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EDUARDA MONTEIRO FIDELIS

EFEITO DE NANOCÁPSULAS CONTENDO CURCUMINA NO COMPORTAMENTO TIPO DEPRESSIVO E NO ESTRESSE OXIDATIVO INDUZIDOS POR β-AMILÓIDE EM CAMUNDONGOS

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Dissertação apresentada ao Programa de Pós-Graduação Stricto sensu em Bioquímica, da Universidade Federal do Pampa (UNIPAMPA, RS), como requisito parcial para a obtenção do Título de **Mestre em Bioquímica.**

Orientadora: Prof.ª Dr.ª Simone Pinton

Coorientadora: Dr.ª Tuane Bazanella Sampaio

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Banca examinadora:

Prof. (a) Dr.(a) Simone Pinton
Orientadora
UNIPAMPA

Prof.(a) Dr.(a) Bibiana Mozzaquatro Gai
UFMT

Dr. Marcelo, Gomes de Gomes
UNIPAMPA

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Minha família

RESUMO

A doença de Alzheimer (DA) é um distúrbio neurodegenerativo classicamente caracterizado pelo comprometimento das funções cognitivas. No entanto, a depressão é uma das alterações comportamentais mais frequentes observadas na sintomatologia da DA. O desenvolvimento de DA inclui inflamação e estresse oxidativo resultante do acúmulo de proteína Aβ no cérebro. A curcumina apresenta propriedades antioxidantes e anti-inflamatórias, mas apresenta baixa biodisponibilidade. Nesse contexto, surge a hipótese do o uso de nanocápsulas de curcumina (NLC C) como uma nova formulação que poderia potencializar os efeitos farmacológicos da curcumina. Assim, o objetivo deste estudo foi investigar os efeitos da NLC C no comportamento depressivo e no estresse oxidativo induzidos por um modelo animal da DA em camundongos. Para isso, camundongos Swiss machos foram divididos em cinco grupos e somente os grupos Aβ, Aβ + NLC C e Aβ + Curcumina receberam injeção intracerebroventricular do agregado $A\beta_{25-35}$. Os grupos Controle e NLC C receberam apenas veículo. A NLC C ou a curcumina não encapsulada foram administradas pela via oral (*gavage*) a uma dose de 10 mg/kg em dias alternados durante 12 dias. Nossos resultados mostraram que a infusão de Aβ induziu um comportamento do tipo depressivo observado no teste de suspensão da cauda e no teste de natação forçada, que foram revertidos pelo tratamento com NLC C. Além disso, o NLC C foi capaz de restaurar o estresse oxidativo gerado pelo Aβ no córtex pré-frontal, evidenciado pela diminuição nos níveis de espécies reativas, atividades de superóxido dismutase e catalase. É importante ressaltar que o NLC C apresentou efeitos superiores à forma livre de curcumina. Assim, demonstramos os efeitos antidepressivos e antioxidantes da NLC C em um modelo de camundongo da DA, sugerindo seu potencial terapêutico para essa desordem.

Palavras-Chave: Doença de Alzheimer; curcumina; depressão; nanocápsulas; estresse oxidativo.

ABSTRACT

Alzheimer's disease is a neurodegenerative disorder classically characterized by impairment of cognitive functions. However, depression is one of the most frequent behavioral changes observed in the Alzheimer's disease symptomatology. AD development includes inflammation and oxidative stress resulting from the Aβ protein accumulation in the brain. Curcumin shows antioxidant and anti-inflammatory properties but it has low bioavailability. In this context, the use of curcumin's nanocapsules (NLC C) emerges. Thus, the aim this study was to investigate the effects of NLC C on the depressive behavior and oxidative stress induced by an animal model of AD. For this, Swiss male mice were divided into five groups and only the Aβ, Aβ+NLC C and Aβ+Curcumin groups received intracerebroventricular injection of the $A\beta_{25-35}$ aggregate. Control and NLC C groups received only vehicle. The NLC C were administered via gavage at a dose of 10 mg/kg in alternate days for 12 days. Our results showed that Aβ infusion induced a depressive-like behavior observed in the tail suspension test and forced swimming test, which were reversed by NLC C treatment. Furthermore, NLC C was able to restore the Aβ-generated oxidative stress in the prefrontal cortex, evidenced by the increase in the reactive species levels, superoxide dismutase and catalase activities. Importantly, NLC C displayed superior effects than the free form of curcumin. Thus, we demonstrated the antidepressant-like and antioxidant effects of NLC C in a mice model of AD, suggesting its therapeutic potential for this disorder.

