.6381$, $p > 0.05$ for acquisition; ANOVA: $F_{4,30} = 0.2552$, $p > 0.05$ for consolidation; ANOVA: $F_{4,30} = 0.3164$, $p > 0.05$ for retrieval).

3.2.2. Step-down inhibitory avoidance task

Fig. 4 shows the effects of QTCA-1 and DON on SCO-induced amnesia in the training and test sessions in the step-down inhibitory avoidance task. During the training session in the step-down inhibitory avoidance, there was no difference in the transfer latency time among groups in the different memory phases (ANOVA: $F_{4,30} = 0.2052$, $p > 0.05$ for acquisition; ANOVA: $F_{4,30} = 1.570$, $p > 0.05$ for consolidation and ANOVA: $F_{4,30} = 0.6652$, $p > 0.05$ of retrieval) (data not shown).

During the test section, SCO decreased the transfer latency time in the memory phases of acquisition, consolidation and retrieval (around 76, 60 and 85%, respectively). QTCA-1 or DON attenuated the reduction in the transfer latency time caused by SCO, when compared to the control group, in the different memory phases (Fig. 4A for acquisition, 4B for consolidation and 4C for retrieval). Mice treated only with QTCA-1 did not change the transfer latency time in the different memory phases (Fig. 4A for acquisition, 4B for consolidation and 4C for retrieval). (ANOVA: $F_{4,30} = 5.586$, $p < 0.01$ for acquisition; ANOVA: $F_{4,30} = 4.446$, $p < 0.05$ for consolidation; ANOVA: $F_{4,30} = 9.979$, $p < 0.001$ for retrieval).

3.4. *Ex vivo* assays: AChE activity

Fig. 5 demonstrates the effects of QTCA-1 and DON in the AChE activity in the cerebral cortices (Fig. 5A) and hippocampus (Fig. 5B) of mice after SCO-induced amnesia.

For the memory acquisition phase, SCO increased the AChE activity in the cerebral cortices (around 147%) and hippocampus (137%) of mice, when compared with the control group (Fig. 5A and 5B, respectively). Treatment with QCTA-1 or DON normalized the AChE activity in the cerebral structures (Fig. 5A for cerebral cortices and 5B for hippocampus). No changes in the activity of the enzyme AChE in the cerebral cortex and hippocampus were observed after *per se* treatment with QCTA-1 (Fig. 5A and 5B, respectively). (ANOVA: $F_{4, 30} = 15.35$, $p < 0.0001$ for cerebral cortices and ANOVA: $F_{4, 30} = 9.047$, $p < 0.001$ for hippocampus).

For the memory consolidation phase, SCO increased the AChE activity in the cerebral cortices (around 123%) and hippocampus (95%) of mice, when compared with the control group (Fig. 5C and 5D, respectively). Treatment with QCTA-1 or DON normalized the AChE activity in the cerebral cortices and hippocampus of mice (Fig. 5C and 5D, respectively). QCTA-1 *per se* did not change the enzyme activity in the cerebral cortex and hippocampus of mice (Fig. 5C for cerebral cortices and 5D for hippocampus). (ANOVA: $F_{4,30} = 24.69$ $p < 0.0001$ for cerebral cortices and ANOVA: $F_{4,30} = 9.546$, $p < 0.001$ for hippocampus).

For the memory retrieval phase, SCO increased the AChE activity in the cerebral cortices (around 74%) and hippocampus (109%) of mice, when compared with the control group (Fig. 5E and 5F, respectively). QCTA-1 or DON treatments normalized the AChE activity in cerebral structures of mice (Fig. 5E for cerebral cortices and 5F for hippocampus). QCTA-1 *per se* did not change the enzyme activity in the cerebral cortices and hippocampus of mice (Fig. 5E and 5F, respectively).

(ANOVA: $F_{4, 30} = 4.678$, $p < 0.05$ for cerebral cortex and ANOVA: $F_{4, 30} = 11.64$, $p < 0.0001$ for hippocampus).

4. Discussion

The main finding of the present study is that QCTA-1 enhanced memory performances in each memory phase (acquisition, consolidation or retrieval) in amnesic mice, and that the administration of compound significantly inhibited the AChE activity in cerebral cortices and hippocampus of mice.

It is well established that AChE inhibitors increased ACh levels in the synaptic cleft and ameliorated cognitive symptoms, enhancing life quality of patients with AD (Howard et al., 2012; Simoni et al., 2017; Sun et al., 2008). In this sense, firstly, we investigated anticholinesterase action of QTCA-1 through *in vitro* inhibition of AChE in the cerebral cortices and hippocampus of mice, in order to investigate whether there are differences in the compound action depending on the brain structure analyzed. Our results demonstrated that QTCA-1 inhibited AChE activity and that inhibitory concentrations in the hippocampus were lower than in the cerebral cortices of mice. Moreover, the Imax values of QCTA-1 in the hippocampus (74%) was higher than cerebral cortices (61%), indicating a greater efficacy of compound in the hippocampus. In fact, this result is important given that brain regions such as hippocampus, that is associated with mental functions such as recognition of sensory stimulus, memory and abstract thinking, are the most affected by biochemical changes caused by AD (Demarin et al., 2011).

Previously, we showed that QTCA-1 is a multi-target drug and it had anti-amnesic action after a subchronic treatment in mice (Luchese et al., 2020). However, considering that (i) memory is a complex process represented by three main stages:

acquisition, consolidation, and retrieval (Izquierdo, 2002); and that (ii) several neurodegenerative diseases impaired one or more phases of cognition (Cieslak et al., 2018), it became essential to investigate the action of QCTA-1 in each memory phase. In this way, we verified that a single dose of QTCA-1 attenuated the impairment in memory phases of acquisition, consolidation and retrieval caused by SCO, similarly to positive control (DON). This is an interesting result, as QCTA-1 increased memory phases, and it could be a candidate for treating the cognitive disturbances observed in the neurodegenerative diseases. In addition, none of the treatments caused changes in the spontaneous locomotor and exploratory activities of mice, indicating, mainly, that the effect of QTCA-1 is not due to nonspecific changes, such as psychostimulant or sedative activities. Hence, this is an important result since psychostimulant and sedative drugs may give a false positive result in animal models (Cryan and Holmes, 2005; Ramos, 2008).

Furthermore, we investigated whether AChE activity would be involved in impairment at different memory phases in amnesic mice. Indeed, our results showed that a single dose of QTCA-1 exhibited anticholinesterase action at different memory phases in cerebral cortices and hippocampus of mice, similarly to positive control (DON). In this context, previously, we demonstrated that subchronic treatment with QCTA-1 normalized SCO-induced cholinergic dysfunction in cerebral structures of mice, and that anticholinesterase effect of QCTA-1 could be a mechanism by which the compound prevented behavioral changes caused by SCO (Luchese et al., 2020). Additionally, quinolines are important anticholinesterase agents and with anti-AD activity (Fronza et al., 2019; Wang et al., 2018). Including, Fronza et al. (2019) demonstrated in the docking study that a quinoline derivative it has binding afinity with AChE through residues of amino acids (Trp84, Tyr130, Phe330 and Phe331) present on

catalytic active site (CAS) and through residues (Tyr121) on peripheral anionic site (PAS) of the enzyme. PAS is located at the entry to the active site gorge and is responsible for extra activities, as induce β-amyloid oligomerization, and CAS is often the connection site for AChE inhibitors (Johnson and Moore, 2006). Besides that structural studies have pointed to the dual inhibition of AChE (catalytic active site CAS and PAS) can reduce the cholinergic deficit (Galimberti and Scarpini, 2016). In this sense, QTCA-1 for having similarity of quinolic structure of molecule studied Fronza et al., (2019), could come to interact similarly with dual binding site cholinesterase inhibitor. This way, QTCA-1 may be an alternative for the treatment of memory deficit, because it reduced AChE and possibly it is by interacting with amino acids present in the enzyme catalytic site.

In conclusion, our results showed that QCTA-1 improvement performance in memory phases of acquisition, consolidation or retrieval in amnesic mice. Moreover, compound presented anticholinesterase action in cerebral cortices and hippocampus of mice, evidencing a major effect in the hippocampus. In this context, the increase of cognitive activity of QTCA-1 might offer a useful therapeutic choice to alleviate memory impairments, especially those related to cholinergic dysfunction.

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Compliance with ethical standards

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

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Legend of Figures

Fig. 1 - Chemical structure 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1).

Fig. 2- Experimental protocol to investigate the effects of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) on acquisition, consolidation , and retrieval of memory in a model of scopolamine-induced memory impairment in mice.

Fig. 3- Effect of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) at different concentrations (1-500 μM) on the acetylcholinesterase (AChE) activity in cerebral cortex (A) and hippocampus (B) of mice. Data are reported as mean ± standard error of the mean (S.E.M.) of 3 independent experiments, performing in different days. AChE activity was expressed as μmol acetylthiocholine/h/mg protein. (*) denotes $p< 0.05$, (**) denotes $p <0.01$, (***) denotes $p< 0.001$ and (****) denotes $p<0.0001$ as compared with the control group (one-way analysis of variance/ Tukey test).

Fig. 4- Effect of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) on scopolamine (SCO)-induced memory deficit on the step-down inhibitory avoidance task in mice. Data are from the test session for (A) acquisition, (B) consolidation, and (C) retrieval. Each column represents mean±SEM from seven animals per group. (*) denotes $p< 0.05$, (**) denotes $p <0.01$, (***) denotes

p< 0.001 as compared with the control group. (#) denotes p< 0.05, (##) denotes p <0.01, (###) denotes p< 0.001 as compared with the scopolamine group (one-way analysis of variance/ Tukey test).

Fig. 5- Effect 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) on acetylcholinesterase (AChE) activity on cerebral cortex and hippocampus of mice with scopolamine (SCO)-induced. Data are from the AChE activity for (5A) cerebral cortex and (5B) for hippocampus for acquisition, (5C) for cerebral cortex and (5D) for hippocampus for consolidation, and (5E) for cerebral cortex and (5F) for hippocampus for retrieval. Each column represents mean±SEM from seven animals per group. (*) denotes p< 0.05, (***) denotes p< 0.001, (****) denotes p <0.0001 as compared with the control group. (#) denotes p< 0.05, (###) denotes p< 0.001, (####) denotes p <0.0001 as compared with the scopolamine group (one-way analysis of variance/ Tukey test).

Figures

Fig. 1.

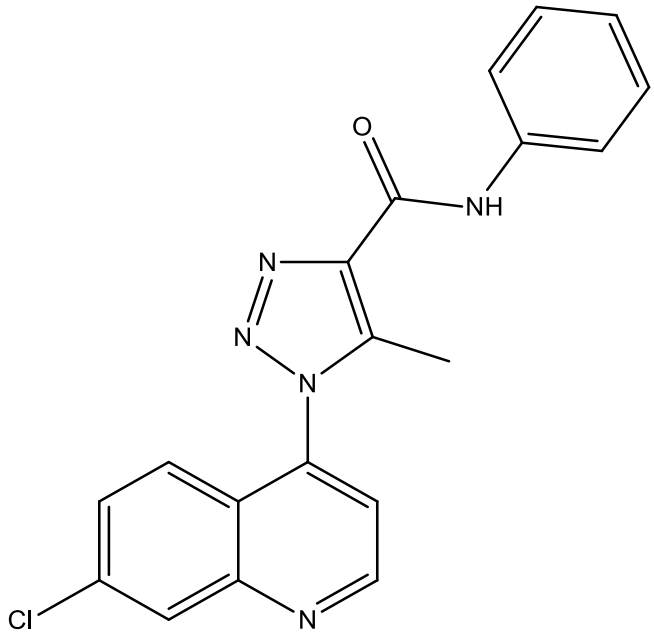


Fig. 2.

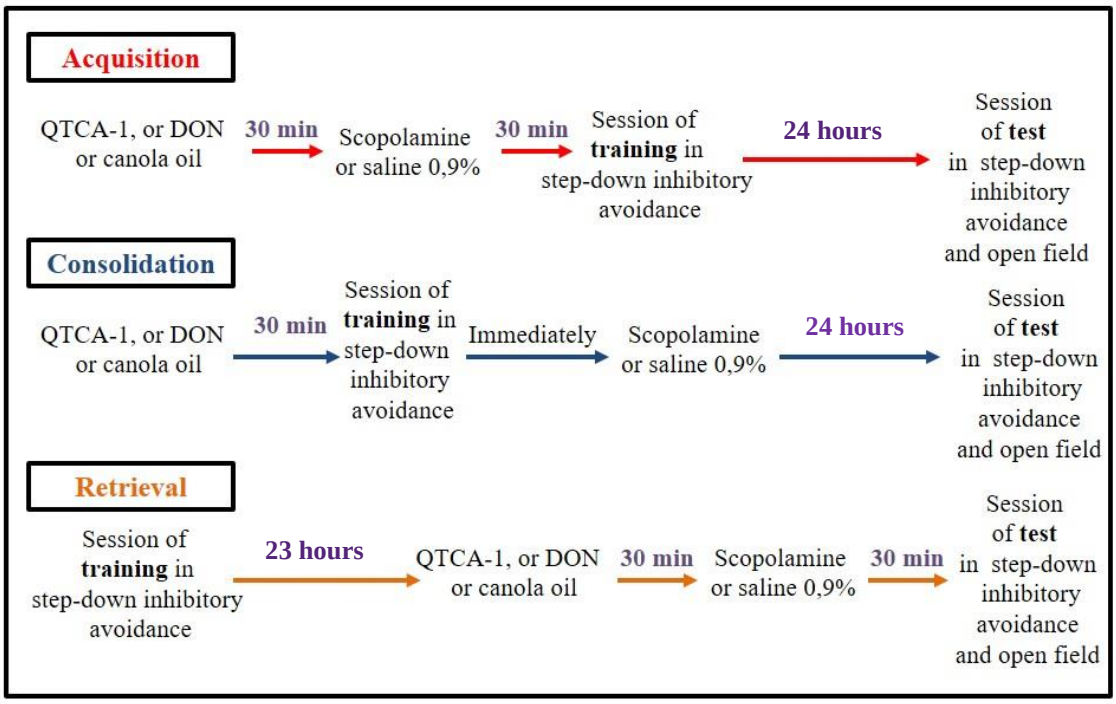
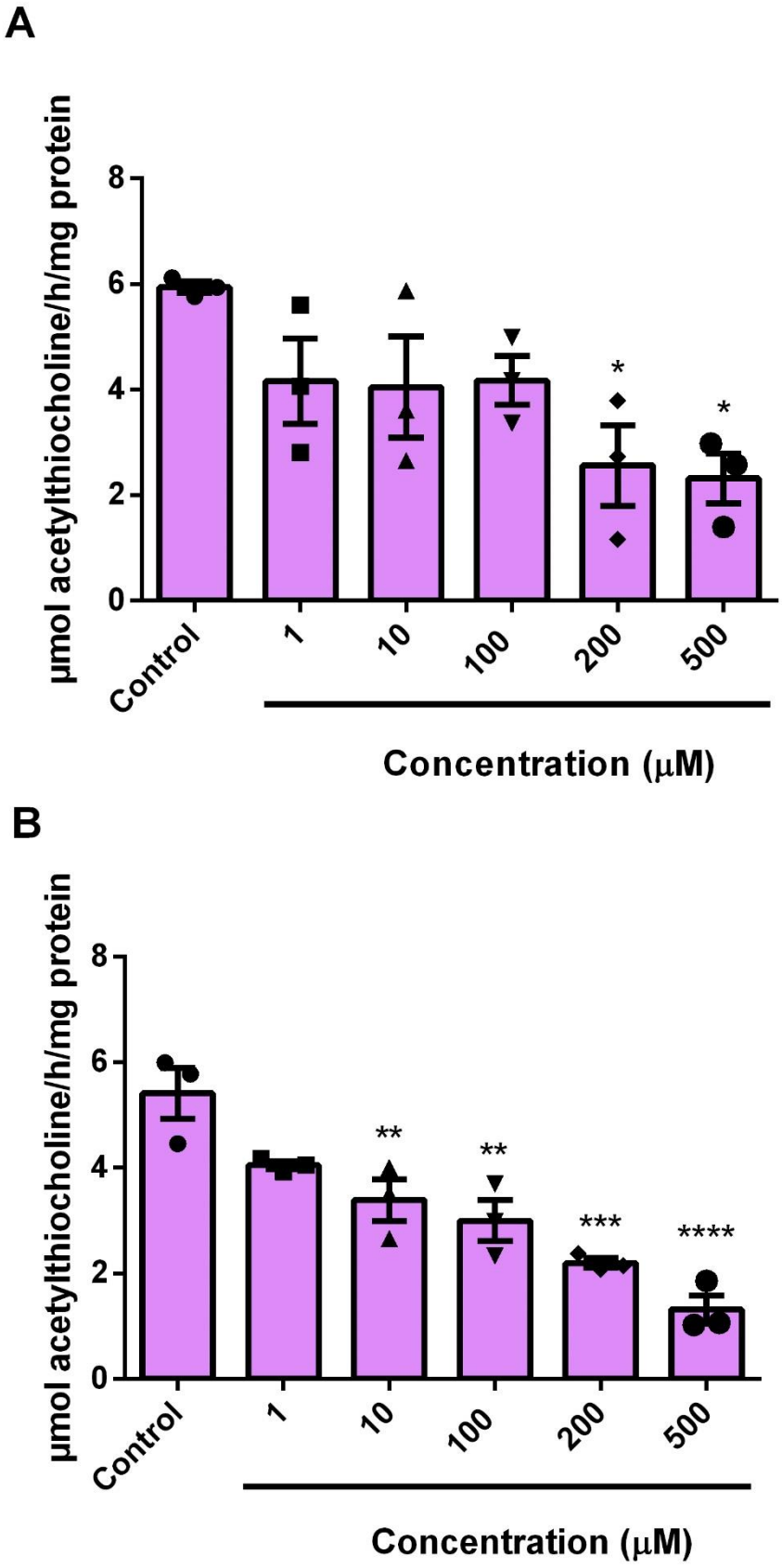

Acquisition
QTCA-1, or DON or canola oil
30 min
Scopolamine or saline 0,9%
30 min
Session of training in step-down inhibitory avoidance
24 hours
Session of test in step-down inhibitory avoidance and open field
Consolidation
QTCA-1, or DON or canola oil
30 min
Session of training in step-down inhibitory avoidance
Immediately
Scopolamine or saline 0,9%
24 hours
Session of test in step-down inhibitory avoidance and open field
Retrieval
Session of training in step-down inhibitory avoidance
23 hours
QTCA-1, or DON or canola oil
30 min
Scopolamine or saline 0,9%
30 min
Session of test in step-down inhibitory avoidance and open field

Fig. 3.


A
µmol acetylthiocholine/h/mg protein
8
6
4
2
0
Control
1
10
100
200
500
*
*
Concentration (µM)
B
µmol acetylthiocholine/h/mg protein
8
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Control
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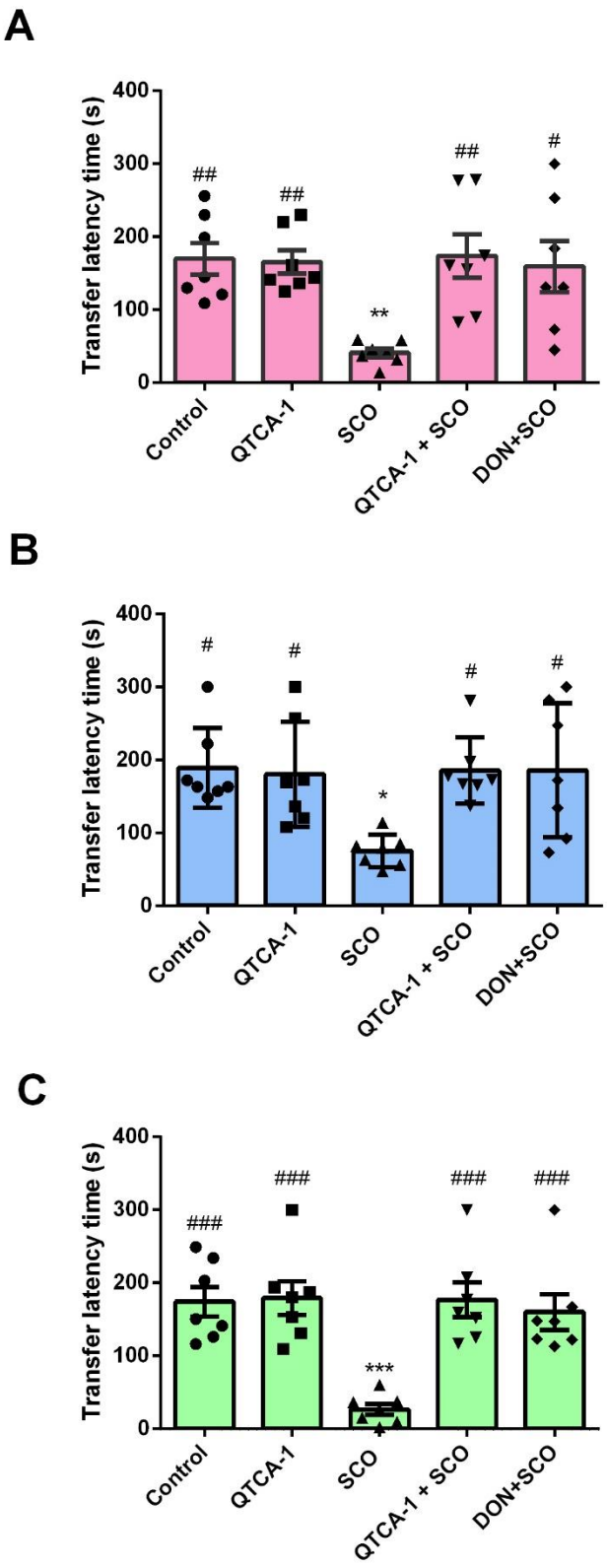
Concentration (µM)

Fig. 4.