Keywords: Alzheimer's disease; curcumin; depression; nanocapsules; oxidative stress.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS E SIGLAS

DA - Doença de Alzheimer

APP- Proteína Precursora de Amilóide

Aβ - Beta-amilóide

PS - Placas Senis

EN - Emaranhados Neurofibrilares

NE - Norepinefrina

DNA - Ácido Desoxirribonucleico

EO - Estresse Oxidativo

EROs - Espécies Reativas de Oxigênio

ATP - Adenosina Trifosfato

SOD - Superóxido Dismutase

CAT - Catalase

GPx - Glutationa Peroxidase

$O_2^{\bullet-}$ - Radical ânions superóxido

H_2O_2 - Peróxido de hidrogênio

OH• - Radical Hidroxila

CA1- Região CA1 do hipocampo dorsal, do latim Cornuammonis (Corno de Ammon)

5-HT- Serotonina

GSH - Glutationa

GSSG - Glutationa Oxidada

GR - Glutationa Redutase

BHE - Barreira Hemato Encefálica

NLC - Nanocápsulas de Núcleo Lipídico

NLC C - Nanocápsula de Curcumina

i.c.v–Intracerebroventricular

SUMÁRIO

1 INTRODUÇÃO

A doença de Alzheimer (DA) é um distúrbio neurodegenerativo complexo que se desenvolve gradualmente e progressivamente, com os sintomas progredindo ao longo do tempo, desde um leve esquecimento até um grave comprometimento mental (MORGAN et al., 2019), sendo a forma mais comum de demência em idosos, o que representa 50 a 75% de todos os casos (CHOI et al., 2014). Também a ocorrência da DA em indivíduos mais jovens, nesse caso mais propensos a uma anormalidade genética (ZVĚŘOVÁ, 2018). Os distúrbios neurodegenerativos refletem um enorme ônus econômico, incluindo o custo do tratamento e também a perda da produtividade dos pacientes e cuidadores, visto que a DA é uma doença neurodegenerativa crônica e desencadeia diversos efeitos cognitivos e neuropsiquiátricos incapacitantes (BOSTANCIKLIOĞLU, 2019).

Esta patologia é sinalizada pela clivagem descontrolada de APP, que provoca aglomeração do peptídeo βA no cérebro, o que gera a formação de placas amiloides extracelulares e emaranhados neurofibrilares (LAKEY-BEITIA et al., 2015). Estudos sugerem que o estresse oxidativo está envolvido nos mecanismos de neurotoxicidade induzida por Aβ na patogênese de DA (PREDIGER et al., 2007; PIERMATINI et al., 2010; SOUZA et al.; 2013). Outra característica proeminente do tecido cerebral na DA é neuroinflamação, sendo que respostas inflamatórias desempenham um papel importante na modulação da progressão da doença (BERNARDI et al., 2012; TAI et al., 2015).

Estudos clínicos que investigam a prevalência de alterações neuropsiquiátricas em pacientes relatam que a depressão ou os sintomas depressivos estão entre os sintomas mais comuns da DA (BERGER et al., 1999; CASTILLA-PUENTES e HABEYCH, 2010; CHUI et al., 2017). Neste sentido, a depressão é um indicador clínico da DA e constitui também um fator de risco da doença (BURHANULLAH et al., 2019).

A curcumina é um composto fenólico de pigmentação amarela, principal constituinte do açafrão, que apresenta atividade antioxidante e anti-inflamatória (FARKHONDEH et al., 2019). Estudos mostram que o uso da curcumina reduz efeitos como a perda da função cognitiva influenciada pela inflamação causada pela DA (AGGARWAL e HARIKUMAR, 2009) e que novas formulações utilizando curcumina atuam como agentes eficazes para prevenir e tratar várias doenças

neurológicas como DA (SOMPARN et al., 2007). No entanto, o uso deste composto é limitado devido à sua baixa biodisponibilidade (KELLOFF et al.,1996).

Frente a este problema, o objetivo do presente estudo foi investigar os efeitos de nanocápsulas poliméricas de curcumina (NLC C) no comportamento tipo depressivo e estresse oxidativo induzido por um modelo de DA em camundongos, visto que novas formulações nanotecnológicas poderiam aumentar a biodisponibilidade da curcumina, tornando-a mais efetiva.

2. REVISÃO DA LITERATURA

2.1 Doença de Alzheimer (DA)

A doença de Alzheimer (DA) é um distúrbio neurodegenerativo que afeta regiões do cérebro que controlam a memória, mudanças no humor, incluindo apatia e depressão (PEREZ e RUIZ, 2012). Essa enfermidade foi descrita pela primeira vez pelo médico *Alois Alzheimer,* especialista em neuropsiquiatria, em 1906. Alois Alzheimer relatou o caso de uma paciente do sexo feminino, com 51 anos de idade e que havia sido internada em um sanatório de psiquiatria em Frankfurt na Alemanha, como uma doença rara que afeta os neurônios do córtex (HARMAN, 1996). Assim, suas contribuições deram origem ao nome desta enfermidade.

O envelhecimento é considerado fator de risco importante para a DA com a incidência e a prevalência aumentando gradualmente com a idade (DEL BO et al, 2009; ITO et al, 2010). Além disso, sabe-se que há outros fatores de risco para o aparecimento da DA como doenças cardiovasculares, lesões traumáticas na cabeça, doenças cerebrovasculares, hábitos pouco saudáveis como sedentarismo, a obesidade e fatores genéticos (CARUSO et al.,2018).