A
Transfer latency time (s)
400
300
200
100
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##
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##
#
Control
QTCA-1
SCO
QTCA-1 + SCO
DON+SCO
B
Transfer latency time (s)
400
300
200
100
0
#
#
*
#
#
Control
QTCA-1
SCO
QTCA-1 + SCO
DON+SCO
C
Transfer latency time (s)
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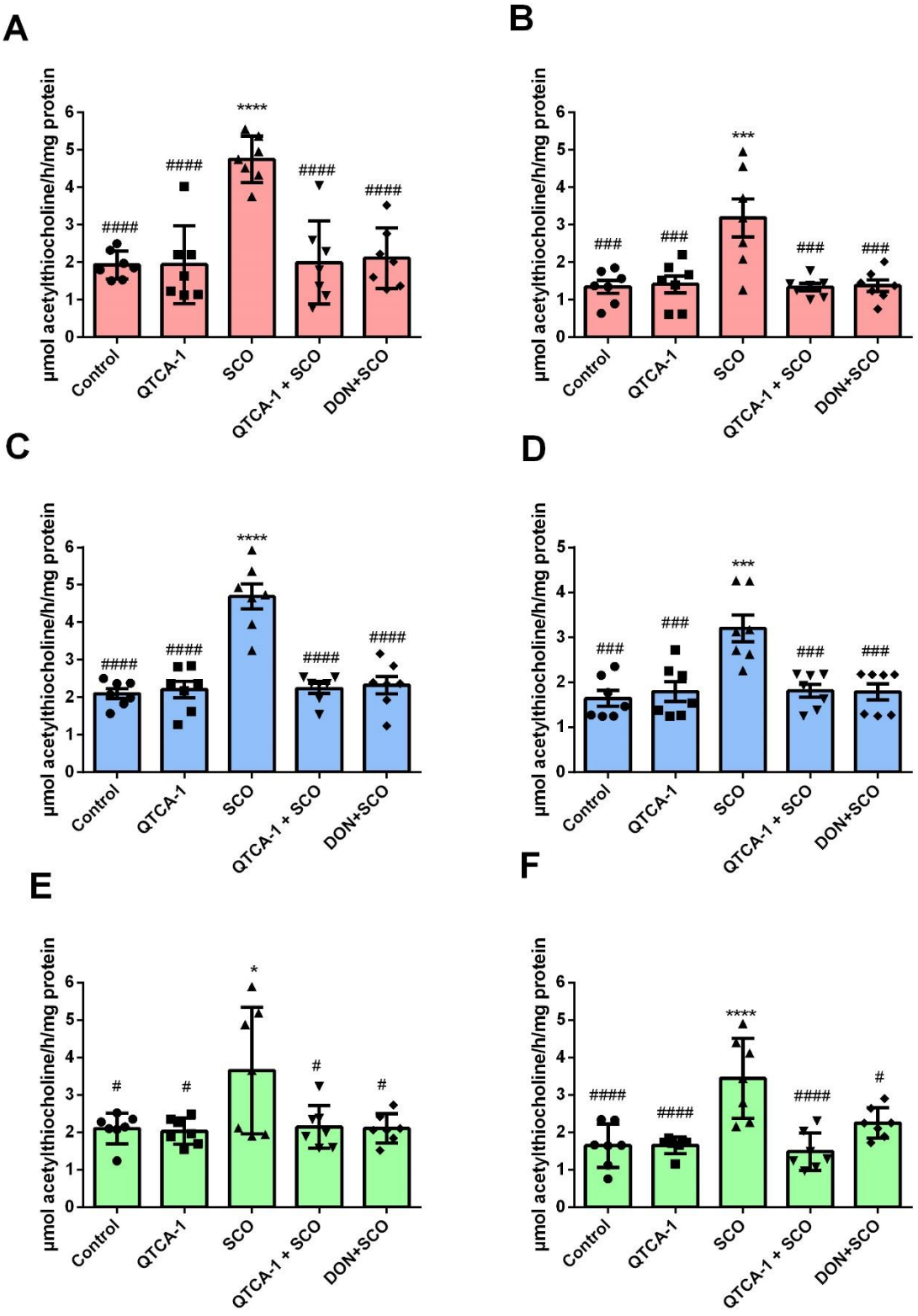
SCO
QTCA-1 + SCO
DON+SCO

Fig. 5.


A
B
C
D
E
F
µmol acetylthiocholine/h/mg protein
Control
QTCA-1
SCO
QTCA-1 + SCO
DON+SCO

Tables

Table 1. Maximal inhibition (I_{max}) value of compound 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) on acetylcholinesterase (AChE) activity in cerebral cortices and hippocampus of mice.

	AChE
	I_{max}
Cerebral cortex	61 ± 7
Hippocampus	74 ± 8

The I_{max} values are reported as mean ± standard error of the mean (SEM).

Table 2. Effects of treatments on the number of crossing and rearing of mice in the open-field test in different memory phases.

	Acquisition		Consolidation		Retrieval	
	Crossing	Rearing	Crossing	Rearing	Crossing	Rearing
Control	93 ± 4	21 ± 3	94 ± 6	24 ± 2	86 ± 7	24 ± 3
QTCA-1	100 ±13	21 ± 5	88 ± 3	24 ± 2	82 ± 7	23 ± 4
SCO	106 ±6	20 ± 3	102 ± 6	22 ± 3	104 ± 5	20 ± 3
QTCA-1 + SCO	98 ±8	17 ± 3	92 ± 5	21 ± 3	99 ± 4	22 ± 4
DON + SCO	99.7±6	16 ± 3	96 ± 9	24.3±3	99 ± 3	19 ± 4

Data are reported as mean ± standard error of the mean (SEM) of 7 mice in each group. QTCA-1 means 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide, SCO means scopolamine and DON means donepezil. Statistical analysis was performed using one- way ANOVA/ *Tukey* test.

CAPÍTULO 2

ARTIGO 1

Amnesia-ameliorative effect of a quinoline derivative through regulation of oxidative/cholinergic systems and Na^+/K^+-ATPase activity in mice.

Manuscrito aceito e publicado pela revista ***Metabolic Brain Disease.***

ANEXO A – Autorização divulgação do artigo na tese.

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ORIGINAL ARTICLE

Amnesia-ameliorative effect of a quinoline derivative through regulation of oxidative/cholinergic systems and Na^+/K^+-ATPase activity in mice

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Abstract

The present study evaluated the anti-amnesic activity of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) against scopolamine (SCO)-induced amnesia in mice. It was evaluated cholinergic dysfunction, oxidative stress and Na^+/K^+-ATPase activity in cerebral cortex and hippocampus of mice. Male Swiss mice were treated with QTCA-1 (10 mg/kg, intragastrically (i.g.), daily) for nine days. Thirty minutes after the treatment with compound, the animals received a injection of SCO (0.4 mg/kg, intraperitoneally (i.p.)). Mice were submitted to the behavioral tasks 30 min after injection of SCO (Barnes maze, open-field, object recognition and location, and step-down inhibitory avoidance tasks) during nine days. In day 9, cerebral cortex and hippocampus of mice were removed to determine the thiobarbituric acid reactive species (TBARS) levels, and catalase (CAT), Na^+/K^+-ATPase and acetylcholinesterase (AChE) activities. SCO caused amnesia in mice for changing in step-down inhibitory avoidance, Barnes maze, and object recognition and object location tasks. QTCA-1 treatment attenuated the behavioral changes caused by SCO. Moreover, SCO increased AChE and CAT activities, decreased Na^+/K^+-ATPase activity and increased TBARS levels in the cerebral structures of mice. QTCA-1 protected against these brain changes. In conclusion, QTCA-1 had anti-amnesic action in the experimental model used in the present study, through the anticholinesterase effect, modulation of Na^+/K^+-ATPase activity and antioxidant action.

Keywords Dementia · Quinoline · Acetylcholinesterase · Antioxidant · Na^+/K^+-ATPase · Scopolamine

Introduction

Dementia is a state of cognitive decline, associated with psychiatric and behavioral disturbances (Ritchie et al. 2015). This syndrome is considered one of the major causes of morbidity and mortality among older people (Van de Vorst et al. 2015). In 2015, dementia affected 47 million people worldwide (or roughly 5% of the world's elderly population), a figure that is predicted to increase to 75 million in 2030 and 132 million by 2050 (WHO 2017). Recent reviews estimate that globally nearly 9.9 million people develop dementia each year (WHO 2017).

Dementias may be difficult to clinically diagnose because of their multifactorial causes and overlapping symptoms with various neurological disorders, such as Alzheimer's disease (AD), resulting in inconsistent clinical presentation and diagnostic challenges (Nelson et al. 2011; Puri et al. 2014). Many of neurological disorders associated with the dementia are often related to deficiencies in cerebral cholinergic neurotransmission (Lobo et al. 2000). Indeed, the blockade of muscarinic acetylcholine (ACh) receptors interrupts the learning and memory functions, given that ACh is an important neurotransmitter involved in regulating of cognitive functions (Kumar et al. 2015). A

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reduction in the ACh levels in cerebro-spinal fluid of AD patients is correlated inversely with the severity of dementia (Lin et al. 2016). Moreover, decreased ACh concentration in brain also results in a diminished ability to learn and form new memories (Lin et al. 2016). Acetylcholinesterase (AChE) is an important regulatory enzyme that modulates cholinergic synapses through the hydrolysis of ACh (Kumar et al. 2015).

Besides the cholinergic system, oxidative stress is another well-known causative factor in the pathogenesis of neurological disorders related to dementia (Cruz et al. 2002). Studies have shown that oxidative stress is related to cognitive impairments (Tao et al. 2015), being also connected to neuronal loss in brain (Azman et al. 2015). In addition, Na^+/K^+-ATPase is a potent neuroprotective modulator against neurological disorders associated dementia, given that oxidative stress modulates memory process through of Na^+/K^+-ATPase activity (Zhang et al. 2013).

Clinically, primary treatments for most forms of dementia in AD approved by Food and Drug Administration (FDA) are AChE inhibitors, such as donepezil, galantamine and rivastigmine (Zemek et al. 2014). However, the usual therapies for dementia treatment in AD provide symptomatic relief (Zemek et al. 2014). These treatments are effective in the early stages of disease and they presented some limitations, such as low efficacy and adverse effects for the long-term use (Libro et al. 2016). Consequently, the development of effective strategies that help to prevent age- and disease-related worsening of brain structure and function may thus provide significant benefits for society and health-care systems.

Several researches have suggested that the quinoline moiety is the pharmacophore of many anti-AD drugs, such as tacrine, clioquinol, methylene blue, berberine derivatives and PMS1339 (Freeman and Dawson 1991; Mancino et al. 2009; Oz et al. 2009; Jiang et al. 2011). In this context, our research group has dedicated attention to study the effect of quinoline derivatives in disorders affecting the central nervous system. Among the quinoline derivatives, 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) showed anticonvulsant activity, mainly due to its lipophilic nature, being cerebral tissue the target of this compound (Wilhelm et al. 2014).

Therefore, the present study evaluated the anti-amnesic activity of QTCA-1 against scopolamine (SCO)-induced amnesia in mice. The effect of QTCA-1 was evaluated on SCO-induced cholinergic dysfunction in terms of AChE activity in hippocampus and cerebral cortex of mice. Moreover, oxidative stress and Na^+/K^+-ATPase activity were estimated in cerebral structures of mice as a plausible mechanism of action for anti-amnesic activity of QTCA-1 in such condition.

Material and methods

Chemicals

QTCA-1 (Fig. 1) was prepared according to the literature method (Wilhelm et al. 2014). Analysis of the ^1H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. QTCA-1 was dissolved in canola oil. (−) Scopolamine hydro bromide, 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) and thiobarbituric acid (TBA) were purchased from the Sigma Chemical Co. (St Louis, Missouri, USA). (−) Scopolamine hydro bromide was dissolved in saline 0.9%. All other chemicals were of analytical grade and obtained from standard commercial suppliers.

Animals and ethical approval

Male adult Swiss mice (25–35 g) from a local breeding colony were used. Animals were kept on a separate room, on a 12 h light/dark cycle, at a temperature of 22 ± 2 °C, with free access to food and water. All animal experiments in the present study were approved by the Committee on Care and Use of Experimental Animal Resources of Federal University of Pelotas, Brazil (CEEA number 1974–2016) and in accordance with the guide of Brazilian National Animal Care Ethical Council (CONCEA), which is based on National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (Publication no. 85–23, revised 1985).

Experimental protocol

Mice were randomly divided into 4 experimental groups (7 animals/group, total of 28 animals): Group I (control) received canola oil + saline, group II (QTCA-1) received QTCA-1 +

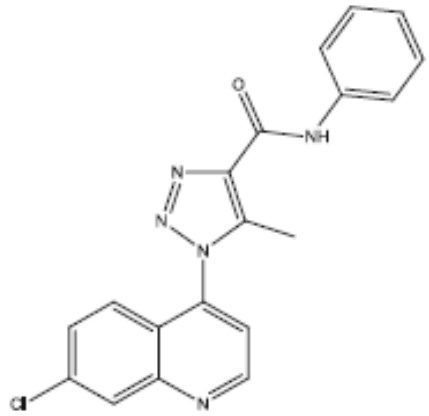

Fig. 1 Chemical structure 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1)

saline, group III (SCO) received canola oil + SCO, and group IV pre (QTCA-1 + SCO) received QTCA-1 + SCO. Initially, groups I and III received, intragastrically (i.g.) via gavage, canola oil (10 ml/kg), while groups II and IV were treated, i.g. via gavage, with QTCA-1 (10 mg/kg). Thirty minutes after treatments, groups III and IV received SCO (0.4 mg/kg, intraperitoneally (i.p.)) (Pahaye et al. 2017) and groups I and II received saline 0.9% (5 ml/kg, i.p.). Treatments were performed daily during the 9-day period. Thirty minutes after SCO or saline injections, animals were subjected to the behavioral tests. In day 9, animals were euthanized, and cerebral cortex and hippocampus were removed for ex vivo experiments (Fig. 2).

The choice of QTCA-1 dose was based on other studies of memory decline induced by SCO (Fukuda et al. 2019; da Silva et al. 2017; Eduviere et al. 2015). In these studies, different drugs exerted pharmacological effect at the dose of 10 mg/kg. Thus, considering the potential of these drugs in improving the memory decline and the necessity to reduce the number of animals used in experiments, based on humanitarian principles of animal experimentation or principle of the 3Rs (Russell and Burch 1959), a curve of dose-response was not tested. We opted by the oral route of administration for QTCA-1 because of the advantages presented, such as, low total dose, low gastrointestinal side effects, reduced dosing frequency, good patient acceptance and compliance, less fluctuation at plasma drug levels, more uniform drug effect, improved efficacy/safety ratio and low cost-effectiveness (Mignani et al. 2013). This has been the route of the preferred for the several authors that search alternative treatments for amnesia (Martini et al. 2018; Souza et al. 2010; Nath et al. 2009). Since it is well described in the literature that intraperitoneal administration of SCO induces amnesia in animals, this route of administration was chosen for the present study (Ngoupaye et al. 2017; Pahaye et al. 2017; da Silva et al. 2017; Habiba et al. 2017).

Behavioral tests

Barnes maze task

Spatial learning and memory were accessed using Barnes maze task (Pompl et al. 1999), with minor adaptations (Souza et al. 2012). This behavioral test was performed from the first day of the experimental protocol. The Barnes maze consists of a flat and circular disk (69 cm diameter) with sixteen circular holes (4.5 cm diameter) at equal distances around the perimeter, and it elevates 50 cm above the floor. The escape box (13 × 29 × 14 cm) was localized under of one hole. Mice learn the location of escape box under hole, using spatial reference points fixed to the wall. Animals were trained in the maze Barnes on days 1, 2, 3 and 4 of experimental protocol. Training consisted of placing the animal in a black box and leaving it for a period of 1 min. Then, black box was placed in the center of the Barnes maze. Black box was removed and then started the training. Mice freely explored the maze to find the escape box. Maximum latency to find out escape box was 180 s. Each mouse was trained three times per day, with 10-min inter-trial interval, during four consecutive days. The latency to reach the escape box and number of wrong holes were measured in four training sessions. Twenty-four hours after the last day of training phase (day 5 of experimental protocol), a probe trial was performed. In the probe trial, each mouse was placed in maze center, it was measured the latency to find the escape box and checked number of holes visited.

Open-field test

The locomotor and exploratory behaviors were assessed in the open-field test (Walsh and Cummins 1976). The open-field was made of plywood and surrounded by 30 cm-high walls. The floor of the open-field, 45 cm long and 45 cm wide, was divided by masking tape markers into 9 squares (3 rows of 3).

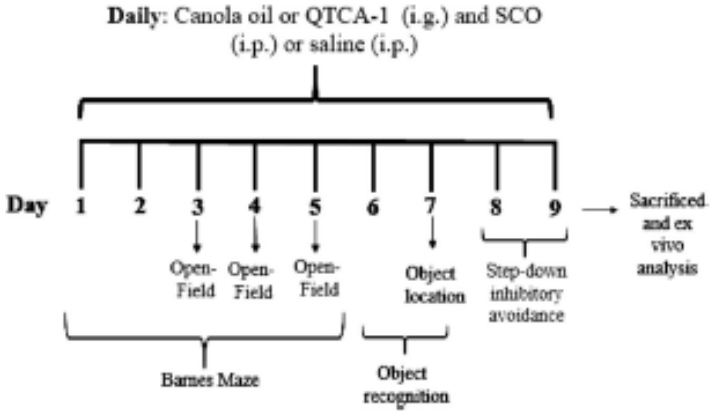

Fig. 2 Scheme of experimental protocol. Thirty minutes before initiating intragastric (i.g.) treatments, mice received scopolamine (SCO) or saline, both intraperitoneally (i.p.). I.g. treatments and i.p. induction were performed every day, until the end of the experimental protocol. From the first day of the experimental protocol, the animals were submitted to the Barnes maze, open-field, object recognition, object location, step-down inhibitory avoidance tasks. On the ninth day, mice were euthanized

Each animal was placed individually at the center of the apparatus and observed for 4 min to record the locomotor (number of segments crossed with the four paws) and exploratory (number of time rearing on the hind limbs). Mice were habituated to the open-field for 3 days (on days 3, 4 and 5 of experimental protocol) after Barnes maze task.

Object recognition task

The object recognition task is used as a measure to evaluate the long-term (LTM) memories (Stangherlin et al. 2009). The object recognition task was performed in an open-field apparatus. On the day of the test (day 6 of the experimental protocol), each animal was submitted to a habituation session in the absence of objects for 5 min. Subsequently, four objects were used: A1, A2, and B. The A1 and A2 objects were two identical balls, the B object was an octagon. Each object had the following color pattern: blue, red and yellow. All objects were made of plastic material, measuring 10 × 10 cm (length×height). During the training, in the day 6 of the experimental protocol, the animals were placed in the arena containing two identical objects (objects A1 and A2) for 5 min. Exploration was defined when the animal directed its nose within 2 cm of the object while looking, sniffing, or touching it. The LTM was performed 24 h after training phase, in the day 7 of the experimental protocol, when mice were placed to explore a familiar object (A1) and a new object (B) for 5 min and the total time spent in exploring each object was determined. Data were expressed as a percentage of the exploratory preference and calculated as follows: Training = (A2 /(A1 + A2)) × 100; LTM = (B/(A1 + B)) × 100.

Object location task

The object location task was performed according to Dix and Aggleton (1999). This test is a hippocampal-dependent spatial memory task. The apparatus used for this test was the same open-field apparatus used in the object recognition task with the LTM objects (object A1 and object B). In this task, four hours after the LTM, object B was moved to a location that was diagonally opposite to object A1, and the mouse was left in the apparatus for 5 min of exploration. The time spent exploring the location of new and familiar objects was recorded. The object location test is realized in the day 7 of protocol experimental. Data were expressed as a percentage of the exploratory preference and calculated as follows: object location task = (B/(A1 + B)) × 100.

Step-down inhibitory avoidance task

Long-term memory was investigated using the step-down inhibitory avoidance task (Sakaguchi et al. 2006), with modifications in the intensity of electric shock and in the exposure time. Animals were trained on step-down inhibitory avoidance in day 8 of experimental protocol. During the training session, each mouse was placed on the platform. When it stepped down and placed its four paws on the grid floor, an electric shock (0.5 mA) was delivered for 2 s. The test (30 min after scopolamine or saline injection) was performed 24 h after training (in day 9). Each mouse was placed again on the platform and the step-down transfer latency time was recorded, but mice no received the aversive stimulus.

Ex vivo assays

In the day 9 of the experimental protocol, mice were anesthetized and euthanized with isoflurane and cerebral structures (cerebral cortex and hippocampus) were immediately removed. Cerebral cortex and hippocampus were separated and washed with cold saline solution (0.9%) to determine AChE, Na^+/K^+-ATPase, and catalase (CAT) activities, as well as, thiobarbituric acid reactive species (TBARS) levels.

Cerebral structures were homogenized in cold 50 mM Tris-HCl, pH 7.4 (1/10, weight/volume), centrifuged at 900 *x*g at 4 °C for 10 min and supernatants were used for determination of enzymes activities (Na^+/K^+-ATPase and CAT) and TBARS levels. Also, cerebral cortex and hippocampus of mice were homogenized in 0.25 M sucrose buffer (1/10, *w/v*), centrifuged at 900 *x*g at 4 °C for 10 min and supernatants were used for determination of AChE activity.

AChE activity

The activity of AChE was determined by a modified method by Ellman et al. (1961), using acetylthiocholine as substrate. An aliquot of the supernatant (protein of 2.8 mg/mL) was pre-incubated for 2 min at 25 °C in a medium containing 100 mM, potassium phosphate buffer, pH 7.5. Enzymatic reaction was initiated by adding DTNB (final concentration of 0.5 mM) and acetylthiocholine (final concentration of 0.8 mM). The rate of hydrolysis of acetylthiocholine iodide was measured at 412 nm through the release of the thiol compound, which when reacted with DTNB produces the color-forming compound thionitrobenzene (TNB). Results were expressed as µmol acetylthiocholine/h/mg protein.

Na^+/K^+ ATPase activity

Supernatant was mixed with 3 mM MgCl, 125 mM NaCl, 20 mM KCl and 50 mM Tris-HCl, pH 7.4. The reaction was initiated by the addition of 3 mM adenosine triphosphate (ATP). Control samples were carried out under the same conditions with the addition of 0.1 mM ouabain. Cerebral cortex and hippocampus protein extracts taken for the assay were 8

and 7 mg/ml, respectively. The samples were incubated at 37 °C for 30 min and incubation was stopped by adding trichloroacetic acid (10%) with 10 mM $HgCl_2$. Na^+/K^+-ATPase activity was calculated by the difference between the two assays. Released inorganic phosphate (Pi) was measured according to the method described by Fiske and Subbarow (1925). Enzyme activity was expressed as nmol Pi/mg protein/min.

TBARS levels

TBARS levels were determined as described by Ohkawa et al. (1979) and used as lipid peroxidation measure. An aliquot of supernatant was added to the reaction mixture containing: 8.1% sodium dodecyl sulfate (SDS), 0.8% thiobarbituric acid and acetic acid buffer (pH 3.4). The system was incubated at 95 °C for 2 h. Absorbance was measured at 532 nm. Results were reported as nmol malondialdehyde (MDA)/mg protein.

CAT activity

Enzyme activity was assayed by the method of Aebi (1984), which involves monitoring the disappearance of H_2O_2 in the homogenate at 240 nm. Enzymatic reaction was initiated by adding of the supernatant and the substrate (H_2O_2) in a medium containing 50 mM potassium phosphate buffer, pH 7.0. The enzymatic activity was expressed as Unit (U) CAT/mg protein (1 U decomposes 1 μmol H_2O_2/min at pH 7 at 25 °C).

Protein quantification

The protein concentration was measured by the method of Bradford (1976), using bovine serum albumin as the standard.

Statistical analysis

Data are expressed as means ± standard error of the mean (SEM). The normality of data was evaluated by the D'Agostino and Pearson omnibus normality test. Statistical analysis was performed using one-way (for data of Barnes maze task in the test phase and for the other behavioral and biochemical tests) or two-way (for data of Barnes maze task in the training phase) ANOVA followed by the Newman-Keuls multiple comparisons test. Main effects are presented only when the higher second order interaction was non-significant. Values of $p < 0.05$ were considered statistically significant.

Results

Behavioral tests

Barnes maze task

In the training phase, the two-way analysis of the latency to find the escape box revealed the main effect of days (ANOVA: $F_{3, 96} = 12.88$, $p < 0.0001$) and treatments (ANOVA: $F_{3, 96} = 6.689$, $p < 0.001$). When the number of holes visited was evaluated in the training phase, the two-way analysis showed significant main effect of days (ANOVA: $F_{3, 96} = 12.66$, $p < 0.0001$) and treatments (ANOVA: $F_{3, 96} = 3.492$, $p < 0.05$). On the first, second and third days of training, no difference between the groups in latency to find the escape box and number of holes visited was observed (Fig. 3A and 4A, respectively).

On the fourth day of training, SCO injection significantly increased (around 102%) the latency to find the escape box (Fig. 3A) and number of holes visited (around 90%) (Fig. 4A). QTCA-1 treatment significantly protected against this

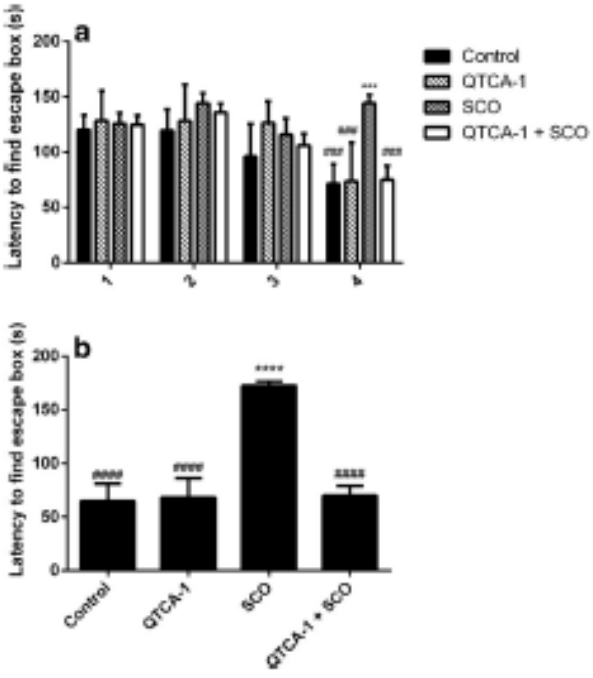

Fig. 3 Effect of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) on scopolamine (SCO)-induced memory deficits in Barnes maze task on the latency to find the escape box (s) (3A) on training and (3B) on the test phases. Data are reported as mean ± standard error of the mean (S.E.M.) of seven animals per group. (***) denotes $p < 0.001$ and (****) denotes $p < 0.0001$ as compared to the control group; (###) denotes $p < 0.001$ and (####) denotes $p < 0.0001$ as compared to the SCO group (two-way analysis of variance/Newman-Keuls test for training and one-way analysis of variance/Newman-Keuls test for probe test)

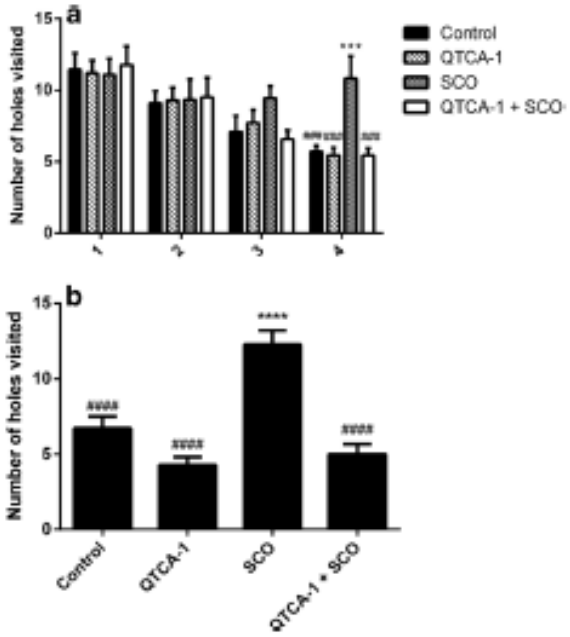

Fig. 4 Effect 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) on scopolamine (SCO)-induced memory deficits in Barnes maze task in the number of holes visited (4A) on training and (4B) on the test phases. Data are reported as mean ± standard error of the mean (S.E.M.) of seven animals per group. (***) denotes $p < 0.001$ and (****) denotes $p < 0.0001$ as compared to the control group; (###) denotes $p < 0.001$ and (#####) denotes $p < 0.0001$ as compared to the SCO group (two-way analysis of variance/Newman-Keuls test for training and one-way analysis of variance/Newman-Keuls test for probe test)

increase, when compared to the control group (Fig. 3A and 4A, respectively).

In the probe test (day 5), SCO increased the latency time to find the escape box (around 166%), and the number of holes visited (around 87%), when compared with the control group (Fig. 3B and 4B, respectively). QTCA-1 attenuated the latency to find the escape box and number of holes visited increased by SCO (Fig. 3B and 4B, respectively) (ANOVA: $F_{3,\ 24} = 15.64$, $p < 0.0001$ for latency and ANOVA: $F_{3,\ 24} = 24.42$, $p < 0.0001$ for number of holes).

Open-field test

The one-way ANOVA followed by Newman-Keuls test demonstrated that treatments did not cause any significant change in the number of crossings in days 1 (ANOVA: $F_{3,24} = 1.956$, $p > 0.05$), 2 (ANOVA: $F_{3,\ 24} = 2.902$, $p > 0.05$) and 3 (ANOVA: $F_{3,24} = 2.431$, $p > 0.05$) (Table 1). One-way ANOVA for the number of rearing in first day (ANOVA: $F_{3,24} = 1.806$, $p > 0.05$), second day (ANOVA: $F_{3,24} = 1.800$, $p > 0.05$) and third day (ANOVA: $F_{3,24} = 0.6916$, $p > 0.05$) revealed no significant difference (Table 1).

Object recognition task

In the training phase of the object recognition task, there was no difference in the exploratory preference of objects among groups (ANOVA: $F_{3,\ 24} = 1.078$, $p > 0.05$) (Fig. 5A). In the probe test, mice treated with SCO had a decrease (around 28% for LTM) in the exploratory preference of the new object, and QTCA-1 prevented this reduction (Fig. 5B) (ANOVA: $F_{3,\ 24} = 8.216$, $p < 0.01$). QTCA-1 alone did not change the exploratory preference for the new object in LTM (Fig. 5B).

Object location task

For the object location task, mice injected with SCO had a reduction (around 33%) in the exploratory preference by the new object location and QTCA-1 significantly prevented this reduction, when compared to the control group (Fig. 5C) (ANOVA: $F_{3,\ 24} = 4.508$, $p < 0.05$). QTCA-1 alone did not change the exploratory preference for the new object location (Fig. 5C).

Step-down inhibitory avoidance

During the training session of the step-down inhibitory avoidance, there was no difference in the transfer latency time among groups (ANOVA: $F_{3,\ 24} = 0.3553$, $p > 0.05$) (Fig. 6). In the test phase, SCO decreased (around 70%) the transfer latency time and QTCA-1 significantly prevented this reduction, when compared to the control group (Fig. 6) (ANOVA: $F_{3,\ 24} = 11.14$, $p < 0.0001$). Mice treated only with QTCA-1 did not change the transfer latency time in the test phase (Fig. 6).

Ex vivo assays

AChE activity

Figure 7A and B illustrate the effects of treatments on AChE activity in hippocampus and cerebral cortex of mice, respectively. Results demonstrated that SCO increased the AChE activity in hippocampus (around 182%) and cerebral cortex (around 235%) of mice, when compared with the control group. QTCA-1 treatment significantly prevented the increase on the AChE activity in cerebral structures caused by SCO (Fig. 7A and B, respectively). QTCA-1 alone did not change the AChE activity in the hippocampus and cerebral cortex (Fig. 7A and B, respectively) (ANOVA: $F_{3,\ 24} = 20.78$, $p < 0.0001$ for hippocampus and ANOVA: $F_{3,\ 24} = 7.555$, $p < 0.001$ for cerebral cortex).

Table 1 Effects of SCO, QTCA-1 and QTCA-1 plus SCO on open-field test in mice

Group	Number of crossing			Number of rearing		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Control	103 ± 3	116 ± 6	121 ± 12	42.6 ± 0.6	44.9 ± 2.8	40.4 ± 1.3
TCQ	110 ± 7	102 ± 10	85 ± 8	34.3 ± 5.0	38.9 ± 1.0	34.6 ± 5.7
SCO	116 ± 8	125 ± 6	109 ± 11	41.7 ± 0.8	46.6 ± 3.3	39.3 ± 3.5
TCQ + SCO	121 ± 3	128 ± 6	107 ± 6	40.4 ± 2.0	42.7 ± 2.4	34.9 ± 2.4

Data are reported as the mean ± S.E.M. of 7 mice in each group. QTCA-1 means 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide. SCO means scopolamine

Na^+/K^+-ATPase activity

SCO induced a reduction on the Na^+/K^+ ATPase activity (around 57%) in hippocampus (Fig. 8A) and cerebral cortex (around 76%) (Fig. 8B) of mice. QTCA-1 treatment protected against the reduction in the Na^+/K^+-ATPase activity caused by SCO in both cerebral structures of mice (Fig. 8A for hippocampus and 8B for cerebral cortex). Treatment only with QTCA-1 did not modify the Na^+/K^+-ATPase activity in hippocampus and cerebral cortex of mice (Fig. 8A and B, respectively) (ANOVA: $F_{3, 24} = 6.307$, $p < 0.01$ for hippocampus and ANOVA: $F_{3, 24} = 4.552$, $p < 0.05$ for cerebral cortex).

TBARS levels

SCO increased the TBARS levels in the hippocampus (around 67%) and in the cerebral cortex (around 83%) (Fig. 9A and B, respectively). QTCA-1 treatment protected against the increase caused by SCO in both cerebral structures (Fig. 9A and B). QTCA-1 alone did not change the TBARS levels in the hippocampus and cerebral cortex (Fig. 9A and B, respectively) (ANOVA: $F_{3, 24} = 14.84$, $p < 0.0001$ for hippocampus and ANOVA: $F_{3, 24} = 11.69$, $p < 0.0001$ for cerebral cortex).

CAT activity

Figure 10A and B illustrate the effects of treatments on the CAT activity in hippocampus and cerebral cortex of mice, respectively. Results demonstrated that SCO increased the CAT activity in hippocampus (around 178%) and the cerebral cortex (around 132%) of mice, when compared to the control group. QTCA-1 treatment significantly prevented the increase in the CAT activity in hippocampus and cerebral cortex caused by SCO (Fig. 10A and B, respectively). Treatment only with QTCA-1 did not modify the CAT activity in hippocampus and cerebral cortex of mice (Fig. 10A and B, respectively) (ANOVA: $F_{3, 24} = 4.035$, $p < 0.05$ for hippocampus and ANOVA: $F_{3, 24} = 4.506$, $p < 0.05$ for cerebral cortex).

Discussion

The present study demonstrated, for the first time, that the administration of QTCA-1 for 9 days mitigated amnesia in SCO-challenged mice. Moreover, QTCA-1 attenuated SCO-induced cholinergic dysfunction, oxidative stress and Na^+/K^+-ATPase activity in hippocampus and cerebral cortex of mice and it did not cause changes in locomotor and exploratory activity in the open-field test. These results may implicate the fact that QTCA-1, could be a potential candidate in the management of AD and other neurological disorders associated with dementia.

Cholinergic dysfunction is one of the responsible factors for amnesia in the pathophysiology of AD (Puri et al. 2014; Kumar et al. 2015). To assess the amnesia in the SCO mouse model, four types of cognitive behavioral tests were conducted. Similar to previous findings, as expected, SCO caused amnesia in mice in terms of changes in step-down inhibitory avoidance, Barnes maze, and object recognition and object location tasks. SCO exhibits amnesia through antagonistic activity on muscarinic receptors in the brain of experimental animals (da Silva et al. 2017; Pahaye et al. 2017).

Blockage of cholinergic neurons by SCO is widely used in the screening of new drugs for the treatment of dementia in neurodegenerative disorders (Chen et al. 2014; Weon et al. 2016; da Silva et al. 2017; Kim et al. 2018). In this context, the same four types of cognitive behavioral tests were performed as screening methods to show anti-amnesic action of QTCA-1. QTCA-1 treatment attenuated spatial learning and reference memory (as shown by reducing the latency to find the escape box and number of holes visited in the Barnes maze task), non-spatial aversive long-term memory (as evidenced by prolonging the latency time in the step-down inhibitory avoidance), LTM (as demonstrated by increasing the exploratory preference for the new object in the object recognition task) and spatial memory (as evidenced by increasing the exploratory preference for the new object location in the object location task) induced by SCO in mice. Our results highlight that QTCA-1 has an anti-amnesic action.

Here, we evaluated the exploratory and locomotory activities of the animals in the open field test for 3 days in order to

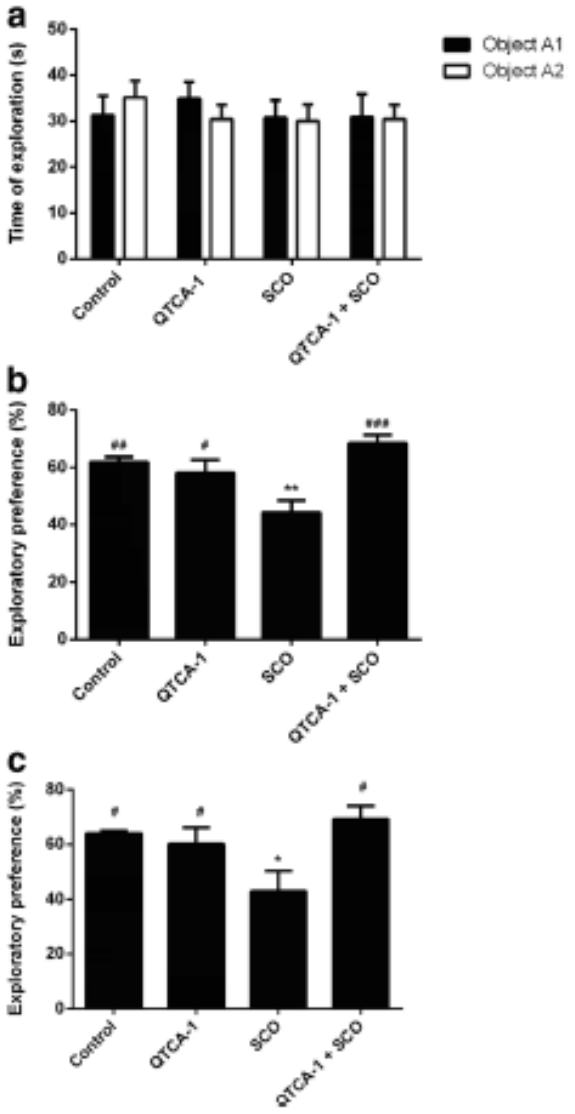

Fig. 5 Effects of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) on scopolamine (SCO)-induced memory deficits in the object recognition task during (5A) training phase, (5B) the long-term (LTM) memories and (5C) location memory. Data are reported as mean ± standard error of the mean (SEM) of seven animals per group. (*) denotes $p < 0.05$ and (**) denotes $p < 0.01$ as compared to the control group; (#) denotes $p < 0.05$, (##) denotes $p < 0.01$ and (###) denotes $p < 0.001$ as compared to the SCO group (one-way analysis of variance/Newman-Keuls test)

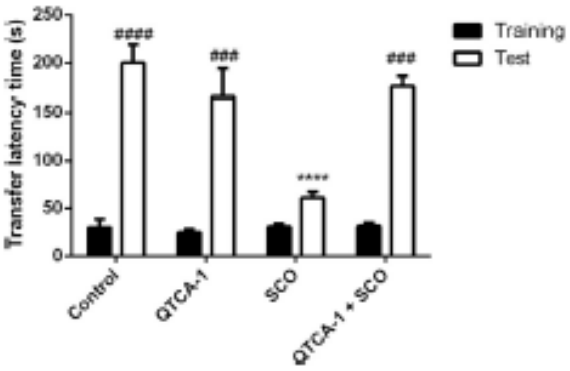

Fig. 6 Effect 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) on scopolamine (SCO)-induced memory deficits in step-down inhibitory avoidance: latency (s) to fall from the platform in the training and test phases. Data are reported as mean ± standard error of the mean (SEM) of seven animals per group. (****) denotes $p < 0.0001$ as compared to the control group; (###) denotes $p < 0.001$ and (####) denotes $p < 0.0001$ as compared to the SCO group (one-way analysis of variance/Newman-Keuls test)

demonstrate that the treatments did not influence in these parameters and to habituate the animals in this environment for the object recognition task. The exploratory and locomotory activities are important for the evaluated tests. Indeed, in the present study the observed effects were not related to the changes in spontaneous locomotor and exploratory activities of mice in the open field test, so treatments were devoid of motor stimulant properties. In addition, the animals presented a good response in the exploratory preference.

Furthermore, SCO induces a cerebral cholinergic dysfunction by decreasing level of ACh and increasing activity of AChE (Guo et al. 2016). Our results showed that SCO increased AChE activity in cerebral cortex and hippocampus of mice. Importantly, QTCA-1 administration protected SCO-induced cholinergic dysfunction in both cerebral structures of animals in the present study. In this context, anticholinesterase effect of QCTA-1 could be a mechanism by which the compound prevented behavioral changes caused by SCO. Indeed, inhibition in the AChE activity is proofed to be an attractive strategy for the treatment of dementia (Parent and Baxter 2004; Kim et al. 2018), given that memory disorders typically exhibit cholinergic deficits (abnormally elevated AChE activity and reduced ACh levels). Therefore, QTCA-1 may proves to be a therapeutic strategy for some neurodegenerative disorders not yet treated, like AD.

In addition to cholinergic dysfunction, the increase in oxidative stress with age is the main risk factor for amnesia in neurodegenerative disorders, mainly AD (Niedzielska et al. 2016; Ferreira-Vieira et al. 2016). The state of brain after SCO injection can be determined by the state of oxidative stress. We determined the redox status of brain by measuring the levels of TBARS and activity of CAT. In the present study, we reported that there was an elevation in the levels of TBARS and in the CAT activity in cerebral cortex and hippocampus of mice caused by SCO. Indeed, SCO has a robust effect on the brain; that is, the redox state imbalance of the cerebral cortex and hippocampus is aggravated (Balaban et al. 2016; Pahaye et al. 2017).