Mesmo ainda não sabendo o fator culminante para o desenvolvimento da DA algumas alterações histopatológicas como a produção exacerbada da proteína precursora de amiloide (APP) provocando o acúmulo no depósito de fragmentos β-amilóide (βA) organizadas em placas senis no cérebro (PS); presença de emaranhados neurofibrilares (EN) decorrente da hiperfosforilação da proteína Tau e o surgimento simultaneamente de sinais de inflamação crônica (LAKEY-BEITIA et al., 2015). Os EN surgem quando a proteína Tau sofre hiperfosforilação, por excesso da atividade das quinases ou redução do desempenho das fosfatases, se desligando dos microtúbulos (CATELLANI et al., 2008), provocando assim neurodegeneração como a perda da função estrutural do citoesqueleto (microtúbulos) mais proteína tau mantendo o fluxo axoplasmático causando disfunção sináptica e provocando morte neural (CHENG e BAI, 2018).

As placas amiloides ou PS são acumulações extracelular composto principalmente de βA que são peptídeos constituído de 40 a 42 aminoácidos provenientes da catálise da APP. A APP é uma glicoproteína transmembrana expressa na superfície celular de neurônios e glia, a sua clivagem é gerada de dois meios: *via amiloidogênica* e *via não-amiloidogênica* (QUERFURTH e LAFERLA, 2010). A via amiloidogênica, é menos comum, a clivagem de APP é realizada por beta secretase (β-secretase), seguida por uma gama-secretase (γ-secretase), gerando o peptídeo tóxico βA (ANDREEVA et al., 2017). Logo, a via não amiloidogênica é considerada a principal, onde APP é clivada pela alfa-secretase (α-secretase) seguida por nova fragmentação pela γ-secretase, não gerando peptídeos βA no espaço extracelular (LAFERLA, GREEN e ODDO, 2007).

Os fragmentos βA em excesso formam agregados com efeitos neurotóxicos e neurodegenerativos (MASTERS et al., 2015; JACK et al., 2016; ANDREEVA et al., 2017). Com a deposição de peptídeos βA há uma redução do número de sinapses, portanto, uma perda seletiva de neurônios colinérgicos nas vias do lobo frontal e hipocampo (GHASEMI et al., 2014; SUN et al., 2018). As alterações degenerativas ocasionadas pela DA ocorrem no giro denteado e na sub-região CA1 do hipocampo, no neocórtex de associação e no córtex entorrinal, em todos os estágios da doença (GÓMEZ et al., 1996; WANG et al., 2006; SCHUFF et al., 1997; CHANTAL et al., 2002). Há outras vias mencionadas na DA que estão interligadas dentre elas, como o mecanismo vascular, mitocondrial, oxidativo e inflamatório (KIVIPELTO e SOLOMON, 2008; MASTERS et al., 2015; JACK et al., 2016; KOZLOV et al., 2017; CARUSO et al., 2018; KUMAR e TSAO, 2018).

Embora a patologia da DA não esteja completamente elucidada atribui-se que as alterações induzidas por βA e EN, desencadeiam cascatas neuropatológicas que envolvem: disfunção e perda sináptica, neuroinflamação, danos oxidativo, alterações no metabolismo energético, resultando em morte neuronal.

2.2 Doença de Alzheimer e depressão

A depressão é uma das doenças neuropsiquiátricas mais disseminadas no mundo, têm sido relatadas ao longo dos séculos fazendo parte de várias abordagens diferentes ao longo da história (RODRIGUES et al., 2014). Embora numerosos estudos de depressão tenham sido realizados através dos campos ambiental, sócio étnico e neurocientífico, sua etiologia, diagnóstico, mecanismos patogênicos e tratamentos ainda não estão completamente elucidado (GRUENBERG et al. 2005).

Distúrbios psiquiátricos apresentam em alguns casos sintomas depressivos e demenciais em idosos, deprimidos regularmente manifestam falhas na memória. A depressão e ansiedade são frequentemente vistos na DA e tem impacto significativo na qualidade de vida do enfermo e da família (ORGETA et al., 2015). A depressão está relacionada ao déficit de monoaminas, particularmente norepinefrina (NE), serotonina (5-HT) e dopamina (STEPHEN et al., 2003), a associação de humor deprimido com um défice de monoaminas, a via mesolímbica da DA é um regulador chave de interesse / prazer e a disfunção da via pode estar subjacente à depressão na DA (SAPOLSKY, 1996). A depressão tem sido associada com declínio cognitivo acelerado e atrofia do lobo frontal e cingulado anterior que podem refletir mecanismo subjacente distinto da patologia de Alzheimer.

Estudos clínicos que investigam a prevalência e a gravidade da depressão associada à demência descobriram que a depressão ou os sintomas depressivos estão entre os sintomas comportamentais e psicológicos mais comuns da DA (BERGER et al., 1999; CASTILLA-PUENTES e HABEYCH, 2010; CHUI et al., 2017). De fato, a depressão é um indicador clínico da DA e constitui também um fator de risco da doença (BURHANULLAH et al., 2019). Neste sentido, diversos modelos biológicos de ensaios pré-clínicos da DA apontam a depressão como uma das alterações comportamentais ligadas aos prejuízos na memória e no aprendizado (AMANI et al., 2019, ROSA et al., 2019; ZHOU et al., 2019)

2.3 Estresse Oxidativo

A produção de espécies reativas ocorre durante processos fisiológicos, essa formação constitui um processo contínuo envolvendo algumas funções biológicas relevantes (BARBOSA et al., 2010), essas espécies podem apresentar-se como radicais livres ou espécies não radicales.

Radicais livres são definidos como qualquer átomo ou molécula que apresente em seu orbital mais externo um ou mais elétrons desemparelhados tornando-o reativo. Os radicais, em excesso podem reagir com moléculas como lipídios, proteínas e DNA provocando a transferência de um elétron da molécula para radical livre o que acaba produzindo uma nova molécula instável. O processo de oxidação danifica a molécula suas funções gerando assim uma reação em cadeia prolongando o dano oxidativo, esse processo é denominado estresse oxidativo (EO).

O sistema nervoso central (SNC) é vulnerável ao EO, uma vez que, elevadas taxas de consumo de oxigênio e considerado conteúdo lipídico, apresenta sistemas antioxidantes pouco eficientes comparativamente a outros tecidos (ZLATKOVIC et al., 2014). O EO, é considerado um dos principais contribuintes para o processo de envelhecimento (MILTON e SWEENEY, 2011) e estudos apontam que a idade é o fator de risco mais aceito da DA e que deve ser o argumento central em qualquer hipótese (HERRUP, 2015; SAMANO et al,. 2016).

As espécies reativas de oxigênio (EROs) são produtos universais do metabolismo aeróbico e podem ser gerados durante a respiração celular (GIORGIO et al.,2007; BURGOYNE et al., 2012), também são responsáveis por processos oxidativos, como a peroxidação lipídica, oxidação proteica e dano no ácido desoxirribonucleico celular (DNA) (AHMAD et al., 2017). As mitocôndrias são as organelas responsáveis pela produção de ATP na célula (fosforilação oxidativa) e são extremamente importantes para o controle de processos celulares dependentes de ATP, essenciais para a viabilidade celular (PUNTEL et al., 2015).

O dano mitocondrial que resulta em aumento da produção de EROs contribui para os estágios iniciais da DA, antes mesmo do início dos sintomas clínicos e da presença patológica de βA (UTTARA et al., 2009), esta ligação já foi demonstrada entre dano oxidativo no hipocampo de ratos e comprometimento da aprendizagem.

Uma marca característica dos neurônios envelhecidos é a aparência da lipofuscina que é o acúmulo de proteínas danificadas oxidativamente e lipídios de membrana que geram níveis crescentes de EROs (SEEHAFER e PEARCE, 2006; TERMAN e BRUNK, 2006). Os resultados dessas alterações podem provocar acúmulo de βA além de morte neuronal e demência (SWERDLOW et al., 2010), às próprias EROs provenientes das mitocôndrias podem fomentar a geração de βA aumentando a via amiloide gênica (LEUNER et al., 2012).

As EROs envolvem ânions superóxido (O_2•-), peróxido de hidrogênio (H_2O_2), radicais hidroxila (OH•) e ânions hidroxila que são fortemente reativos derivados de radicais livres de espécies de oxigênio que ocorrem como um subproduto natural do metabolismo celular, principalmente a respiração mitocondrial (MILTON e SWEENEY, 2012). Além da geração de EROs mitocondrial, a homeostase anormal de metais bioativos, como o Ferro (Fe), Cobre (Cu), Zinco (Zn), entre outros, pode estar envolvida na formação de radicais livres e no estresse oxidativo que atua na agregação de βA (GREENOUGH et al., 2013).

Conforme envelhecemos a capacidade do organismo de fornecer antioxidantes endógenos diminui, por isso muitas pesquisas têm buscado potenciais antioxidantes exógenos que atuem na proteção e prevenção da DA (LIGUORI et al., 2018).

A primeira defesa endógena é impedir a formação das espécies reativas, principalmente pela inibição das reações em cadeia com o ferro e o cobre, que são compostos capazes de agir aos ataques das EROs, impossibilitando sua formação ou sequestram-nas de forma a não permitir sua interação com alvos celulares (ROVER JÚNIOR et al., 2001; BARBOSA et al. 2010).

As defesas antioxidantes que são produzidas pelo nosso próprio organismo, incluem as enzimas Superóxido Dismutase (SOD), Catalase (CAT), Glutamina Peroxidase (GPx) (Fig.1) essas enzimas junto com antioxidantes não enzimáticos endógeno como, vitaminas C e E e Glutationa (GSH) (GEORGIEVA et al., 2017), atuam controlando a formação de EROs, convertendo-as em moléculas mais estáveis.

As SODs são uma família de enzimas que catalisam a dismutação de O_2•- em oxigênio e H_2O_2. Existem 3 isoformas: a citosólica (Cu/Zn-SOD), a mitocondrial (MnSOD) e outra Cu/Zn-SOD localizada no espaço extracelular. A CAT catalisa a conversão de H_2O_2 em água e oxigênio, principalmente quando níveis de H_2O_2 estão baixos, é uma proteína homotetramérica contendo um grupamento heme de ferro, localizada nos peroxissomos. As GPxs são uma família de isoenzimas dependentes de selênio que catalisam a redução de H_2O_2 e hidroperóxidos orgânicos em água e álcoois através da oxidação de glutationa (GSH) em glutationa oxidada (GSSG). Glutationa redutase (GR) é responsável por converter GSSG em GSH à custa de NADPH (MARROCCO et al., 2017).