In this context, it is well documented that antioxidants molecules can be promising therapeutic strategies for treating

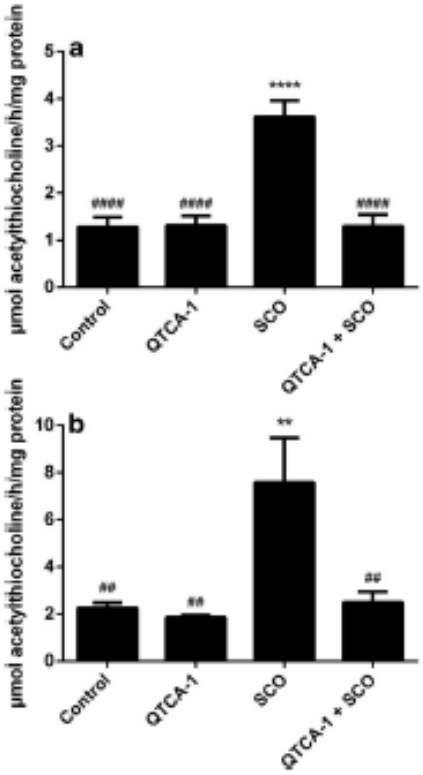

Fig. 7 Effect of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) on acetylcholinesterase (AChE) activity in (7A) hippocampus and (7B) cerebral cortex of mice after induction with scopolamine (SCO). Data are reported as mean ± standard error of the mean (SEM) of seven animals per group. (**) denotes $p < 0.01$, (***) denotes $p < 0.001$ and (****) denotes $p < 0.0001$ as compared to the control group; (##) denotes $p < 0.01$ (###) denotes $p < 0.001$ and (####) denotes $p < 0.0001$ as compared to the SCO group (one-way analysis of variance/Newman-Keuls test)

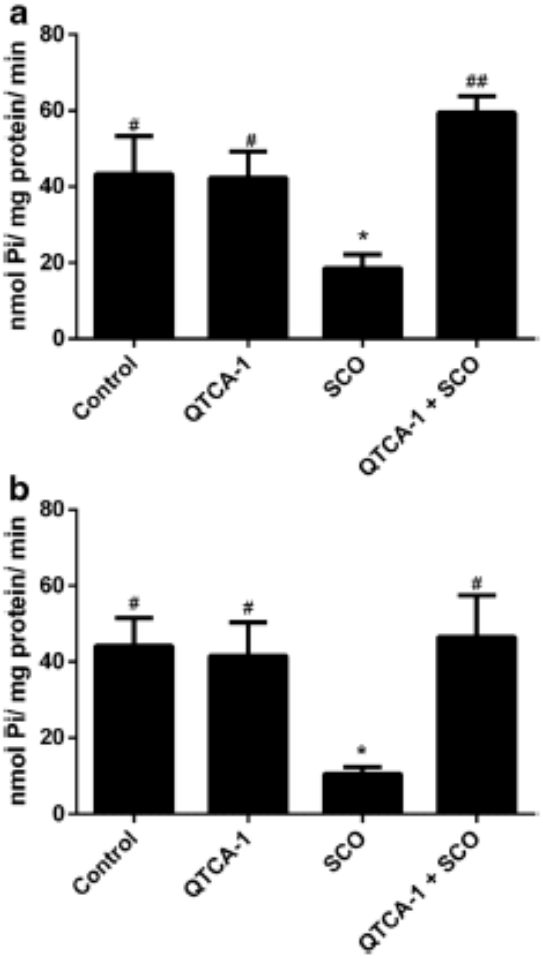

Fig. 8 Effect of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) on Na^+/K^+-ATPase activity in (8A) hippocampus and (8B) cerebral cortex of mice after induction with scopolamine (SCO). Data are reported as mean ± standard error of the mean (SEM) of seven animals per group. (*) denotes $p < 0.05$ as compared to the control group; (#) denotes $p < 0.05$ and (##) denotes $p < 0.01$ as compared to the SCO group (one-way analysis of variance/Newman-Keuls test)

amnesia and dementia in some neurodegenerative disorders (Mattson et al. 1999; Pinz et al. 2018; Sambon et al. 2019; Mazumder et al. 2019). Here, we verified that treatment with QTCA-1 protected against increasing TBARS levels and CAT activity in cerebral structures of mice injected with SCO.

Na^+/K^+-ATPase is an important neuromodulator against AD, given that the deficiency of Na^+/K^+-ATPase causes learning and memory deficits (Zhang et al. 2013). In this study, we found a reduction in the Na^+/K^+-ATPase activity in cerebral cortex and hippocampus of animals treated with SCO. The reduction in the enzyme activity by SCO has a central role in memory process (Gutierres et al. 2014; da Silva et al. 2017) and pathogenesis of neurodegenerative diseases, like AD (Gutierres et al. 2014). Thus, we can relate the decrease in Na^+/K^+-ATPase activity with the amnesia caused by SCO.

In line with this, QTCA-1 protected from the reduction in Na^+/K^+-ATPase activity induced by SCO in cerebral structures of mice. In this way, this finding indicates that anti-amnesic action of compound could be related with protective effect in Na^+/K^+-ATPase activity. In accordance, Ali and Arafa (2011) reported an increase in the Na^+/K^+-ATPase activity by anti-amnesic drugs. Furthermore, antioxidant effect of QTCA-1 could be involved in the beneficial effect of compound on the Na^+/K^+-ATPase activity, because it has been established that oxidative stress modulates memory process in AD through of Na^+/K^+-ATPase activity (Zhang et al. 2013).

In this sense, this study demonstrated that QTCA-1 had anti-amnesic action by protecting the AChE and Na^+/K^+-ATPase

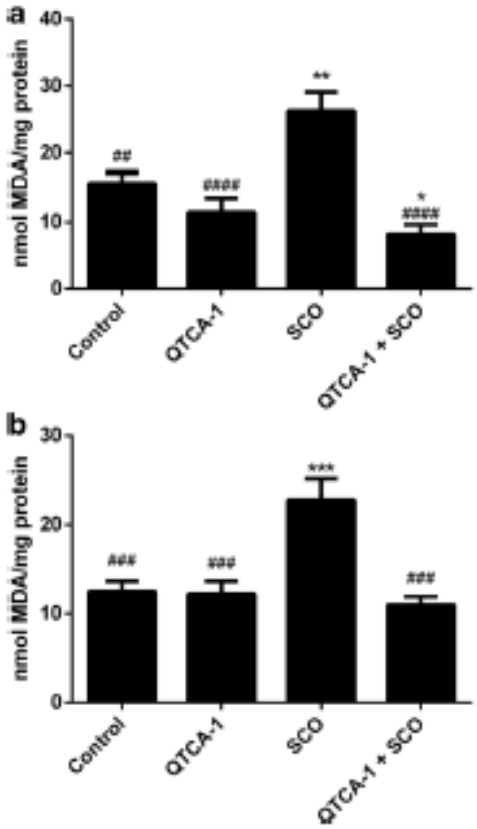

Fig. 9 Effect of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) on thiobarbituric acid reactive species (TBARS) levels in (9A) hippocampus and (9B) cerebral cortex of mice after induction with scopolamine (SCO). Data are reported as mean ± standard error of the mean (SEM) of seven animals per group. (*) denotes $p < 0.05$, (**) denotes $p < 0.01$ and (***) denotes $p < 0.001$ as compared to the control group, (##) denotes $p < 0.01$, (###) denotes $p < 0.001$, and (####) denotes $p < 0.0001$ as compared to the SCO group (one-way analysis of variance/Newman-Keuls test)

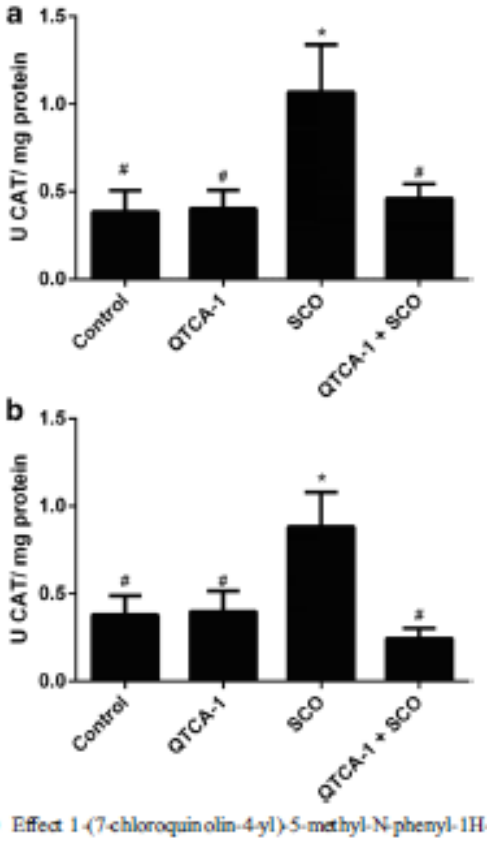

Fig. 10 Effect 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) in the catalase (CAT) activity in (10A) hippocampus and (10B) cerebral cortex of mice after induction with scopolamine (SCO). Data are reported as mean ± standard error of the mean (SEM) of seven animals per group. (*) denotes $p < 0.05$ as compared to the control group; (#) denotes $p < 0.05$ as compared to the SCO group (one-way analysis of variance/Newman-Keuls test)

activities changed by SCO, and also by its antioxidant effect. Therefore, this compound has been shown to be a pharmacological alternative for the treatment of dementia related to some neurodegenerative disorders, mainly AD. Moreover, more studies are needed to elucidate the other mechanisms of QTCA-1, mainly to deepen its action on the cholinergic system.

Although promising results have been obtained here, it is important to note that one of the limitations of this study is the evaluation of QTCA-1 using only the dose of 10 mg/kg. A dose–response curve could be evaluated, but because it was necessary to reduce the number of animals used in experiments, based on humanitarian principles of animal experimentation or principle of the 3Rs (Russell and Burch 1959), it was not done. In addition, it is important to highlight that due to the small amount of samples of cerebral cortex and hippocampus other assays can not performed. Also, mouse model used is not a disease model with permanent amnesia. Thus, further studies are necessary to evaluate the pharmacological potential of QTCA-1 using different model of induction of amnesia.

Although QTCA-1 exerts an important pharmacological action, the evaluation of toxicological effects of acute and repeated administrations of QTCA-1 should be elucidated in further studies.

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Compliance with ethical standards

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

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CAPÍTULO 3

MANUSCRITO 2

QCTA-1, a quinoline derivative, ameliorates pentylenetetrazole-induced kindling and memory comorbidity in mice: Involvement of antioxidant system of brain.

Manuscrito submetido à revista **Pharmacology Biochemistry and Behavior.**

ANEXO B – Comprovante de submissão do manuscrito**.**

QCTA-1, a quinoline derivative, ameliorates pentylenetetrazole-induced kindling and memory comorbidity in mice: Involvement of antioxidant system of brain.

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Highlights

- QCTA-1 may be a promising tool for the treatment of epileptogenesis.
- QTCA-1 may be a promising tool for the treatment epilepsy-associated comorbidity (memory impairment).
- QCTA-1 to prevent alterations by PTZ-kindling model may involve the reduction of oxidative stress.
- QCTA-1 to prevent alterations by PTZ-kindling model may involve the normalization of Na^+/K^+-ATPase activity.

Abstract

The present study evaluated the therapeutic effect of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) in seizure severity, oxidative stress, and memory disorder in a pentylenetetrazol (PTZ)-kindling model in mice. Male Swiss mice were treated with QTCA-1 (10 mg/kg, intragastrically (i.g.)) or phenobarbital (PHEN) (10 mg/kg; i.g.), thirty minutes before the injection of PTZ (35 mg/kg, intraperitoneally (i.p.)). Treatments with QCTA-1 or PHEN and PTZ were performed once every 48 h (on the 1st, 3rd, 5th, 7th, 9th and 11th days). After each PTZ injection, the animals were observed for 30 min to assess the stage of seizure intensity. Behavioral parameters were evaluated from the 12th day until the 16th day of the experimental protocol. On the 16th day, mice were euthanized, and the cerebral cortex and hippocampus of mice were removed to determine the thiobarbituric acid reactive species (TBARS) and reactive species (RS) levels, and superoxide dismutase (SOD), Na^+/K^+-ATPase and acetylcholinesterase (AChE) activities. Our results demonstrated that QTCA-1 significantly decreased the seizure stage score in PTZ-kindled mice. QCTA-1 protected against memory impairment induced by PTZ. QTCA-1 normalized oxidative stress and Na^+/K^+-ATPase activity in the cerebral structures of PTZ-kindled mice. The effect of QTCA-1 treatment was similar to the positive control used in this study (PHEN). AChE activity did not change in the cerebral structures in PTZ- kindling mice. In conclusion, that QCTA-1 may be a promising tool for the treatment of epileptogenesis and epilepsy-associated comorbidity (memory impairment). QCTA-1 to prevent these alterations may involve the reduction of oxidative stress and normalization of Na^+/K^+-ATPase activity.

Keywords: Seizure, Kindling, Memory impairment, Quinoline, Oxidative stress.

1. Introduction

Epilepsy is a life-shortening progressive chronic neurological disorder, affecting around 50 million people worldwide (WHO, 2019), being characterized by recurrent seizures (Fisher et al., 2014). However, the cause of epilepsy in many patients is unknown, seizures can be the result of almost any insult that disturbs brain function. These insults include acquired causes, for instance after stroke or traumatic brain injury, autoimmune and infectious diseases, or genetic mutations (Devinsky, 2018). Moreover, dysfunctions in the chemical balance of neurotransmitters may be the main cause of development and maintenance of epileptiform electrical activity (Janet Robertson, 2015).

Although seizures are the primary symptom, they are not the only aspect of epilepsy that affects a patient's quality of life. Several comorbities, such as cognitive and memory deficits, also contribute to diminish quality of life, in addition life quality to the poor prognosis associated with the disease (Aldenkamp and Arends, 2004; Dodrill, 2004) (England et al., 2012; Fisher et al., 2014). In this way, several memory complaints and deficits are commonly seen in patients with epilepsy, where memory-related brain structures are directly affected by seizures (Tramoni-Negre et al., 2017).

Some epileptic patients are considered pharmacoresistant to the currently available anti-seizure drugs, while a considerable proportion of patients have seizure relapses during the treatment (Laxer et al., 2014). Epilepsy treatment remains a challenge since there are high rates of refractoriness to current therapy. Furthermore, currently available anti-seizure drugs are also known to produce a variety of adverse cognitive, psychiatric and motor effects (George et al., 2015; Zaccara et al., 2004) (Tian et al., 2018). In this context, the study of new promising drugs for the treatment of

epilepsy and that do not carry the potential to increase the adverse effects liability is extremely important.

In this sense, quinoline derivatives are important classes of bioactive heterocyclic compounds in the pharmaceutical field, and posses diverse pharmacological properties (Upadhyay et al., 2018; Wang et al., 2018; Zhang et al., 2018). Our research group has dedicated attention to studying the effect of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1), a quinoline derivative, in neurologic disorders (Luchese et al., 2020; Wilhelm et al., 2014). In fact, QTCA-1 showed anti- amnesic (Luchese et al., 2020), anticonvulsant (Wilhelm et al., 2014) and anti-inflammatory (Wilhelm et al., 2014) properties.

Thus, this study aimed at investigating the therapeutic effect of QTCA-1 in seizure severity, oxidative stress, and memory disorder in pentylenetetrazol (PTZ)-kindling model in mice. Additionally, we determined the possible role of Na^+/K^+-ATPase and AChE activities in cerebral structures of mice.

2. Materials and methods

2.1. Chemicals

QTCA-1 (Fig. 1) was prepared according to the method in literature (Wilhelm et al., 2014). Analysis of the ^1H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of QTCA-1 (99.9%) was determined by gas chromatography-mass spectrometry (GC/MS). QTCA-1 was dissolved in canola oil. Phenobarbital (PHEN) **was** purchased from Sanofi (standard commercial). PTZ was purchased from Sigma Chemical Co. (St Louis, Missouri, USA). PTZ and PHEN were dissolved in saline

0.9%. All other chemicals were of analytical grade and obtained from standard commercial suppliers.

2.2. Animals and ethical approval

Male adult Swiss mice (25–35 g) from a local breeding colony were used. Animals were kept in a separate room, on a 12 h light/dark cycle, at a temperature of 22 ± 2°C, with free access to food and water. All animal experiments were approved by the Committee on Care and Use of Experimental Animal Resources of the Federal University of Pelotas, Brazil (CEEA number 5445-2017) and they are in accordance with the guide of Brazilian National Animal Care Ethical Council (CONCEA), which is based on the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (Publication no. 85–23, revised 1985). Every effort was made to minimize the number of animals used and their discomfort.

2.3. Experimental protocol

Mice were randomly divided into 5 experimental groups (7 animals/group): Group I (control) received canola oil and saline; group II (QTCA-1) received QTCA-1 and saline; group III (PTZ) received canola oil and PTZ; group IV (QTCA-1 + PTZ) received QTCA-1 and PTZ; and group V (PHEN + PTZ) received PHEN and PTZ. Mice received canola oil (10 ml/kg, intragastrically (i.g.) via gavage) or QTCA-1 (10 mg/kg, i.g. via gavage) or PHEN (10 mg/kg; i.g., via gavage) thirty minutes before the injection of PTZ (35 mg/kg, intraperitoneally (i.p.)) or saline (0.9%, 5 ml/kg, i.p.). The PTZ dose chosen is considered sub-convulsant and it was based on previous studies (Abdel-Zaher et al., 2017). Treatments with canola oil/QCTA-1/PHEN or PTZ/saline were performed once every 48 h on the 1st, 3rd, 5th , 7th, 9th and 11th days (Fig. 2). After each PTZ injection, the animals were observed for 30 min to assess the stage of seizure

intensity (Fig 2). Mice behavior was classified according to the Racine scale (Racine et al., 1972), modified by (Itzhak and Martin, 2000), that define: stage 1, normal behavior; stage 2, hyperactivity; stage 3, repeated 'vertical' movements which may represent stereotypical-like behavior; stage 4, forelimb clonus and rearing; and stage 5, generalized clonic-tonic seizures with fall. Behavioral parameters were evaluated from the 12th day, which corresponds to 24 h after the last treatment with canola oil/QCTA-1/PHEN or PTZ/saline (Fig 2). On the 16th day, animals were euthanized, and the cerebral cortices and hippocampi of mice were removed for *ex vivo* experiments (Fig. 2).

2.4. Behavioral tests

2.4.1. Open-field test

The place for the open-field test was made of plywood and surrounded by 30 cm high walls. The floor of the open-field (40 cm length × 40 cm width) was divided into 9 squares (3 rows of 3). Each animal was placed individually at the center of the apparatus and observed for 4 min to record the locomotor (number of segments crossed with the four paws) and exploratory activities (expressed by the number of times it reared on the hind limbs) (Walsh and Cummins, 1976). The open-field test was performed on the 12th day of the experimental protocol.

2.4.2. Object recognition task (ORT)

ORT was used to assess the short-term (STM) and long-term (LTM) memories of mice (Stangherlin et al., 2009). ORT was performed in an open-field apparatus. On the 12th day of experimental protocol, each animal was submitted to a habituation session, in the absence of objects, for 5 min. Subsequently, four objects were used: A1, A2, B and C. Each object had the following color pattern: blue, red and yellow. All

objects were made of plastic, measuring 10 × 10 cm (length x height). During the training session (on the 12th day of the experimental protocol), animals were placed (into an arena containing two identical objects (objects A1 and A2) for 5 min. Exploration was defined when the animal directed its nose within 2 cm of the object while looking, sniffing, or touching it. The STM (on the 12th day of the experimental protocol) of mice was evaluated 1.5 h after the training session in the presence of a familiar object (A1) and a new object (B). Total time spent in exploring each object was determined during 5 min to measure the learning and recognition memory. The LTM was performed 24 h after training section, on the 13th day of the experimental protocol. Mice were placed in the testing box to explore a familiar object (A1) and a new object (C) for 5 min and the total time spent in exploring each object was determined. In order to analyze the cognitive performance, the exploratory preference was calculated and data were expressed as percentage as follows: Training = (A2 / (A1 + A2)) × 100; STM= (B / (A1 + B)) × 100; LTM= (C / (A1 + C)) × 100).

2.4.3. Y-maze task

The Y-maze task was used as a measure of working memory, being performed as described by (Sarter et al., 1988). The Y-maze apparatus consisted of a three-arm horizontal maze (40 cm long and 3 cm wide with walls 12 cm high), in which the three arms at 120° angles to each other, radiated out from a central point. The Y-maze task was performed on the 14th day of the experimental protocol. Mice were initially placed within one arm (A), and the arm entry sequence (e.g. ABCCAB, where letters indicate arm codes) and the number of arm entries were recorded manually for each mouse over an 8 min period. Alternation was determined from successive entries into the three arms on overlapping triplet sets in which three different arms are entered. An actual alternation was defined as entries into all three arms consecutively (i.e. ABC, CAB or

BCA but not BAB). An entry was defined as placing all four paws within the boundaries of the arm.

2.4.4. Step-down inhibitory avoidance

The step -down inhibitory avoidance task was performed to evaluate aversive and non-spatial LTM (Sakaguchi et al., 2006). Animals were trained on step-down inhibitory avoidance on the 15th day of the experimental protocol. During the training session, each mouse was placed on the platform. When it stepped down and placed its four paws on the grid floor, an electric shock (0.5mA) was delivered for 2 s. The test was performed 24 h after the training session (on the 16th day) (Fig 2). Each mouse was placed again on the platform and the step-down transfer latency time was recorded, but mice no received the aversive stimulus. The maximum transfer latency time was 300 s.

2.5. Ex vivo assays

On the 16th day of the experimental protocol, mice were anesthetized with isoflurane and cerebral structures (cerebral cortices and hippocampus) were immediately removed. Cerebral cortices and hippocampus were separated and washed with cold saline solution (0.9%) to determine the thiobarbituric acid reactive species (TBARS) and reactive species (RS) levels, as well as, superoxide dismutase (SOD), Na^+/K^+-ATPase and acetylcholinesterase (AChE) activities.

Cerebral structures were homogenized in cold 50 mM Tris-HCl, pH 7.4 (1/10, weight (w)/volume (v)), centrifuged at 900 *x*g at 4°C for 10 min and supernatants were used to determine the enzyme activities (Na^+/K^+-ATPase and SOD), TBARS content and RS levels. Also, cerebral structures of mice were homogenized in 0.25 M sucrose buffer (1/10, w/v), centrifuged at 900 *x*g at 4°C for 10 min and supernatants were used to determine AchE activity.

2.5.1. TBARS levels

TBARS levels were determined as described by (Ohkawa et al., 1979) and used as lipid peroxidation measure. An aliquot of supernatant was added to the reaction mixture containing: 8.1% sodium dodecyl sulfate (SDS), 0.8% thiobarbituric acid and acetic acid buffer (pH 3.4). The system was incubated at 95°C for 2 h. Absorbance was measured at 532 nm Results were reported as nmol malondialdehyde (MDA)/mg protein.

2.5.2. RS Levels

The RS levels were determined with a spectrofluorimetric method, with a 2′,7′-dichlorofluorescein diacetate (DCHFDA) assay according to (Loetchutinat et al., 2005). Supernatant was incubated with DCHF-DA (1 mmol/L) and Tris-HCl (10 mmol/L) (pH 7.4). The oxidation of DCHF-DA to fluorescent dichlorofluorescein (DCF) was measured for the detection of intracellular RS. The DCF fluorescence intensity emission was recorded at 525 nm (with 488 nm excitation), 60 min after the addition of DCHF-DA to the medium (Shimadzu RF-5301PC fluorometer). RS levels were expressed as arbitrary units of fluorescence.

2.5.3. SOD activity

This method is based on the capacity of SOD to inhibit autoxidation of epinephrine. SOD activity was measured spectrophotometrically according to (Misra and Fridovich, 1972). Supernatant was added to a 0.05 mol/L Na_2CO_3 buffer, and the enzymatic reaction was started by adding epinephrine. The color reaction was measured at 480 nm. One unit of enzyme was defined as the amount of enzyme required to inhibit

the rate of epinephrine autoxidation by 50% at 26°C. The enzymatic activity was expressed as U/ mg protein.

2.5.4. Na^+/K^+-ATPase activity

Supernatant was mixed with 3 mM MgCl, 125 mM NaCl, 20 mM KCl and 50 mM Tris-HCl, pH 7.4. The reaction was initiated by the addition of 3 mM adenosine triphosphate (ATP). Control sampling was performed under the same conditions with the addition of ouabain.. Cerebral cortex and hippocampus protein extracts taken for the assay were 8 and 7 mg/ml, respectively. The samples were incubated at 37°C for 30 min and incubation was stopped by adding trichloroacetic acid (10%) with 10 mM $HgCl_2$. Na^+,K^+-ATPase activity was calculated by the difference between the two assays. Released inorganic phosphate (Pi) was measured according to the method described by (Fiske and Subbarow, 1925). Enzyme activity was expressed as nmol Pi/mg protein/min.

2.5.5. AChE activity

The activity of AChE was determined using a modified method by (Ellman et al., 1961), with acetylthiocholine as substrate. An aliquot of the supernatant was pre-incubated for 2 min at 25°C in a medium containing 100 mM potassium phosphate buffer, pH 7.5. Enzymatic reaction was initiated by adding DTNB (final concentration of 0.5 mM) and acetylthiocholine (final concentration of 0.8 mM). The rate of hydrolysis of acetylthiocholine iodide was measured at 412 nm through the release of the thiol compound, which when reacted with DTNB produces the color-forming compound thionitrobenzene (TNB). Results were expressed as μmol acetylthiocholine/h/mg protein.

2.5.6. Protein quantification

The protein concentration was measured by the method of (Bradford, 1976), using bovine serum albumin as the standard.

2.6. Statistical analysis

Seizure stage score was analyzed by two-way ANOVA followed by Fisher's LSD test with "day" as within subjects' factor and treatment as between subjects' factor. Data from behavioral tests and *ex vivo* assays are expressed as means ± standard error of the mean (SEM). The normality of data was evaluated by the D'Agostino and Pearson omnibus normality test. Statistical analysis was performed using one-way ANOVA followed by the Tukey multiple comparisons test. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Effect of QTCA-1 on the seizure stage score in mice submitted to PTZ-induced kindling

Two-way ANOVA revealed a significant main effect of "treatments" (ANOVA $F_{4,180} = 31.36$, $p < 0.0001$). Repeated treatment of mice with PTZ increased the seizure score from day 1 to 11 of the experimental protocol (around 72% for day 1, around 86% for day 3, around 186% for day 5, 100% for day 7, around 143 % for day 9, around 214 % for day 11), when compared with the control group (Fig. 3). Treatment with QCTA-1 and PHEN reached stage 1 seizures on every analyzed day, attenuating the seizure score induced by PTZ (Fig. 3). QTCA-1 alone did not change the seizure score, when compared to the control group (Fig. 3). None of the animals treated died in the protocol (data not shown).

3.2. Effect of QCTA-1 on the locomotor and exploratory activities, and memory deficit in mice submitted to PTZ-induced kindling

3.2.1. Open-field test

Fig. 3 illustrates the effects of treatments on the number of crossings (Fig. 4A) and rearing (Fig. 4B) of mice in the open-field test. No changes were demonstrated in the number of crossings (ANOVA: $F_{4,30} = 0.3658$, $p > 0.05$) and rearings (ANOVA: $F_{4,30} = 0.6243$, $p > 0.05$) after treatments (Fig.s 4A and B, respectively).

3.2.2. ORT

In the training phase of ORT, there was no difference in the exploratory preference of objects among groups (ANOVA: $F_{4,30} = 0.4602$, $p > 0.05$) (Fig. 5A). In the probe test, PTZ reduced the exploratory preference of the new object in the STM (around 54%) (Fig. 5B) and LTM (around 34%) (Fig. 5C), when compared with the control group. Treatment with QTCA-1 was able to attenuate this reduction in the STM (Fig. 5B) and LTM (Fig. 5C), similarly to PHEN (positive control). QTCA-1 alone did not change the exploratory preference for the new object in STM and LTM (Fig. 5B and 5C, respectively) (ANOVA: $F_{4,30} = 47.97$, $p < 0.0001$ for STM, ANOVA: $F_{4,30} = 18.13$, $p < 0.0001$ for LTM).

3.2.3. Y-maze task

Figure 6 demonstrates the effects of treatments in the Y- maze task**.** PTZ (around 25%) the spontaneous alternation behavior, when compared with the control group (Fig. 6A). QTCA-1 attenuated the reduction of spontaneous alternation behavior, when compared with the PTZ group, similarly to PHEN (positive control) (Fig. 6A). QTCA-1 alone did not change the spontaneous alternation behavior of mice (Fig. 6A). (ANOVA: $F_{4,30} = 7.051$, $p < 0.001$).

The treatments did not cause any significant change in the number of arm entries in the Y-maze task (ANOVA $F_{4,30} = 1.639$, $p > 0.05$) (Fig. 6B).

3.2.4. Step-down inhibitory avoidance

In the training phase of step-down inhibitory avoidance, there was no difference in the transfer latency time among groups (ANOVA: $F_{4, 30} = 0.05297$, $p > 0.05$) (data not shown). In the test phase, PTZ decreased (around 56%) the transfer latency time, when compared with the control group (Fig. 7). QTCA-1 significantly prevented this reduction, similarly to PHEN (positive control) (Fig. 7). Mice treated only with QTCA-1 did not change the transfer latency time in the test phase (Fig. 7). (ANOVA: $F_{4,30} = 23.15$, $p < 0.0001$).

3.3. Effect of QCTA-1 in parameters of oxidative stress in cerebral structures of mice submitted to PTZ-induced kindling

3.3.1. TBARS levels

PTZ increased the TBARS levels in the cerebral cortex (around 144%) and in the hippocampus (around 127%) of mice, when compared with the control group (Fig. 8A and 8B, respectively). QTCA-1 treatment normalized the increase caused by PTZ in cerebral cortex and hippocampus, similarly to PHEN (positive control) (Fig. 8A and 8B, respectively) QTCA-1 alone did not change the TBARS levels in the cerebral cortex and hippocampus (Fig. 8A and 8B, respectively). (ANOVA: $F_{4, 30} = 27.41$, $p < 0.0001$ for cerebral cortex and ANOVA: $F_{4, 30} = 9.934$, $p < 0.001$ for hippocampus).

3.3.2. RS Levels

An increase in the levels of RS was detected in the cerebral cortex (48%) and hippocampus (50%) of mice after administration of PTZ, when compared with the control group (Fig. 9A and 9B, respectively). QTCA-1 treatment normalized the increase caused by PTZ in the cerebral cortex and hippocampus, similarly to PHEN (positive control) (Fig. 9A and 9B, respectively). QTCA-1 alone did not change the RS levels in the cerebral structures (Fig. 9A for cerebral cortex and 9B for hippocampus). (ANOVA: $F_{4, 30} = 6.193$, $p < 0.01$ for cerebral cortex; ANOVA: $F_{4, 30} = 15.66$, $p < 0.0001$ for hippocampus).

3.3.3. SOD activity

As shown in Fig. 10, PTZ administration increased the SOD activity in the cerebral cortex (42%) (Fig. 10A) and in the hippocampus (100%) (Fig. 10B) of mice, when compared with the control group. QTCA-1 treatment normalized the increase in SOD activity caused by PTZ in cerebral structures, similarly to PHEN (positive control) (Fig. 10A for cerebral cortex and 10B for hippocampus). QTCA-1 alone did not change the SOD activity in the cerebral cortex and hippocampus (Fig. 10A and 10B, respectively). (ANOVA: $F_{4,30} = 12.62$, $p< 0.0001$ for cerebral cortex; ANOVA: $F_{4,30} = 14.73$, < 0.0001 for hippocampus).

3.4. Effect of QCTA-1 in Na^+/K^+- ATPase activity in the cerebral structures of mice submitted to PTZ-induced kindling

Figure 11 shows that PTZ inhibited Na^+/K^+ ATPase activity in the cerebral cortex (around 41%) (Fig. 11A) and hippocampus (around 50%) (Fig. 11B) of mice, when compared with the control group. QTCA-1 treatment significantly normalized the inhibition of Na^+/K^+-ATPase activity caused by PTZ in both cerebral structures of mice

Fig. 11A for cerebral cortex and 11B for hippocampus), similarly to PHEN (positive control). Treatment only with QTCA-1 did not modify the Na^+/K^+-ATPase activity in the cerebral cortex and hippocampus of mice (Fig. 11A and 11B, respectively). (ANOVA: $F_{4, 30} = 20.73$, $p < 0.0001$ for cerebral cortex and ANOVA: $F_{4, 30} = 16.28$, $p < 0.0001$ for hippocampus).

3.5. Effect of QCTA-1 in AChE activity in cerebral structures of mice submitted to PTZ-induced kindling

AChE activity in the cerebral cortices (ANOVA: $F_{4, 30} = 2.493$, $p > 0.05$) and in the hippocampus (ANOVA: $F_{4, 30} = 0.7320$, $p > 0.05$) of mice were not altered by treatments (Fig. 12A and 12B, respectively).

4. Discussion

In this study, we demonstrated that QTCA-1 attenuated the epileptogenesis and memory deficit induced by PTZ-kindling. Moreover, QTCA-1 normalized oxidative stress and Na^+/K^+-ATPase activity in the cerebral cortex and hippocampus of kindled mice. These results suggest that QTCA- 1 is a promising tool for the management of seizure and epilepsy-related memory comorbities.

Kindling has been widely used as a model for studying epilepsy, since it resembles the progressive psychiatric and neurological changes that accompany epilepsy (Mishra and Goel, 2012). In chemical kindling, repeated exposure to sub-convulsive doses of a pro-convulsant agent leads to an increase in seizure activity,, resulting in generalized seizures (Gupta et al., 2003). PTZ-induced kindling is a model is one model widely used for the screening of anticonvulsant/anti-kindling drugs (Kola et al., 2017; Tahmasebi et al., 2020; Tawfik et al., 2018). In the current study, we confirmed that PTZ caused a kindling model increasing the seizure stage score of mice

during the evaluated time interval. PTZ is a non-competitive GABA-A antagonist, but in the kindling protocol, it causes a broad dysfunction in glutamatergic transmission, inducing the behavioral changes characteristic of epilepsy (Asadi-Shekaari et al., 2014; Zhu et al., 2016).

An important finding of the present study was that QTCA-1 significantly decreased seizure severity in PTZ-kindled mice, as demonstrated by reduction in the seizure stage score. Previously, (Wilhelm et al., 2014) showed that a single dose 100 mg/kg of QCTA-1 presented anticonvulsant action in young rats by abolishing seizures and death induced by PTZ. Moreover, the effect in the seizure stage score was similar to PHEN, a positive control used in this study. PHEN is an anticonvulsant drug originating in barbiturate, which potentiates the GABA pathway in synapses, and also antagonizes the glutamatergic pathway, acting on the central nervous system (CNS). In developing countries, PHEN is a drug indicated by the World Health Organization (WHO) as a first line treatment for epilepsy due to low cost (Blumstein and Friedman, 2007).

PTZ kindling induces behavioral alterations in rodents such as memory impairment, revealing that this animal model also mimics comorbidities of epilepsy (Abdel-Zaher et al., 2017; Kaur et al., 2016; Kaur et al., 2015; Mishra and Goel, 2012; Taiwe et al., 2015; Zhao et al., 2014; Zhen et al., 2014). As expected, our results demonstrated that PTZ caused a loss of working memory, STM, LTM, and aversive and non-spatial LTM, which were evaluated in different behavioral tests. Importantly, the main finding of this study was that QCTA-1 protected against memory impairment induced by PTZ in mice, and the beneficial effects were in all types of memory studied. Accordingly, previously, we demonstrated that QTCA-1 mitigated amnesia in SCO-challenged mice, with anti-amnesic action resulting from different mechanisms

(Luchese et al., 2020). Importantly, our results showed that QTCA-1 treatment was able to attenuate the behavior changes similarly to a positive control used in this study (PHEN). In addition, none of the treatments caused changes in the spontaneous locomotor and exploratory activities of mice, indicating, mainly, that the effect of QTCA-1 is not due to nonspecific changes, such as psychostimulant or sedative activities. Hence, this is an important result since psychostimulant and sedative drugs may give a false positive result in animal models (Cryan and Holmes, 2005; Ramos, 2008).

In order to clarify the mechanism by which QTCA-1 exerts an effect on attenuated behavioral changes caused by PTZ, we investigated the possible involvement of oxidative stress in the cerebral cortex and hippocampus of mice. We verified that PTZ caused oxidative stress in cerebral structures of kindled mice, as evidenced by increased lipid peroxidation, RS content and SOD activity. There is increasing evidence suggesting that oxidative stress plays a key role in the development and progression of kindling through increasing the brain vulnerability to free radicals by elevating the oxidative metabolism and low antioxidant enzyme levels (Agarwal et al., 2011; Dhir et al., 2007). We can infer that an increase in RS levels induced by PTZ-kindling favors the process of lipid peroxidation and changes in antioxidant defenses in cerebral structures. Moreover, oxidative stress also plays a key role in the pathophysiology of epileptogenesis and its associated comorbities (Gupta et al., 2003) , and we also evidenced it.

In the present study, oxidative changes induced by PTZ-kindling were reversed by QTCA-1, as evidenced by normalization of the RS content, lipid peroxidation and activity of the SOD antioxidant enzyme, with an action similar to PHEN (positive control). Our results indicate the additional benefit of QTCA-1 to the restoration of

brain oxidative homeostasis. Indeed, we have previously demonstrated that QTCA-1 modulated oxidative stress in an amnesia model induced by SCO in mice (Luchese et al., 2020). However, we cannot say whether QTCA-1 acts directly in the redox state or whether it modulates other pathways/systems, reducing the process of cerebral oxidative stress induced by PTZ-kindling. We can assume that QTCA-1 has a beneficial effect against behavioral changes caused by PTZ-kindling through the modulation of oxidative stress. In addition, some studies indicated the protective effect of antioxidant molecules in the treatment of seizures (Drion et al., 2018; Júnior et al., 2018; Kola et al., 2017; Moezi et al., 2019).

Complementarily, we investigated the Na^+/K^+-ATPase enzyme aiming to expand the action mechanisms involved in effects already demonstrated by the QCTA-1. This study demonstrated that PTZ-induced kindling inhibited the Na^+/K^+-ATPase activity in the cerebral cortex and hippocampus of mice. Na^+/K^+-ATPase is a marker of neurogenesis and an important neuromodulator, since the deficiency/inhibition in enzyme has been described by causing learning and memory deficits (Zhang et al., 2013). Moreover, studies showed an inhibition in the Na^+/K^+-ATPase activity in the brain of rodents after acute seizures induced by PTZ (Marquezan et al., 2013; Oliveira et al., 2004).

As regards inhibition in the Na^+/K^+-ATPase activity by PTZ-kindling, while it may directly inhibit enzyme activity, it is also possible that indirect mechanisms may account for it, such as the production of reactive species, which cause oxidative stress and enzyme damage. In fact, the present study revealed that PTZ- kindling caused a decrease in Na^+/K^+-ATPase activity with a concomitant increase in the levels of oxidative stress markers (TBARS, RS, SOD). Our results also revealed that the PTZ-induced kindling caused different seizure levels assessed on Racine's scale, suggesting

that an alteration in the lipid oxidation, increased RS levels together with increased SOD and decreased Na^+/K^+-ATPase activity may be correlated with seizure development. In fact, the results presented in this report showed that QTCA-1 is a powerful antioxidant in the CNS (Luchese et al., 2020), which was effective in preventing behavioral seizures induced by PTZ in this model of epilepsy.

The administration of this compound also protected against the increase in the levels of oxidative stress markers (RS, TBARS and SOD) and Na^+/K^+-ATPase activity inhibition. These experimental findings suggest that a dysregulation of both inhibitory and excitatory neurotransmission following seizures is responsive to antioxidant treatment. Besides that, previous studies demonstrated that the injection of PTZ-induced convulsive activity is accompanied by reactive species generation and inhibition of local Na^+/K^+- ATPase activity (Oliveira et al., 2004; Ribeiro et al., 2005) reinforcing the assumption that the inhibition of some selected targets for free radicals increases cellular excitability (Jamme et al., 1995).

In this context, the QTCA-1 effect was further confirmed through normalization in the Na^+/K^+-ATPase activity in the cerebral cortex and hippocampus of mice, similarly to PHEN (positive control). Stimulators of the Na^+/K^+-ATPase activity could be used as neuromodulator agents against memory impairment, given that this enzyme modulates memory in mice (Moseley et al., 2007; Zhang et al., 2013). In addition, the antioxidant effect of QTCA-1 could be involved in the beneficial effect of compound on the Na^+/K^+-ATPase activity, given that oxidative stress modulates the memory process through Na^+/K^+-ATPase activity (Zhang et al., 2013).

We also investigated the activity of AChE in an attempt to correlate with memory impairment caused by PTZ-kindling. AChE is the major enzyme which appears to contribute to memory (Hampel et al., 2018). However, our results showed no

changes in the AChE activity in the cerebral cortex and hippocampus of mice induced by PTZ-kindling. Thus, in the present study, we believe that oxidative stress is primarily responsible for the characteristic pathogenesis of memory.

The findings of this study are highly important, since using a single drug, QCTA-1, two diseases can be treated, epileptogenesis and its comorbidity, memory impairment. Importantly, QCTA-1 presents a lipophilic nature, which lead us to infer that the brain tissue is targeted by this compound for action. Thus, our results are in agreement with (Wilhelm et al., 2014) that the QCTA-1 may modulate brain processes.

In conclusion, our results evidenced that QCTA-1 may be a promising tool for the treatment of epileptogenesis and epilepsy-associated comorbidity (memory impairment). The mechanism of QCTA-1 to prevent these alterations may involve the reduction of oxidative stress and normalization of Na^+/K^+-ATPase activity. We are motivated to continue investigating the potential of this compound in search of new mechanisms linked to its effect.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

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Legend of Figures

Fig. 1- Chemical structure of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1).

Fig. 2- Scheme of experimental protocol. Thirty minutes after initiating intragastric (i.g.) treatments, mice received pentylenetetrazole (PTZ) or saline, both intraperitoneally (i.p.). I.g. and i.p. treatments were performed once every alternate day,

(1th, 3rd, 5th, 7th, 9th and 11th days) and seizure was evaluated. From the 12th day of the experimental protocol, the animals were submitted to the open-field, object recognition, Y-maze and step-down inhibitory avoidance tasks. On the 16th day, mice were euthanized.

Fig. 3- Effect 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) and phenobarbital (PHEN) on seizure stage score in mice submitted to pentylenetetrazole (PTZ)-induced kindling. Data are reported as mean ± standard error of the mean (SEM) of seven animals per group. (****) denotes $p < 0.0001$ as compared to the control group; (####) denotes $p < 0.0001$ as compared to the PTZ group (two-way ANOVA followed by Fisher's LSD test).

Fig. 4- Effect of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) and phenobarbital (PHEN) on the (3A) number of crossings and (3B) number of rearings in the open-field test in mice submitted to pentylenetetrazole (PTZ)-induced kindling. Data are reported as mean ± standard error of the mean (SEM) of seven animals per group (one-way ANOVA followed by Tukey's test).

Fig. 5- Effects of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) and phenobarbital (PHEN) on (5A) training, (5B) short-term (STM) and (5C) long-term (LTM) memories in the object recognition task in mice submitted to pentylenetetrazole (PTZ)-induced kindling. Data are reported as mean ± standard error of the mean (SEM) of seven animals per group. (****) denotes $p < 0.0001$ as compared to the control group; (####) denotes $p < 0.0001$ as compared to the PTZ group (one-way ANOVA followed by Tukey's test).

Fig. 6- Effects of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) and phenobarbital (PHEN) on the (5A) spontaneous alternation

behavior and (5B) number of arm entries in the Y- maze task in mice submitted to pentylenetetrazole (PTZ)-induced kindling. Data are reported as mean ± standard error of the mean (SEM) of seven animals per group. (***) denotes $p < 0.001$ as compared to the control group; (##) denotes $p < 0.01$ and (###) denotes $p < 0.001$ as compared to the PTZ group (one-way ANOVA followed by Tukey's test).

Fig. 7- Effects of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) and phenobarbital (PHEN) on the latency (s) to fall from the platform in the test phase in the step-down inhibitory avoidance task in mice submitted to pentylenetetrazole (PTZ)-induced kindling. Data are reported as mean ± standard error of the mean (SEM) of seven animals per group. (****) denotes $p < 0.0001$ as compared to the control group; (####) denotes $p < 0.0001$ as compared to the PTZ group (one-way ANOVA followed by Tukey's test).

Fig. 8- Effects of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) and phenobarbital (PHEN) on thiobarbituric acid reactive species (TBARS) levels in (7A) cerebral cortex and (7B) hippocampus of mice submitted to pentylenetetrazole (PTZ)-induced kindling. Data are reported as mean ± standard error of the mean (SEM) of seven animals per group. (***) denotes $p < 0.001$ and (****) denotes $p < 0.0001$ as compared to the control group; (###) denotes $p< 0.001$ and (####) denotes $p < 0.0001$ as compared to the PTZ group (one-way ANOVA followed by Tukey's test).

Fig. 9- Effects of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) and phenobarbital (PHEN) on reactive species (RS) levels in (8A) cerebral cortex and (8B) hippocampus of mice submitted to pentylenetetrazole (PTZ)-induced kindling. Data are reported as mean ± standard error of the mean (SEM) of seven animals per group. (**) denotes $p < 0.01$ and (****) denotes $p < 0.0001$ as

compared to the control group; (##) denotes p< 0.01 and (####) denotes p < 0.0001 as compared to the PTZ group (one-way ANOVA followed by Tukey's test).