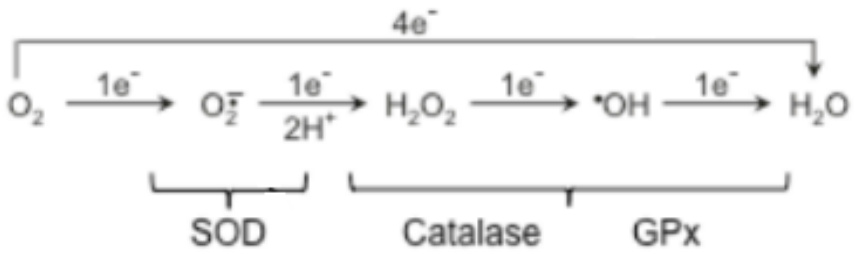

Figura 1: Produção de defesas antioxidantes (Medeiros, 2014).

Os antioxidantes não enzimáticos agem sem degradar diretamente os radicais livres. A vitamina E reage com radicais peroxil lipídicos que são produzidos durante o processo de peroxidação lipídica que perpetuam a reação em cadeia de dano lipídico. Dessa maneira são efetivos em bloquear o seguimento dessa reação e impedem o aumento do dano oxidativo. A vitamina C reage com o superóxido e a radical hidroxila, formando o radical ascorbato. Ao entrar em contato com outro radical ascorbato, forma dehidroascorbato mais ascorbato, eliminando dessa forma o radical livre do meio intracelular.

Quando os antioxidantes endógenos não são suficientes para suprimir o estresse oxidativo fontes exógenas de defesa podem ser suplementadas para buscar a homeostase celular com antioxidantes exógenos, principalmente os advindos da dieta, que tem ganho espaço no âmbito da pesquisa (SIES, 1997; HALLIWELL & GUTTERIDGE, 2007). Alimentos que contêm antioxidantes naturalmente, mas não são ricos em calorias, ou seja, frutas, legumes e grãos, ajudam a manter a saúde e retardar o início de doenças (HALLIWELL e GUTERIDGE, 2010; RAHAL et al., 2014). Nesse contexto, a investigação tem começado a centrar-se sobre os produtos naturais como alternativas para o tratamento da DA (LAKEY-BEITIA et al., 2015).

2.4 Injeção intracerebral do βA_{25-35} : Um modelo animal da DA

A utilização de peptídeo βA sintéticos em modelos de animais, vem sendo aplicado em muitos protocolos como alternativa de menor custo. O uso do peptídeo sintético de 11 aminoácidos βA_{25-35} (GSNKGAIIGLM) (Fig.2), via injeção intracerebral exerce toxicidade similar peptídeo original βA_{1-42} que representa a região ativa, contribuiu para os avanços na percepção dos mecanismos envolvidos na neurotoxicidade (VAN DAN e DE DEYN, 2006).

Na literatura é observado que a injeção intrahipocampal de βA_{25-35} promove a neurodegeneração acompanhada de uma deterioração na memória de curto e longo prazo, danos na aprendizagem além de causar morte neural (DÍAZ et al., 2014; 2018), disfunções colinérgicas e estresse oxidativo (STEPANICHEV et al, 2006). O interesse nesse peptídeo cresceu principalmente porque induz a atrofia de neurites, assim como a plasticidade sináptica semelhante a βA_{1-40} e βA_{1-42} (YAMADA, 2000), mas apresentando melhor solubilidade e eficiência (ZUSSY et al., 2013). Várias pesquisas propõem o uso deste neurotóxico como modelo experimental para o estudo da DA (MILLUCCI i et al., 2010).

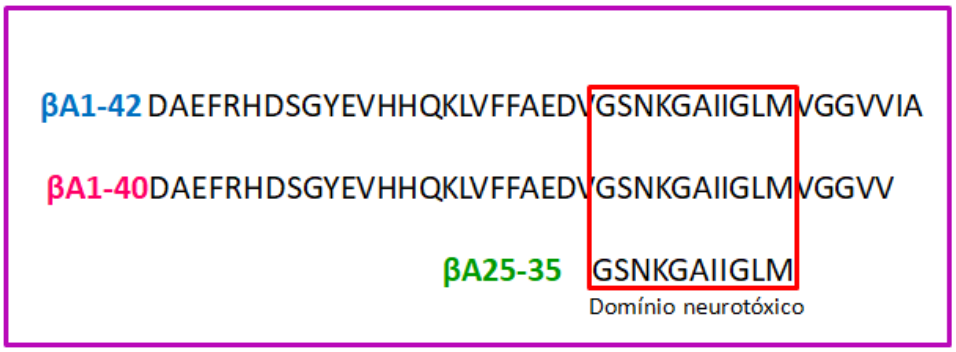

Figura 2: Região biologicamente ativa do peptídeo βA_{1-42} representada pelo peptídeo sintético de 11 aminoácidos βA_{25-35}. (Adaptado de YAMADA e NABESHIMA, 2000).

2.5 Nanopartículas

Os medicamentos usados na sua maioria contêm moléculas pequenas de fármacos que alcançam a corrente sanguínea assim percorrendo todo o corpo, chegam tanto ao seu alvo quanto a outros lugares do organismo não desejáveis.