Fig. 10- Effects of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) and phenobarbital (PHEN) effects.on the superoxide dismutase (SOD) activity in (9A) cerebral cortex and (9B) hippocampus of mice submitted to pentylenetetrazole (PTZ)-induced kindling. Data are reported as mean ± standard error of the mean (SEM) of seven animals per group. (**) denotes p < 0.01 and (****) denotes p < 0.0001 as compared to the control group; (##) denotes p< 0.01 and (####) denotes p < 0.0001 as compared to the PTZ group (one-way ANOVA followed by Tukey's test).

Fig. 11- Effects of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) and phenobarbital (PHEN) on Na^+/K^+-ATPase activity in (10A) cerebral cortex and (10B) hippocampus of mice submitted to pentylenetetrazole (PTZ)-induced kindling. Data are reported as mean ± standard error of the mean (SEM) of seven animals per group. (****) denotes p < 0.0001 as compared to the control group; (####) denotes p < 0.0001 as compared to the PTZ group (one-way ANOVA followed by Tukey's test).

Fig. 12- Effects of 1-(7-chloroquinolin-4-yl)-5-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide (QTCA-1) and phenobarbital (PHEN) on acetylcholinesterase (AChE) activity in (11A) cerebral cortex and (11B) hippocampus of mice submitted to pentylenetetrazole (PTZ)-induced kindling. Data are reported as mean ± standard error of the mean (SEM) of seven animals per group (one-way ANOVA followed by Tukey's test).

Figures

Fig. 1.

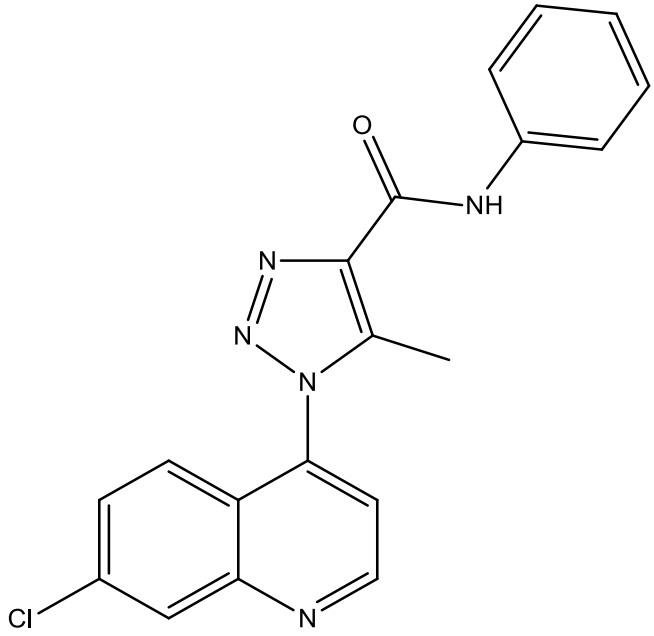


Fig. 2.

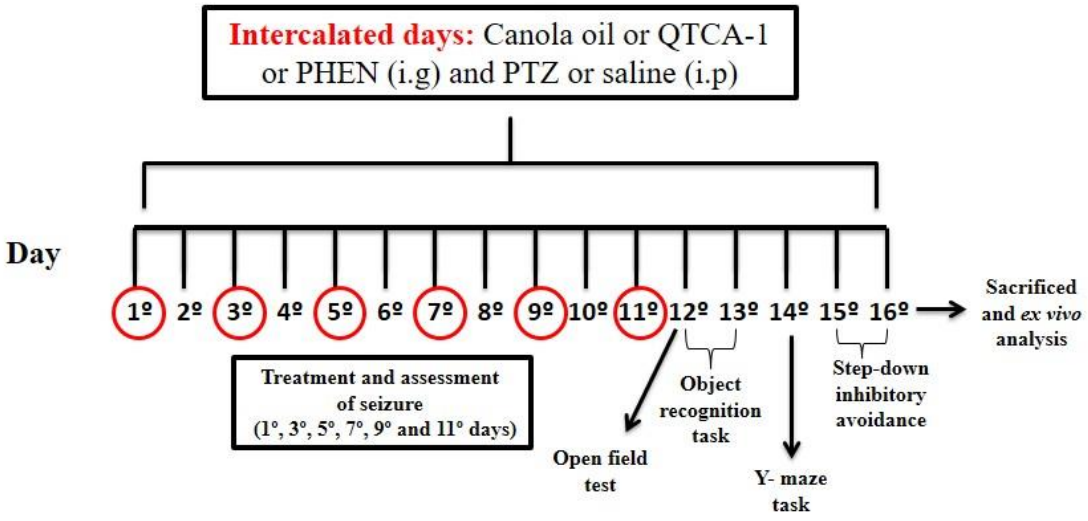

Intercalated days: Canola oil or QTCA-1 or PHEN (i.g) and PTZ or saline (i.p)
Day
1º 2º 3º 4º 5º 6º 7º 8º 9º 10º 11º 12º 13º 14º 15º 16º
Sacrificed and ex vivo analysis
Treatment and assessment of seizure (1°, 3°, 5°, 7°, 9° and 11° days)
Open field test
Object recognition task
Y- maze task
Step-down inhibitory avoidance

Fig. 3.

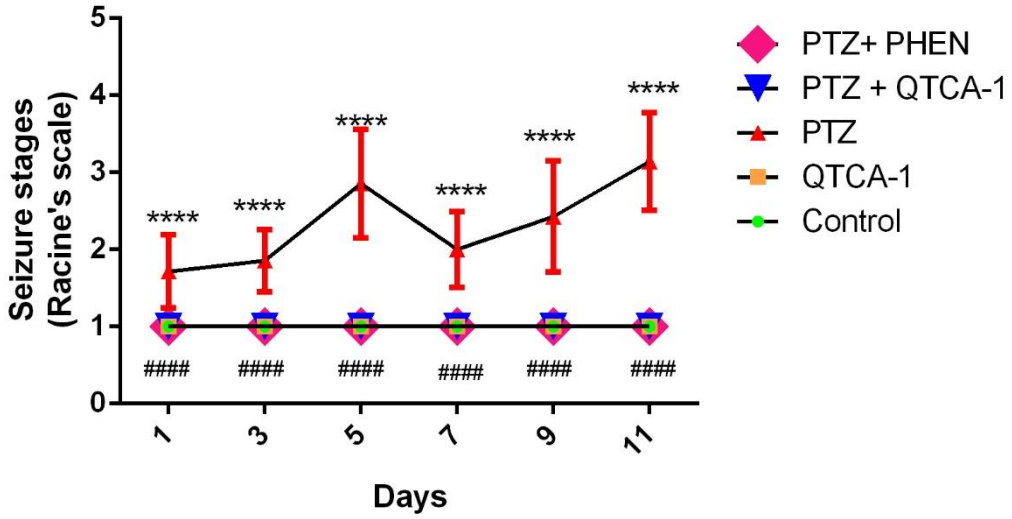

Seizure stages
(Racine's scale)
5
4
3
2
1
0
****
****
****
****
****
****
####
####
####
####
####
####
1
3
5
7
9
11
Days
PTZ+ PHEN
PTZ + QTCA-1
PTZ
QTCA-1
Control

Fig. 4.

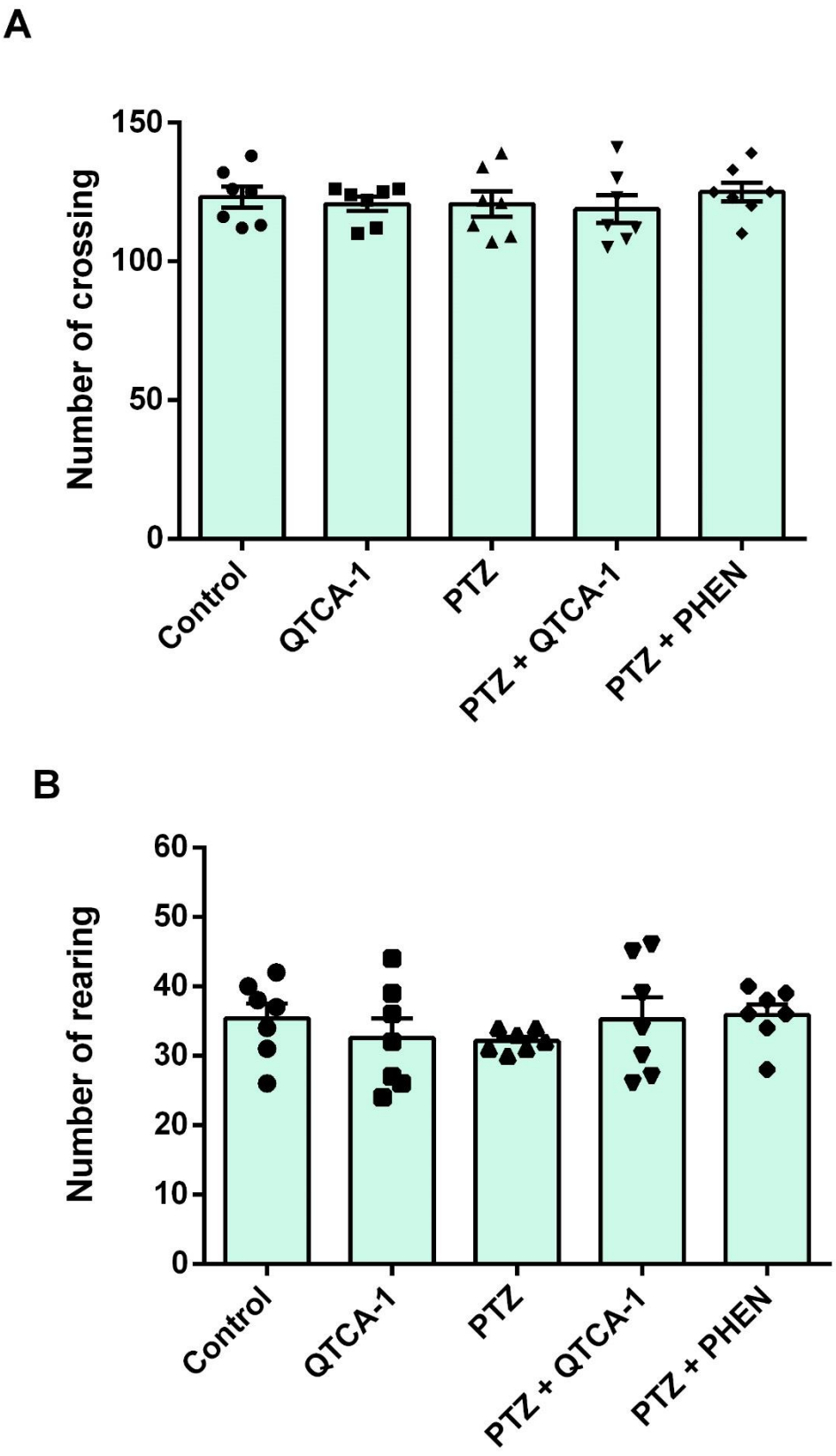

A
Number of crossing
150
100
50
0
Control
QTCA-1
PTZ
PTZ + QTCA-1
PTZ + PHEN
B
Number of rearing
60
50
40
30
20
10
0
Control
QTCA-1
PTZ
PTZ + QTCA-1
PTZ + PHEN

Fig. 5.

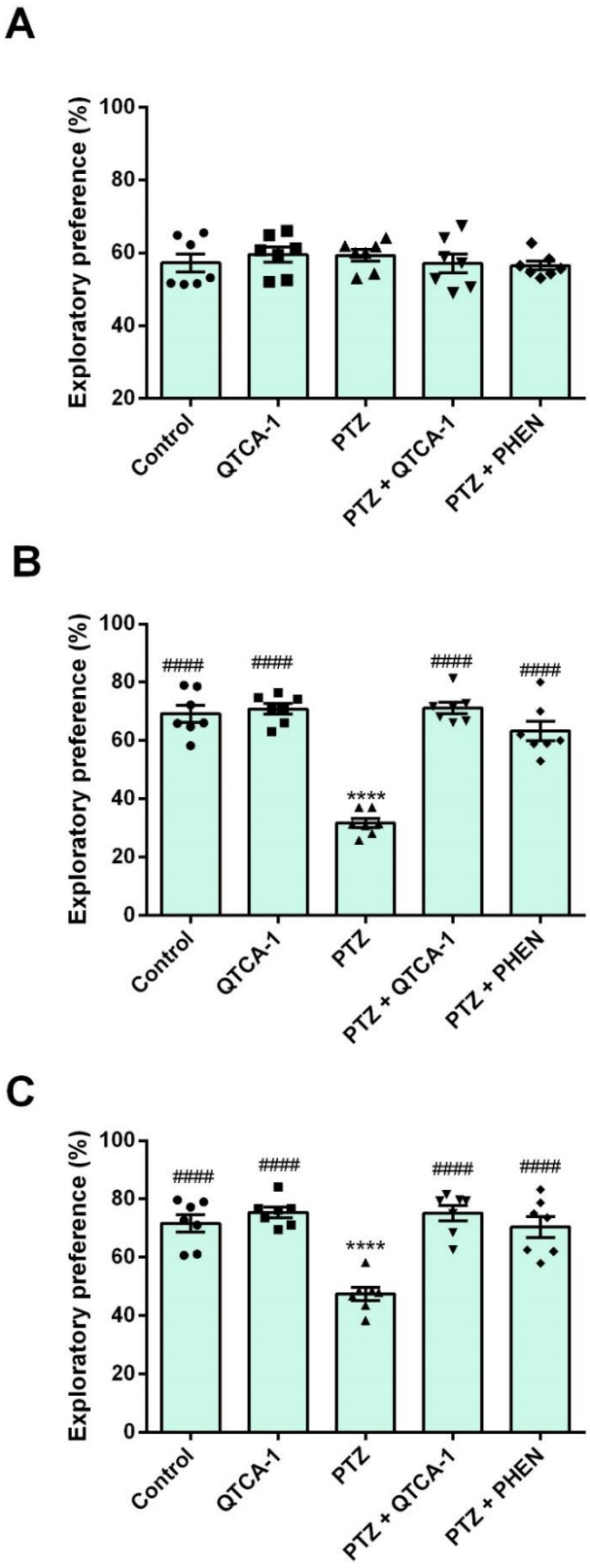

A
Exploratory preference (%)
100
80
60
40
20
Control
QTCA-1
PTZ
PTZ + QTCA-1
PTZ + PHEN
B
Exploratory preference (%)
100
80
60
40
20
0
####
####
****
####
####
Control
QTCA-1
PTZ
PTZ + QTCA-1
PTZ + PHEN
C
Exploratory preference (%)
100
80
60
40
20
0
####
####
****
####
####
Control
QTCA-1
PTZ
PTZ + QTCA-1
PTZ + PHEN

Fig. 6.

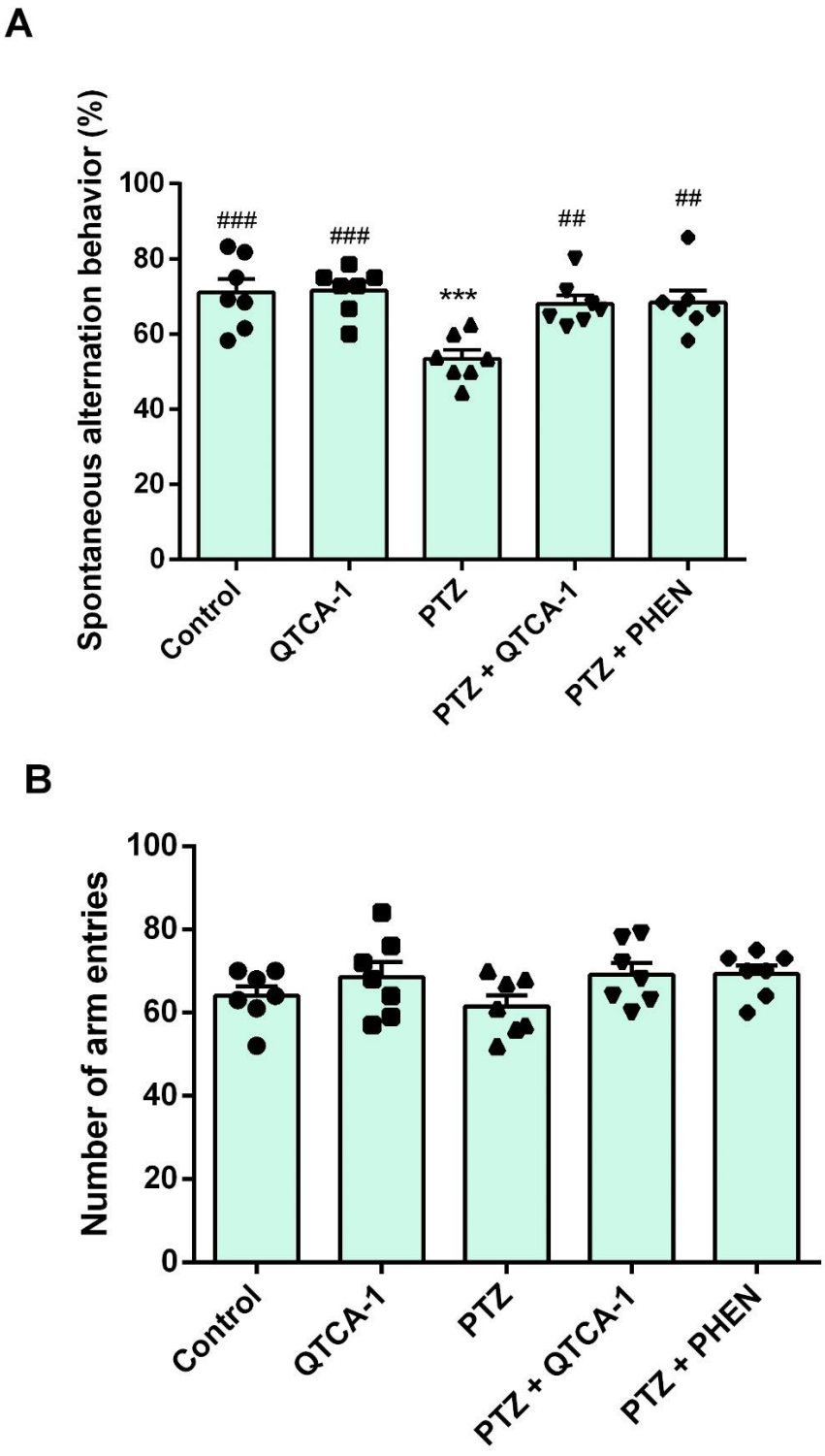

A
Spontaneous alternation behavior (%)
100
80
60
40
20
0
###
###
***
##
##
Control
QTCA-1
PTZ
PTZ + QTCA-1
PTZ + PHEN
B
Number of arm entries
100
80
60
40
20
0
Control
QTCA-1
PTZ
PTZ + QTCA-1
PTZ + PHEN

Fig. 7.

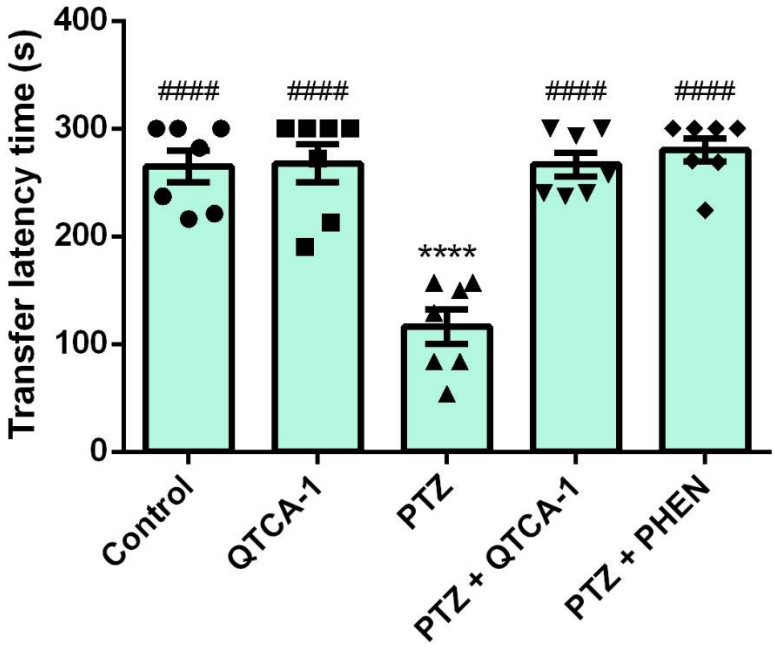

Transfer latency time (s)
400
300
200
100
0
####
####
****
####
####
Control
QTCA-1
PTZ
PTZ + QTCA-1
PTZ + PHEN

Fig. 8.

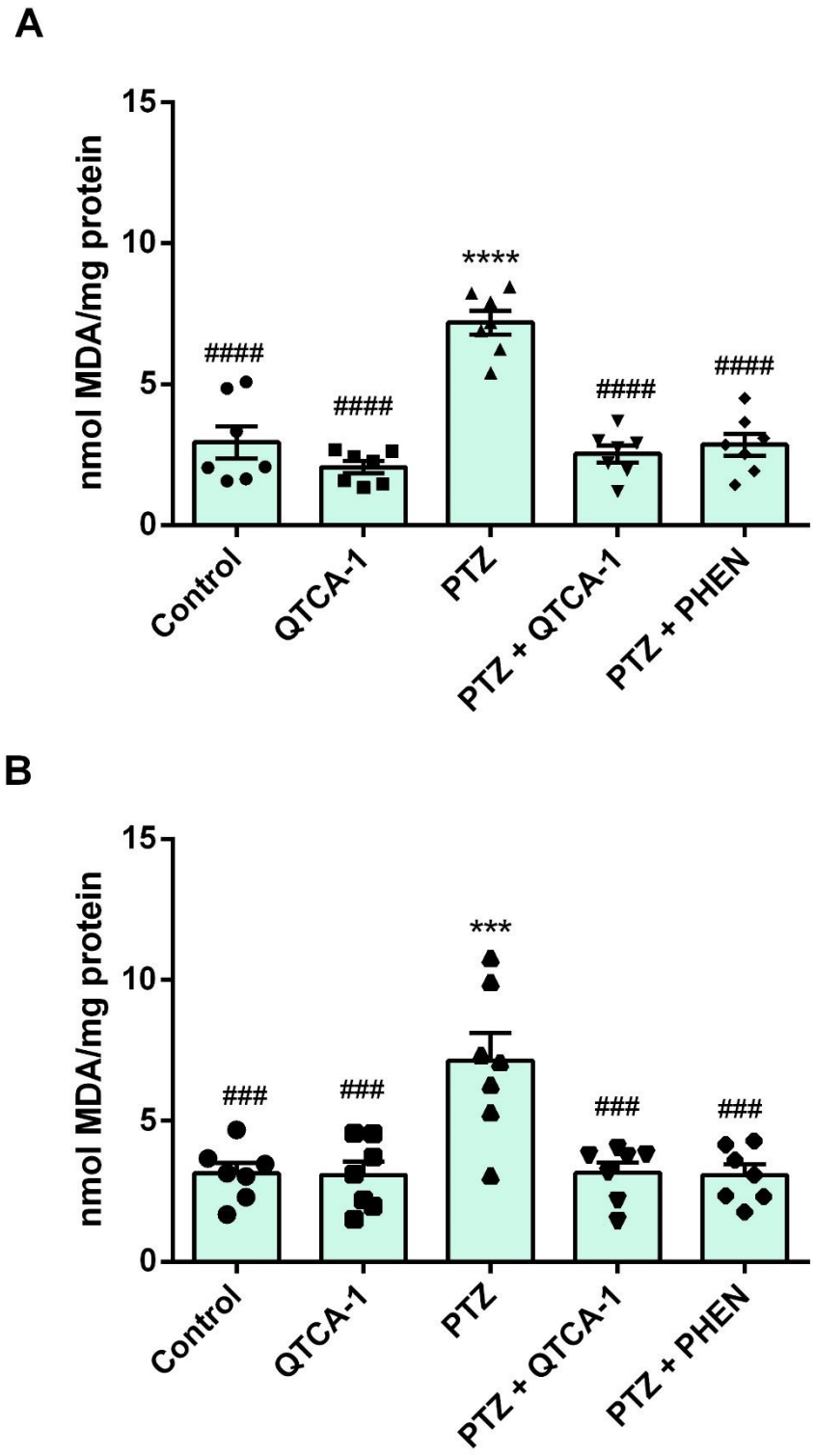
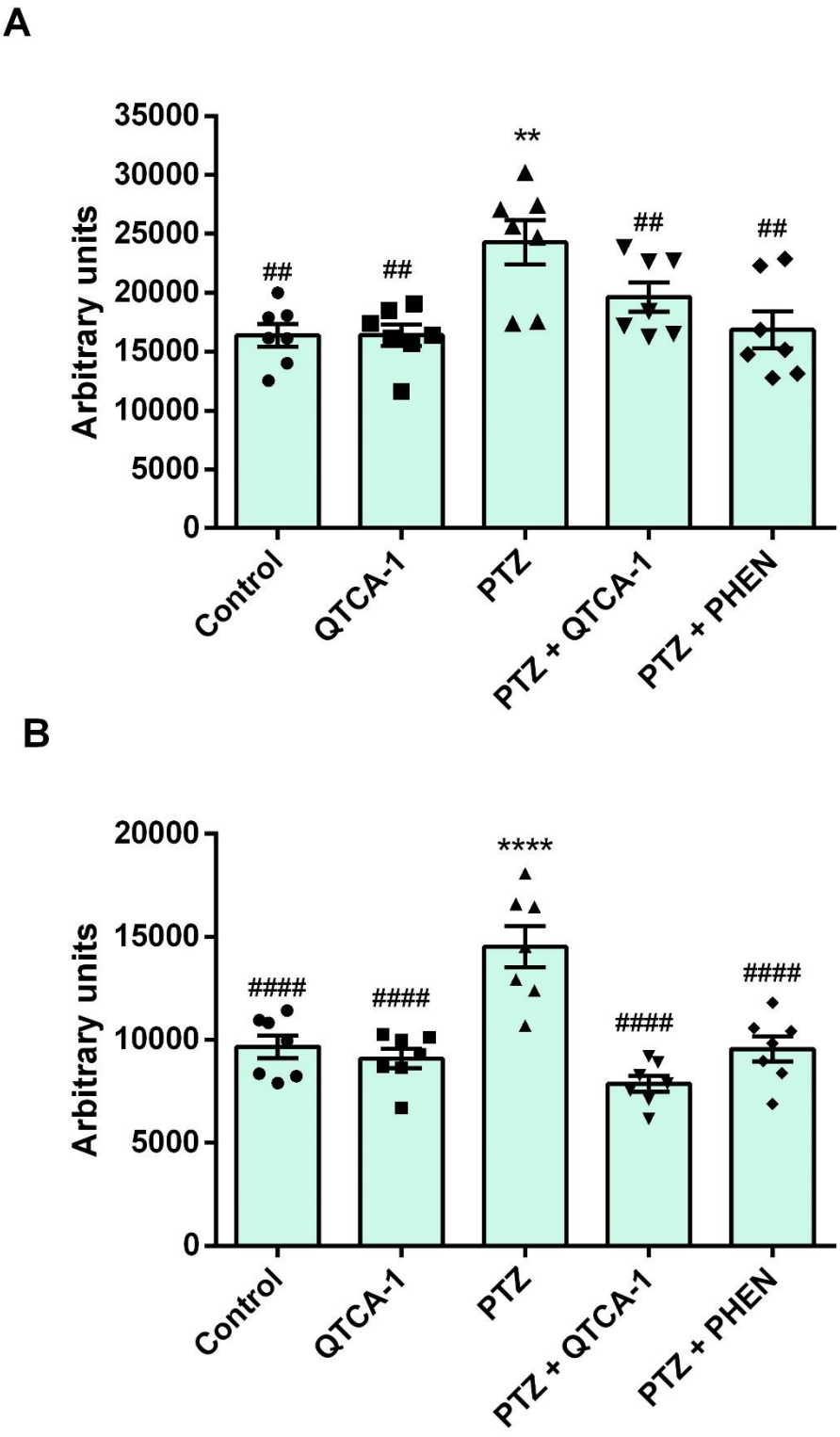

A
nmol MDA/mg protein
15
10
5
0
****
####
####
####
####
Control
QTCA-1
PTZ
PTZ + QTCA-1
PTZ + PHEN
B
nmol MDA/mg protein
15
10
5
0
***
###
###
###
###
Control
QTCA-1
PTZ
PTZ + QTCA-1
PTZ + PHEN

Fig. 9.


A
Arbitrary units
35000
30000
25000
20000
15000
10000
5000
0
**
##
##
##
##
Control
QTCA-1
PTZ
PTZ + QTCA-1
PTZ + PHEN
B
Arbitrary units
20000
15000
10000
5000
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####
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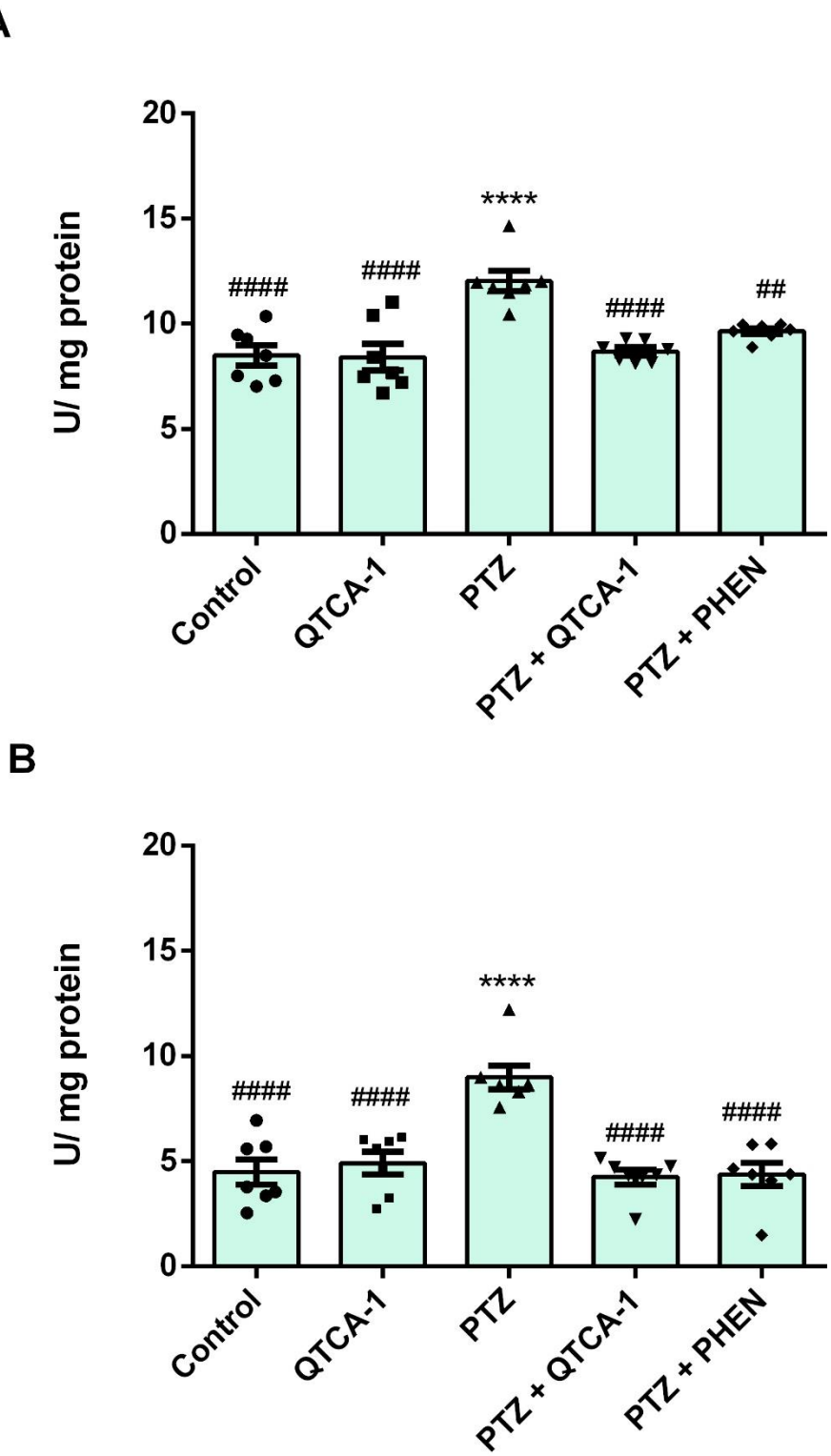
Control
QTCA-1
PTZ
PTZ + QTCA-1
PTZ + PHEN

Fig. 10.


A
U/ mg protein
20
15
10
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####
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Control
QTCA-1
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PTZ + QTCA-1
PTZ + PHEN
B
U/ mg protein
20
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Control
QTCA-1
PTZ
PTZ + QTCA-1
PTZ + PHEN

Fig. 11.

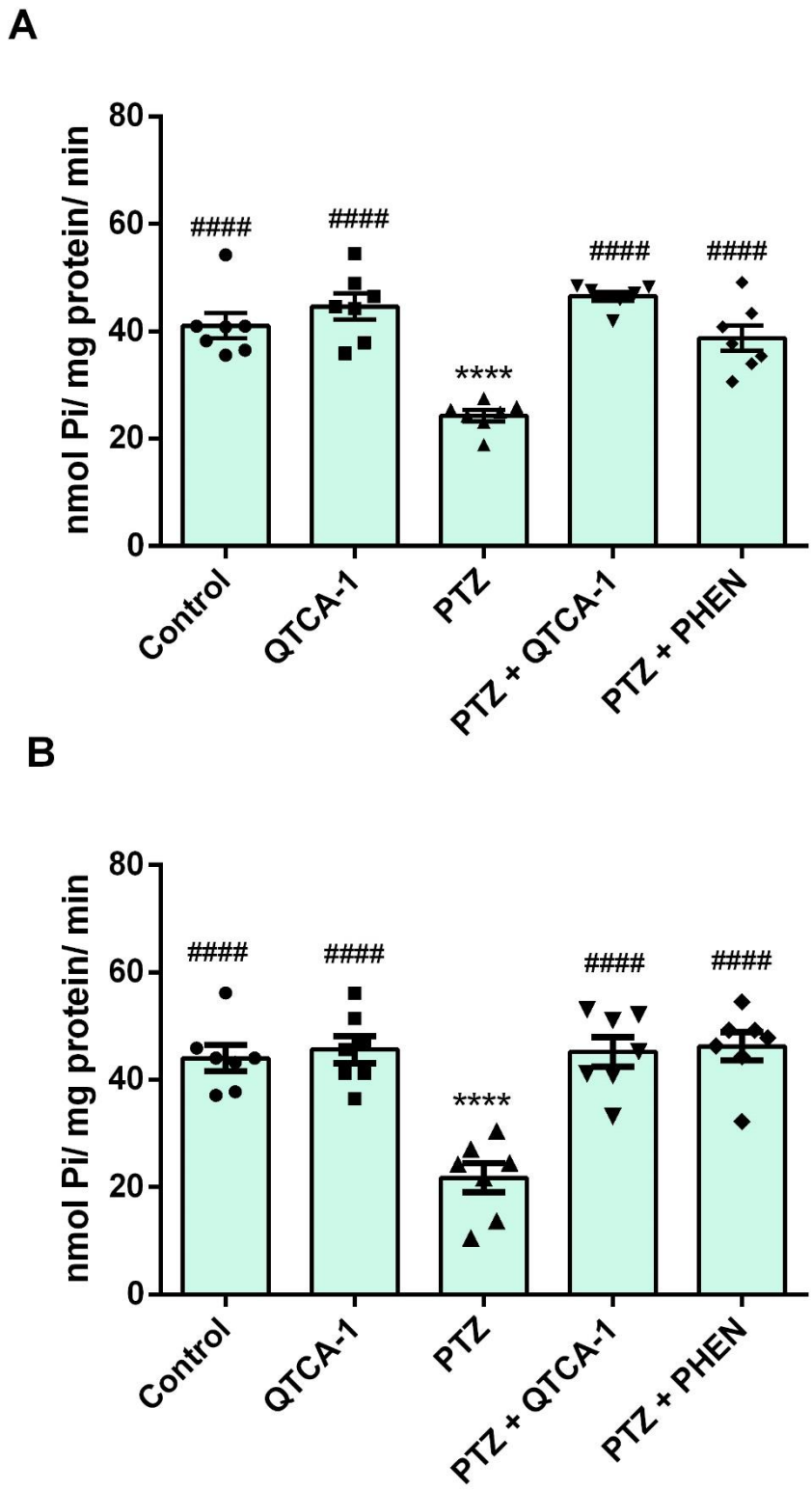
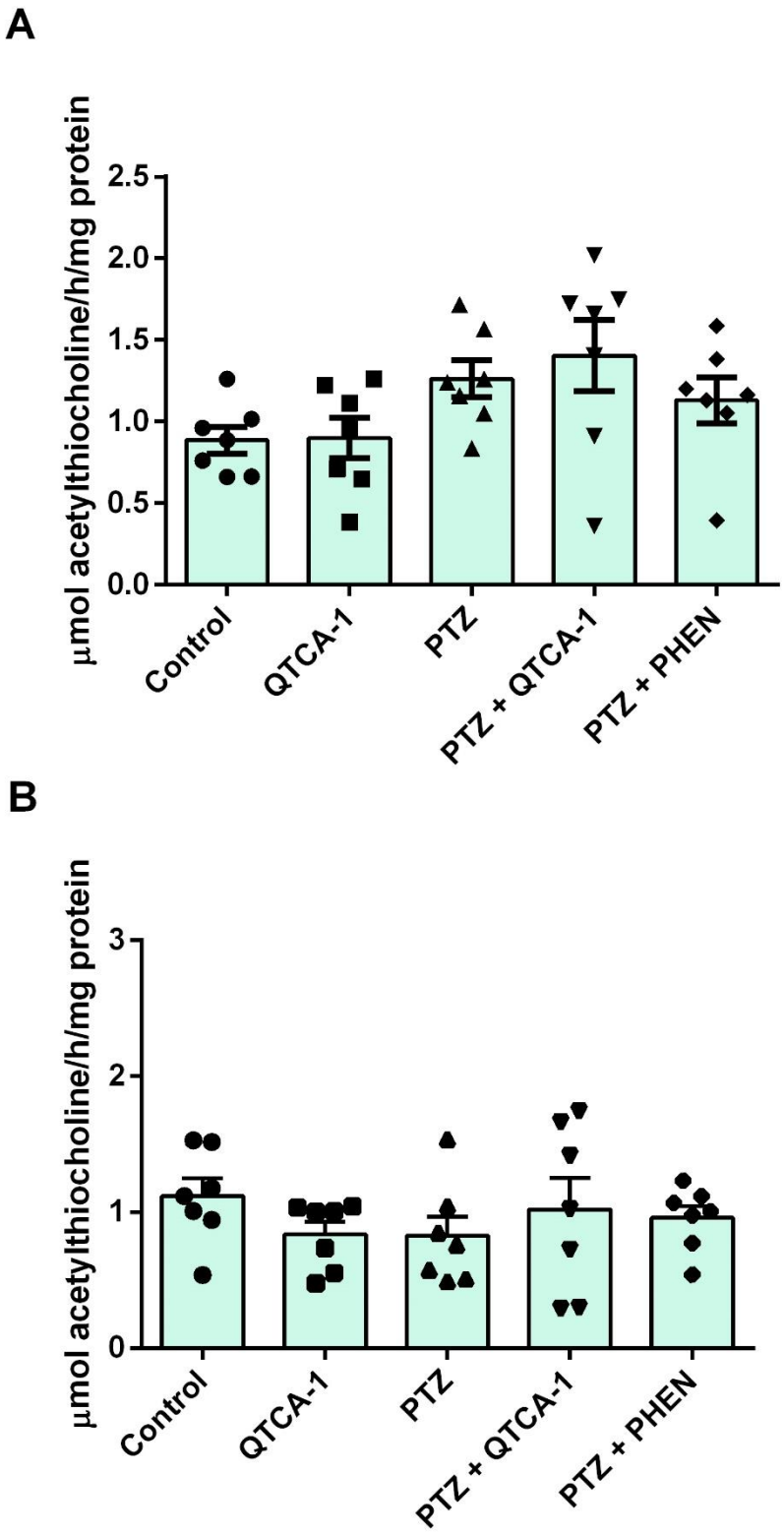

A
nmol Pi/ mg protein/ min
80
60
40
20
0
####
####
****
####
####
Control
QTCA-1
PTZ
PTZ + QTCA-1
PTZ + PHEN
B
nmol Pi/ mg protein/ min
80
60
40
20
0
####
####
****
####
####
Control
QTCA-1
PTZ
PTZ + QTCA-1
PTZ + PHEN

Fig. 12.


A
µmol acetylthiocholine/h/mg protein
2.5
2.0
1.5
1.0
0.5
0.0
Control
QTCA-1
PTZ
PTZ + QTCA-1
PTZ + PHEN
B
µmol acetylthiocholine/h/mg protein
3
2
1
0
Control
QTCA-1
PTZ
PTZ + QTCA-1
PTZ + PHEN

4. DISCUSSÃO

Estima-se que centenas de milhões de pessoas em todo o mundo são afetados por distúrbios neurológicos, atingindo cerca de 10,2% da população (ALESSANDRINI et al., 2019). A maioria das doenças neurológicas como a DA, doença de Huntington, epilepsia e doença de Parkinson são caracterizados pela perda progressiva da função neuronal e estrutural, que eventualmente levam à morte dos neurônios no SN e consequentemente, a perda de memória (VOGEL e WOOD et al., 2014, KARNATI et al., 2015). Sendo assim, a investigação de novos agentes para o tratamento destas desordens neurológicas e suas comorbidades é de extrema importância. Neste contexto, o presente estudo avaliou o possível efeito neuroprotetor do QTCA-1, um derivado de quinolina, no déficit de memória e na epileptogênese em camundongos.

Primeiramente foi investigado a ação anticolinesterásica do QTCA-1, bem como se o composto tem impacto nas diferentes fases da memória (aquisição, consolidação e recuperação) em camundongos amnésicos (**Manuscrito 1**). Está bem descrito na literatura que a enzima AChE é um alvo amplamente conhecido para a identificação de potenciais inibidores contra doenças que causem déficits de memória, principalmente a DA (KHAN et al., 2018).

Nesse sentido, o presente estudo demonstrou que o QTCA-1 exibiu efeito inibidor da enzima AChE *in vitro* nas estruturas cerebrais de camundongos (córtex cerebral e hipocampo) (**Manuscrito 1**). Dados da literatura demonstram que os derivados de quinolina são potentes inibidores da atividade da AChE *in vitro*, dessa forma, atenuando o déficit cognitivo (UMAR et al., 2018, YANG et al., 2018, MAHDAVI et al., 2019).

No presente estudo investigamos a ação anticolinesterásica do QTCA-1 por meio da inibição *in vitro* da AChE no córtex cerebral e hipocampo de camundongos, a fim de investigar se há diferenças na ação do composto em função da estrutura cerebral analisada (**Manuscrito 1**). Nossos resultados demonstraram que o QTCA-1 inibiu a atividade da AChE e que as concentrações inibitórias no hipocampo foram menores do que no córtex cerebral de camundongos. Além disso, os valores de inibição máxima do

QCTA-1 no hipocampo foram maiores do que no córtex cerebral, indicando uma maior eficácia do composto no hipocampo (**Manuscrito 1**). Na verdade, esse resultado é importante dado que regiões cerebrais, como hipocampo estão associadas a funções mentais como reconhecimento de estímulos sensoriais, memória e pensamento abstrato, sendo a mais afetadas pelas alterações bioquímicas causadas pela DA (DEMARIN et al., 2011).

Além disso, considerando que a memória é um processo complexo representado por três principais estágios: aquisição, consolidação e recuperação (IZQUIERDO, 2002), investigou-se o efeito do QCTA-1 em cada fase da memória em camundongos. Os resultados demonstraram que uma única dose de QTCA-1 atenuou o prejuízo causado pela ESC nas fases da memória de aquisição, consolidação e recuperação em camundongos (**Manuscrito 1**). Neste contexto, o efeito da melhora da memória pela administração aguda do QTCA-1 tem grande relevância, tendo em vista que diversas doenças neurodegenerativas prejudicam uma ou mais fases da cognição (CIESLAK et al., 2017).