A liberação de fármacos em sítios de ação específicos através da utilização de condutores, capazes de permitir a otimização da velocidade e do regime de dosagem das substâncias, tem sido uma área de intensa pesquisa nos últimos 16 anos (SHAFFAZICK e GUTERRES, 2003). Para superar essas limitações, um caminho promissor para entrega de fármacos ao SNC é o emprego de sistemas nanoparticulados.

O prefixo *nano* provém da palavra grega ‘anão’, sendo um termo técnico utilizado em qualquer unidade de medida equivalente a um bilionésimo de metro *nm* (GUTERRES et al,. 2005). O interesse em pesquisas relacionadas à nanociência e nanotecnologia torna esta área um novo patamar do conhecimento, com imensos impactos científicos e econômicos (POHLMANN et al., 2012). Diversas abordagens nanotecnológicas vem sendo aplicadas à biomédicas, os nanocarreadores apresentam algumas exclusividades como a distribuição do fármaco direto no local de ação.

Às nanopartículas poliméricas inclui as nanocápsulas e as nanoesferas (Fig.2) que são sistemas carreadores de fármacos que alternam de 10 a 1000 nm, elas diferem entre si dependendo da composição e organização estrutural podendo ser administradas em diversas vias (COUVREUR et al, 2002). As nanoesferas são formadas por uma matriz polimérica na qual o fármaco pode estar disperso na matriz (SCHAFFAZICK et al, 2003). Já as nanocápsulas são constituídas por um invólucro polimérico disposto ao redor do núcleo, geralmente oleoso. Nanopartículas poliméricas têm sido utilizadas a nível terapêutico, apresentando a aplicação mais promissora no tratamento experimental da DA em modelos de animais, uma vez que podem transpassar junções celulares, sendo capazes de atravessar a barreira hemato encefálica (BHE) e alcançar o SNC (MODI et al., 2010).

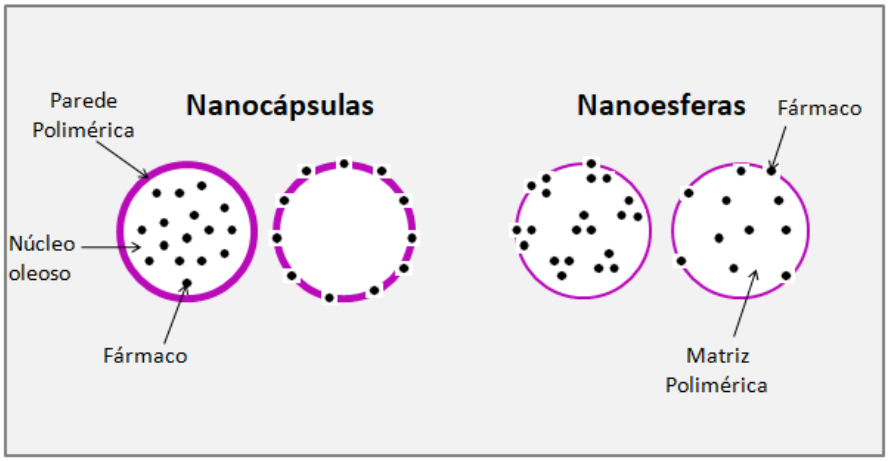

Figura 3. Representação esquemática de nanocápsulas e nanoesferas poliméricas (Adaptado de SCHAFFAZICH e GUTERRES, 2003).

Uma das modificações mais comuns é a funcionalização ou a adição de ligantes à superfície das nanopartículas para que possam interagir diretamente com as células endoteliais, como é o caso da transferrina, insulina e tiamina, que têm receptores específicos na BHE (TOSI et al., 2017). Estudos *in vivo,* mostram a alta solubilidade de fármacos lipofílicos em óleo possibilitam a diminuição da toxicidade (DA COSTA et al., 2014), o aumento da eficácia e biodisponibilidade (HAAS et al., 2014) e permitindo o *delivery* cerebral de fármacos (BENVEGNU et al., 2011; 2012). Diante do exposto, estes sistemas podem contribuir no tratamento de neuropatologias, facilitando a penetração cerebral e amenizar os efeitos adversos.

2.6 Curcumina

A curcumina é um composto fenólico extraído da raiz ou rizoma de açafrão indiano *Curcuma longa* (SHARMA, 1976) (Fig.3), que impede a oxidação ao agir como um limpador de radicais livres (MORAN et al., 2016).

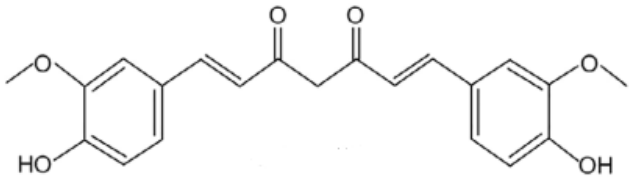

Figura 4. Estrutura química da curcumina (BHAT et al., 2019)

A molécula curcumina é pertencente a uma classe de β-dicetonas de ocorrência natural, que pode atuar como ligante, a funcionalidade central da β-dicetona é flanqueada por dois grandes grupos –CH=CH-C_6H_4(OH) (OMe) -3,4 contendo grupos fenólicos –OH, que são centros reativos (MORALES et al., 2019).

Recentemente, foi relatado que a curcumina possui ampla gama de atividades farmacológicas, como anti-inflamatória, antivírus, antimicrobiana, antioxidante, anti-parasitária, anti-mutagênica e anticâncer, com baixa ou nenhuma toxicidade intrínseca (YANG,2014; HANI,2013; LV,2013). Estudos mostram que o uso da curcumina reduz efeitos como a perda da função cognitiva influenciada pela inflamação causada pela DA (AGGARWAL e HARIKUMAR, 2009).

Como relatado por TETER e colaboradores 2019, a curcumina conseguiu restaurar a expressão gênica de risco para DA melhorando patogênese de Alzheimer. A curcumina tem mostrado prevenir a morte de neurônios em modelos animais de doenças neurodegenerativas e parece estimular o desenvolvimento da neurogênese hipocampal (KIM et al., 2008).

Outros efeitos da curcumina é a prevenção do dano causado pela βA, atenuando a disfunção sináptica (MORALES et al., 2014). Pesquisas mostraram efeitos de nanomicelas carregadas com curcumina na inibição da amilóidegênese prevenindo a formação e acúmulo de fibrilas amilóides e assim suprimindo a DA (MIRZAIE et al., 2019).

Investigações relataram que a curcumina tem efeito protetor contra o estresse oxidativo. Um estudo observou os efeitos protetores da curcumina contra o estresse oxidativo no cérebro, fígado e rins, induzido em um modelo de estresse por contenção, a curcumina além de atenuar significativamente o estresse oxidativo, preveniu a apoptose e aumento da atividade do mecanismo de defesa antioxidante nos tecidos (SAMARGHANDIAN et al., 2017).

Coletivamente, esses resultados fortalecem a característica neuroprotetora da curcumina. No entanto, a curcumina tem baixa solubilidade na água e seu rápido metabolismo e excreção estão relacionados a uma baixa biodisponibilidade o que limita seus benefícios potenciais em organismos vivos (HEGER, et al 2014). Portanto, a incorporação da curcumina em nanocápsulas é uma ferramenta valiosas na tentativa de aumentar sua biodisponibilidade (HUAN et al., 2018). Exposto isso, o uso terapeutico de nanocápsulas do núcleo lipídico (NLC) surge devido à capacidade de melhorar o direcionamento de drogas no cérebro (CARRENÕ et al, 2016., SONVICO et al, 2017).

3 OBJETIVOS

3.1 Objetivo Geral

O objetivo geral do presente estudo foi investigar os efeitos de nanocápsulas poliméricas de curcumina (NLC C) no comportamento tipo depressivo e no estresse oxidativo induzido por um modelo da DA em camundongos.

3.2 Objetivos Específicos

Os objetivos específicos desta dissertação incluíram:

- Determinar o efeito protetor das diferentes formulações de curcumina (NLC C e não encapsulada) no comportamento tipo depressivo induzido pelo agregado βA_{25-35}nos camundongos;
- Avaliar a atividade locomotora espontânea dos camundongos tratados com NLC C e/ou curcumina e/ou expostos ao peptídeo $A\beta_{25-35}$;
- Investigar o efeito das diferentes formulações de curcumina e da infusão i.c.v do peptídeo $A\beta_{25-35}$ sobre os níveis de RS e NPSH no córtex pré-frontal e hipocampo dos camundongos;
- Estimar o efeito da NLC C e da curcumina não encapsulada na atividade das enzimas do sistema de defesa antioxidante, a CAT e a SOD, no córtex pré-frontal e hipocampo de animais submetidos a infusão i.c.v do peptídeo $A\beta_{25-35}$;

4 JUSTIFICATIVA

A DA é um distúrbio neurodegenerativo classicamente caracterizado pelo comprometimento das funções cognitivas e depressão. O desenvolvimento de DA inclui inflamação e estresse oxidativo resultante do acúmulo de proteína Aβ no cérebro, embora a DA seja estudada há muitos anos, os tratamentos para essa enfermidade são limitados e a falta de fármacos empregados para o tratamento e a prevenção da DA, que associam uma alta eficácia e baixos efeitos colaterais, têm estimulado a pesquisa por novos agentes que possam representar uma alternativa terapêutica. A curcumina apresenta propriedades antioxidantes e anti-inflamatórias, mas apresenta baixa biodisponibilidade. Nesse contexto, o estudo das propriedades farmacológicas de nanocápsulas de curcumina (NLC C) poderia ser uma alternativa interessante para potencializar os efeitos farmacológicos da curcumina e podendo auxiliar no tratamento das alterações neurocomportamentais e neurobiológicas presentes na DA.

5 ARTIGO CIENTÍFICO

A metodologia utilizada e os resultados obtidos neste trabalho serão apresentados no formato de um manuscrito científico intitulado "**CURCUMIN NANOCAPSULES REVERSES THE DEPRESSIVE-LIKE BEHAVIOR AND OXIDATIVE STRESS INDUCED BY β-AMYLOID IN MICE**", a ser submetido à revista ***Neuroscience*** *(Elsevier*), cuja formatação segue as normas para submissão no periódico e estão disponíveis no anexo I.