Com o objetivo de esclarecer o mecanismo pelo qual o QTCA-1 exerce efeito na prevenção de alterações comportamentais nas diferentes fases da memória, foi investigado no presente estudo o envolvimento do sistema colinérgico por meio da determinação da atividade da AChE (**Manuscrito 1**). Verificou-se que o QTCA-1 modulou o sistema colinérgico, uma vez que protegeu contra o aumento na atividade da AChE causado pela ESC no córtex cerebral e hipocampo dos camundongos nas diferentes fases da memória (**Manuscrito 1**). Esses resultados corroboram com os encontrados na literatura, que demonstraram que os derivados de quinolinas são importantes agentes anticolinesterásicos e com atividade anti-AD (WANG et al., 2018, FRONZA et al., 2019).

Os processos cognitivos mediados pelo córtex e hipocampo são afetados pela disfunção colinérgica que ocorrem na maioria das doenças neurológicas (SPECK-PLANCHE et al., 2012). Sendo assim, a neurotransmissão colinérgica prejudicada contribui para a disfunção cognitiva (SPECK-PLANCHE et al., 2012; AHMED et al., 2013). Os resultados do presente estudo indicam que a inibição da AChE pelo QTCA-1 poderia agir promovendo um aumento dos níveis de ACh na fenda sináptica dos neurônios,

contribuindo para a melhora da memória nas diferentes fases avaliadas. Importante destacar que o efeito do QTCA-1 na atividade da AChE foi semelhante ao controle positivo usado no estudo (DON) (**Manuscrito 1**).

Além disso, QTCA-1 por ter similaridade de estrutura quinolínica e um anel triazólico com o composto QTC4-MeOBnE em estudo descrito por Fronza e colaboradores (2019), o QCTA-1 poderia vir a interagir de forma semelhante, atuando nos mesmos sítios da AChE (possuindo afinidade de ligação com AChE no sítio ativo catalítico (CAS) e no sítio aniônico periférico (PAS) da enzima). Dessa forma, o QTCA-1 pode ser uma alternativa para o tratamento do déficit de memória (**Manuscrito 1**), visto que estudos estruturais têm apontado que a dupla inibição da AChE (sítio ativo catalítico CAS e PAS) pode reduzir o déficit colinérgico (GALIMBERTI et al., 2016, FRONZA et al., 2019). Neste contexto, pode-se sugerir que QCTA-1 poderia inibir a atividade da AChE de forma semelhante ao QTC-4-MeOBnE.

Considerando os achados do **manuscrito 1**, o composto derivado de quinolina QTCA-1 pode ser uma ferramenta importante para proteger contra o comprometimento de memória, tendo em vista que o composto melhora o desempenho nas fases de aquisição, consolidação e recuperação em camundongos amnésicos e apresenta ação anticolinesterásica no córtex cerebral e hipocampo de camundongos, importantes estruturas relacionadas com o processamento da memória. Desta forma, o aumento da atividade cognitiva do QTCA-1 observado no **manuscrito 1** pode oferecer uma escolha terapêutica útil para aliviar os prejuízos da memória, especialmente aqueles relacionados à disfunção colinérgica.

Como os resultados obtidos no **manuscrito 1** apresentaram dados muito relevantes relacionados ao tratamento agudo do QTCA-1 no aumento da atividade cognitiva nas diferentes fases da memória com contribuição da via colinérgica, foi dada a continuidade da pesquisa deste possível agente terapêutico para o tratamento da demência (**Artigo 1**).

Nesse sentido, apesar da demência ser considerada uma das principais causas de morbimortalidade entre idosos (PURI et al., 2014), e que em torno de 50 milhões de pessoas sofrem de algum tipo de demência no mundo (OMS, 2017), o cenário de tratamento ainda é limitado, com poucas opções disponíveis e baixas taxas de aprovação para novos medicamentos

(PANKEVICH et al., 2014). A demência é caracterizada como uma síndrome neurológica, que leva ao aparecimento de diversos sintomas, dentre um dos mais característicos a amnésia (JANCA et al., 2006). Desta forma, para investigar o efeito do composto QTCA-1 na demência, foi realizado um modelo sub- crônico de amnésia induzida pela ESC e avaliada a relação do efeito do derivado de quinolina com o estresse oxidativo, e com as atividades da Na^+/K^+ - ATPase e AChE (**Artigo 1**).

No presente estudo foi demonstrado que a administração de QTCA-1 protegeu contra a amnésia induzida pela ESC em camundongos (**Artigo 1**). Pode-se observar que o tratamento com QTCA-1 atenuou o prejuízo cognitivo induzido pela ESC nos diferentes tipos de memória avaliados (memória espacial e de longo prazo, memória de referência e memória de longo prazo aversiva não espacial) (**Artigo 1**). Dados da literatura demonstram que a demência esta associada principalmente a dificuldades na aprendizagem e na memória (AGRAWAL et al., 2011) e que a neurodegeneração que ocorre nesse processo acaba interferindo no funcionamento ocupacional e nas atividades sociais (REITZ et al., 2011).

Nesse sentido, tem crescido a busca por novos compostos capazes de combater o aparecimento da demência e também elucidar os mecanismos pelos quais exercem seus efeitos (KULKARNI et al., 2010, BARTH et al., 2019, de OLIVEIRA et al., 2019). Como demonstrado no no **artigo 1,** o QTCA-1 modula a via colinérgica e a modulação desta via pode estar relacionada com a melhora da memória observada nos testes comportamentais. Isso pode ser observado pelo efeito anticolinesterásico que o QTCA-1 apresentou (ou seja, o mesmo protegeu contra o aumento da atividade da AChE induzida por ESC) em ambas as estruturas cerebrais (córtex e hipocampo) dos camundongos. Ainda, neste contexto, esses achados corroboram com os encontrados no **manuscrito 1**, tendo em vista que o QCTA-1 também evitou o prejuízo nas diferentes fases da memória após uma única dose por modular a atividade da enzima AChE.

Além da disfunção colinérgica, o aumento do estresse oxidativo com a idade é um dos principais fatores de risco para o aparecimento da demência (FERREIRA-VIEIRA et al. 2016). Mudanças que possam ocorrer com a idade, aumentando as taxas pró-oxidantes e ou diminuindo os mecanismos de defesa

antioxidante, podem ser a explicação para muitas das alterações orgânicas que ocorrem na demência (De SOUZA e BOHR, 2002).

Para investigar se o QTCA-1 reduz o dano oxidativo e assim melhora a demência induzida pela ESC, foi avaliado o status redox do cérebro através dos níveis de espécies reativas do ácido tiobarbitúrico (TBARS) e da atividade da CAT (artigo 1). Os resultados demonstraram que o QTCA-1 protegeu contra o dano oxidativo (protegendo contra o aumento dos níveis de TBARS e aumento na atividade da CAT) nas estruturas cerebrais de camundongos induzidos com ESC. Desta forma, ainda não se sabe o exato mecanismo para explicar a ação antioxidante do QTCA-1, entretanto com os resultados apresentados no **Artigo** 1 pode-se supor que o composto pode agir em diferentes linhas de defesa antioxidante (modulando defesas antioxidantes enzimáticas e protegendo contra a peroxidação lipídica).

Além disso, esta documentado na literatura que a enzima Na^+/K^+-ATPase é um importante neuromodulador contra doenças neurológicas como a demência, visto que a deficiência na Na^+/K^+-ATPase causa déficits de aprendizagem e memória (ZHANG et al. 2013), e que a redução da atividade enzimática pela ESC tem um papel central no processo de memória (GUTIERRES et al. 2014; da SILVA et al. 2017) e na patogênese de doenças neurológicas como a DA (GUTIERRES et al. 2014). Os resultados do presente estudo demonstraram que o QTCA-1 normalizou a atividade da Na^+/K^+-ATPase induzida pela ESC nas estruturas cerebrais dos camundongos. Desta maneira, os achados **no artigo 1** indicam que ação anti-amnésica do composto poder estar relacionada com o efeito protetor contra a diminuição na atividade Na^+/K^+-ATPase. Esses resultados corroboram com estudo na qual foi descrito o aumento na atividade da Na^+/K^+-ATPase por compostos utilizados no tratamento da demência (ALI e ARAFA, 2011). Além disso, o efeito antioxidante de QTCA-1 demonstrado no **artigo 1** parece estar envolvido no efeito benéfico do composto na atividade enzima Na^+/K^+-ATPase, tendo em vista que o estresse oxidativo modula o processo da memória nas doenças neurológicas como a DA por meio da atividade da Na^+/K^+-ATPase (ZHANG et al. 2013) e enzimas antioxidantes.

Desta forma, os resultados encontrados no **artigo 1**, corroboram com os encontrados no **manuscrito 1**, uma vez que demonstrou-se que o QTCA-1

protege contra os déficits cognitivos induzidos pela ESC tanto em um modelo de tratamento sub-crônico como em um modelo de tratamento agudo, respectivamente. Além disso, pode-se destacar a ação anti-amnésica pela proteção das atividades da AChE, observada tanto no **Artigo 1**, como no **Manuscrito 1**. Além disso, a modulação da atividade da enzima Na^+/K^+-ATPase e o efeito antioxidante parecem estar envolvidos na atividade protetora do composto QTCA-1 (**Artigo 1**). Portanto, o derivado de quinolina QTCA-1 poderia vir a ser uma alternativa farmacológica para o tratamento da demência.

Tendo em vista que o QCTA-1 (i) apresentou efeito anti-aminésico em camundongos (**Manuscrito 1** e **Artigo 1**), (ii) demonstrou efeito anticonvulsivante em ratos jovens (Wilhelm et al., 2014), e (iii) apresenta natureza lipofílica e isso contribui para que o tecido cerebral seja o alvo deste composto, foi verificado se o QCTA-1 apresenta efeito na epileptogênese e na comorbidade de memória em um modelo *kindling* induzido por PTZ em camundongos (**Manuscrito 2**).

Paralelamente as doenças neurológicas relacionadas a disfunção colinérgica, a epilepsia é uma doença que afeta aproximadamente 50 milhões de pessoas em todo o mundo (WILLEMS et al., 2018). No presente estudo foi demonstrado que QTCA-1 atenuou a epileptogênese causada por *kindling* induzido por PTZ, conforme demonstrado pela redução na pontuação do estágio de convulsão (**Manuscrito 2**). Além disso, o efeito do QTCA-1 no escore utilizado para avaliar a convulsão foi semelhante ao fenobarbital, um controle positivo utilizado neste estudo (**Manuscrito 2**). Os achados encontrados no **Manuscrito 2** relacionados a atenuação da epileptogênese pelo QCTA-1 estão de acordo com os descritos anteriormente por Wilhelm e colaboradores (2014), onde foi demonstrado que o QCTA-1, em dose única (100 mg/kg), apresentou ação anticonvulsivante em ratos jovens abolindo as convulsões e morte induzidas por PTZ.

Outro achado muito importante do presente estudo foi que o QCTA-1 protegeu contra o comprometimento da memória (na memória de trabalho, memória de curto e longo prazo, e a memória de longo prazo aversiva não espacial) causada pelo modelo de indução de *kindling* por PTZ nos camundongos (**Manuscrito 2**), demonstrando que QTCA-1 pode ser uma alternativa promissora para o tratamento das comorbidades da epilepsia. De

fato, tem crescido a busca por alternativas para o tratamento das comorbidades envolvidas com a epilepsia, principalmente aquelas ligadas ao déficit de memória (ARJUNE et al., 2020).

Nesse sentido, os achados do **manuscrito 2** estão em concordância com os encontrados no **artigo 1** e **manuscrito 1**, demonstrando que o composto QTCA-1 atua na melhora da memória. Além disso é importante ressaltar que os resultados mostraram que o tratamento com QTCA-1 foi capaz de atenuar as mudanças de comportamento de forma semelhante ao controle positivo (fenobarbital) (**Manuscrito 2**).

Com o objetivo de esclarecer o mecanismo pelo qual o QTCA-1 exerce efeito na atenuação das alterações comportamentais causadas pelo PTZ, investigou-se o possível envolvimento do estresse oxidativo no córtex cerebral e no hipocampo de camundongos (**Manuscrito 2**). Foi observado no presente estudo que o QTCA-1 protegeu contra o aumento nos níveis de TBARS e ERs, bem como o aumento na atividade da SOD causados por *kindling* induzidos pelo PTZ.

Adicionalmente, para buscar elucidar outros mecanismos de ação envolvidos nos efeitos já demonstrados pelo QCTA-1, foi demonstrado que este composto derivado de quinolina protege contra a inibição na atividade da enzima Na^+/K^+-ATPase causada por *kindling* induzido por PTZ (**Manuscrito 2**). De fato, estudos demonstraram que uma inibição na atividade da Na^+/K^+-ATPase no cérebro de roedores após convulsões agudas induzidas por PTZ estão relacionadas ao déficit de memória em camundongos (MARQUEZAN et al., 2013). Ainda pode-se inferir que o QTCA-1 protege contra o comprometimento de memória no **manuscrito 2**, através da modulação na atividade da Na^+/K^+-ATPase. De fato, estimuladores da atividade da Na^+/K^+-ATPase podem ser usados como agentes neuromoduladores contra o comprometimento da memória, visto que esta enzima modula a memória em camundongos (ZHANG et al., 2013).

Em contraponto o presente estudo não demonstrou alteração na atividade da AChE no córtex cerebral e hipocampo de camundongos induzidos por PTZ-*kindling* (**Manuscrito 2**). Assim, no presente estudo, acreditamos que o estresse oxidativo seja o principal responsável pela patogênese característica da memória (**Manuscrito 2**). Com isso, no **manuscrito 2**, foi evidenciado que o

QCTA-1 pode ser uma ferramenta promissora para o tratamento da epileptogênese e comorbidades associadas à epilepsia (comprometimento da memória), e o seu mecanismo de ação parece envolver a redução do estresse oxidativo e a normalização da atividade da Na^+/K^+ -ATPase.

Através do conjunto de resultados obtidos com o **artigo 1** e os **manuscritos 1** e **2** foi demonstrado no presente estudo que o composto QTCA-1 apresenta um promissor efeito neuroprotetor, tendo como tecido alvo o cérebro. Este derivado de quinolina parece exercer a ação neuroprotetora na melhora da memória e epileptogênese principalmente a partir de seu efeito antioxidante, relacionada a modulação da atividade da Na^+/K^+-ATPase. Além disso, o efeito do QTCA-1 relacionado a melhora da memória em diferentes modelos, parece estar ligada a atividade anticolinesterásica do composto. Desta forma, com os achados no presente estudo poderíamos especular que o QTCA-1 apresenta características de moléculas multi-alvo, podendo vir a ser eficaz no tratamento de diversas doenças neurológicas (Figura 10). Sendo assim, estamos motivados a continuar investigando o potencial desse composto em busca de novos mecanismos ligados ao seu efeito.

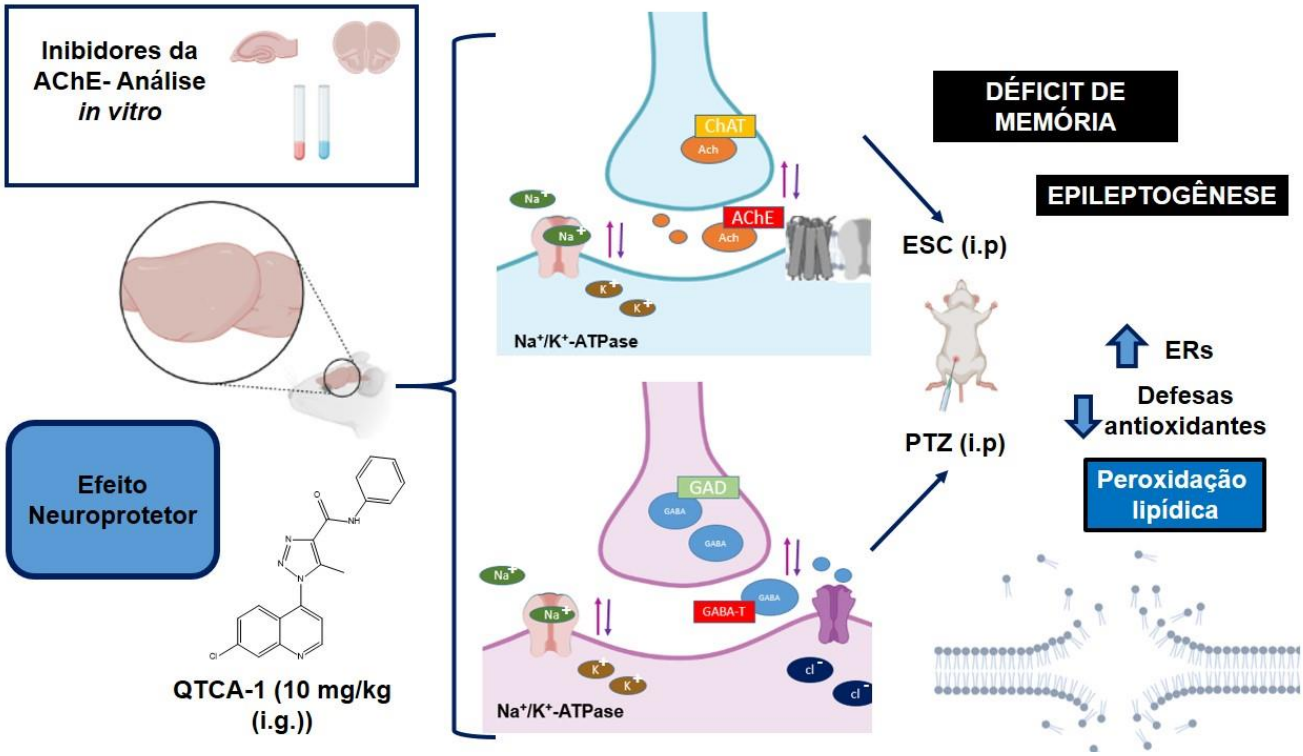


Figura 10. Resumo gráfico dos eventos ocasionados pelas induções com escopolamina (ESC) e/ou pentilenotetrazol (PTZ), e dos possíveis mecanismos de ação do 1-(7-cloroquinolin-4-il)-5-metil-N-fenil-1H-1,2,3-triazol-4 carboxamida (QTCA-1) em camundongos. Abreviações: Acetilcolina (ACh), Acetilcolinesterase (AChE), Ácido gama-aminobutírico (GABA), Cloro (Cl^-) colina acetiltransferase (ChAT), GAD (glutamato descarboxilase), Intragástrica (i.g.), Intraperitoneal (i.p.), Potássio (K^+), Sódio (Na^+).

5. CONCLUSÃO

- O QTCA-1 apresentou atividade modulatória sobre o sistema colinérgico, através da inibição da atividade da AChE *in vitro* observada no córtex cerebral e hipocampo dos camundongos.

- O tratamento com este derivado quinolínico protegeu contra as alterações causadas pela ESC nas diferentes fases da memória: aquisição, consolidação e evocação, não causando alteração na atividade locomotora e exploratória dos animais.

- Uma única administração de QTCA-1 exibiu ação anticolinesterásica nas diferentes fases da memória no córtex cerebral e no hipocampo de camundongos, de forma semelhante ao controle positivo (DON).

- QTCA-1, um derivado de quinolina, demonstrou efeito anti-amnésico, através da proteção das atividades da AChE e Na^+/K^+-ATPase modificadas pela ESC, e também pelo seu efeito antioxidante.

- QTCA-1 atenuou a epileptogênese e o déficit de memória causada por indução de *kindling* por PTZ. Além disso, o QTCA-1 normalizou o estresse oxidativo e a atividade Na^+/K^+-ATPase no córtex cerebral e no hipocampo de camundongos com *kindling*.

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ANEXOS

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Corresponding Author: Dr Cristiane Luchese
Co-Authors: Ane Gabriela Vogt; Renata Leivas de Oliveira; Guilherme Teixeira Voss; Gustavo Bierhals Blödorn; Diego Alves; Ethel Antunes Wilhelm
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ANEXO C e D

Carta de aprovação do protocolo experimental pelo Comitê de Ética em Experimentação Animal da Universidade Federal de Pelotas.

UFPel
CEEA
Comissão de Ética em Experimentação Animal

Pelotas, 08 de agosto de 2017

Certificado

Certificamos que a proposta intitulada **"Avaliação do efeito neuroprotetor de um derivado de quinolina em um modelo de epilepsia e déficit cognitivo induzido por pentilenotetrazol em camundongos"**, registrada com o nº **23110.005445/2017-91**, sob a responsabilidade de **Cristiane Luchese** - que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e recebeu parecer **FAVORÁVEL** a sua execução pela Comissão de Ética em Experimentação Animal, em reunião de 10/07/2017.

Finalidade	(X) Pesquisa ( ) Ensino
Vigência da autorização	Início: 08/2017 Término: 08/2020
Espécie/linhagem/raça	Mus musculus / Swiss
N° de animais	130
Idade	60 dias
Sexo	Masculino
Origem	Biotério Central da UFPel

Solicitamos, após tomar ciência do parecer, reenviar o processo à *CEEA*.

Salientamos também a necessidade deste projeto ser cadastrado junto ao *COBALTO* para posterior registro no *COCEPE* (código para cadastro nº **CEEA 5445-2017**).

M.V. Dra. Anelize de Oliveira Campello Felix
Presidente da CEEA

Pelotas, 16 de maio de 2016

Certificado

Certificamos que a proposta intitulada "**Avaliação do efeito de compostos orgânicos sintéticos na memória e no déficit cognitivo em camundongos**", registrada com o nº23110.001974/2016-34, sob a responsabilidade de **Cristiane Luchese** - que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), recebeu parecer **FAVORÁVEL** a sua execução pela Comissão de Ética em Experimentação Animal, em reunião de 09/05/2016.

Finalidade	(X) Pesquisa ( ) Ensino
Vigência da autorização	17/05/2016 a 01/04/2019
Espécie/linhagem/raça	*Mus musculus*/Swiss
N° de animais	2474
Idade	60 dias
Sexo	Machos
Origem	Biotério Central - UFPel

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