CURCUMIN NANOCAPSULES REVERSES THE DEPRESSIVE-LIKE BEHAVIOR AND OXIDATIVE STRESS INDUCED BY β-AMYLOID IN MICE

Eduarda Monteiro Fidelis[1], Anne Suély Pinto Savall[1], Edina da Luz Abreu[1], Felipe Carvalho[2], Flávia Elizabete Guerra Teixeira[2], Sandra Haas[2], Tuane Bazanella Sampaio[3], Simone Pinton[1]*

[1] Programa de Pós-Graduação em Bioquímica, Universidade Federal do Pampa - Campus Uruguaiana, Uruguaiana, CEP 97500-970, RS, Brazil.

[2] Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal do Pampa, Campus Uruguaiana, Uruguaiana, CEP 97500-970, RS, Brazil.

[3] Programa de Pós-Graduação em Farmacologia, Centro de Ciências da Saúde, Universidade Federal de Santa Maria, Santa Maria, CEP 97105-900, RS, Brazil.

*Correspondingauthor:
Simone Pinton,
Universidade Federal do Pampa - Campus Uruguaiana, Uruguaiana, CEP 97500-970, RS, Brazil
Phone: 55-55-3911-0200
E-mail: sipinton@gmail.com

Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder classically characterized by impairment of cognitive functions. However, depression is one of the most frequent behavioral changes observed in the Alzheimer's disease symptomatology. AD development includes inflammation and oxidative stress resulting from the Aβ protein accumulation in the brain. Curcumin shows antioxidant and anti-inflammatory proprieties but it has low bioavailability. In this context, the use of curcumin's nanocapsules (NLC C) emerges. Thus, the aim this study was to investigate the effects of NLC C on the depressive behavior and oxidative stress induced by an animal model of AD. For this, Swiss male mice were divided into five groups and only the Aβ, Aβ+NLC C and Aβ+Curcumin groups received intracerebroventricular injection of the $A\beta_{25-35}$ aggregate. Control and NLC C groups received only vehicle. The NLC C were administered via gavage at a dose of 10 mg/kg in alternate days for 12 days. Our results showed that Aβ infusion induced a depressive-like behavior observed in the tail suspension test and forced swimming test, which were reversed by NLC C treatment. Furthermore, NLC C was able to restore the Aβ-generated oxidative stress in the prefrontal cortex, evidenced by the increase in the reactive species levels, superoxide dismutase and catalase activities. Importantly, NLC C displayed superior effects than the free curcumin. Thus, we demonstrated the antidepressant-like and antioxidant effects of NLC C in a mice model of AD, suggesting its therapeutic potential for this disorder.

Keywords: Alzheimer's disease; curcumin; depression; nanocapsules; oxidative stress.

Introduction

Alzheimer´s disease (AD) is a neurodegenerative disorder classically characterized by cognitive functions impairments, extracellular amyloid-β peptide (Aβ) plaques and the intracellular neurofibrillary tangles (Perez and Ruiz, 2012). The augment in the Aβ production is a result of the amyloid precursor protein (APP) cleavage, which also is elevated in AD (Joachin and Selkoe, 1992; Tuppo and Arias, 2005; Cuell, 2017). On the other hand, intracellular neurofibrillary tangles are formed by tau hyperphosphorylation (Cheng and Bai, 2018). As result, there is loss of structural function of the cytoskeleton which compromises the axonal transport, generating synaptic dysfunction and neuronal death related to neuroinflammation and oxidative stress processes (Iqbal et al., 2010; Giraldo et al., 2014; Sun and Chen, 2015; Cheng and Bai, 2018).

In this sense, these molecular changes are mainly linked to cognitive disruption observed in the AD patients. However, it is known that changes in the mood, including apathy and depression, also are present in the AD symptomatology (Perez and Ruiz, 2012). Indeed, depression is one of the most frequent behavioral changes observed in the AD (Benoit et al., 2012). Moreover, the high incidence of depressive signals in AD patients is associated with increased risk of morbidity and mortality (Steck et al., 2018).

Regarding molecular changes, extracellular Aβ can generate reactive species and lipid peroxidation products that develop mitochondria damage leading to cell death by apoptosis (Chauhan and Chauhan, 2006). Due to the elevated consumption of oxygen, the brain becomes vulnerable to the oxidative damage. Really, the increase of free radicals formation and the changes in the activities of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) were reported in the central nervous system and peripheral tissues of AD carriers (Omar et al., 1999).

Curcumin (Fig. 1), a natural phenolic compound extracted from the root or rhizome of Indian saffron (*Curcuma longa*) (Sharma, 1976), displays antioxidant (Samarghandian et al., 2017), anti-inflammatory (Teter et al., 2019), and cytoprotective properties (Panda et al., 2017). Importantly, Aggarwal and Harikumar (2009) demonstrated that the curcumin reduces the cognitive deficit associated to inflammation caused by an AD mouse model.

However, curcumin has low biodisponibility (HUAN et al., 2018). In this context, the use of lipid core nanocapsules (NLC) emerges because of its ability to improve the targeting of drugs to brain (Carrenõ et al, 2016; Sonvico et al, 2017). Thus, the aim of the present study was to investigate the effects of curcumin polymeric nanocapsules (NLC C) in the depressive-like behavior and oxidative stress induced by a mice model of AD.

Materials and Methods

Drugs

Firstly, Aβ peptides (fragment 25-35) were dissolved in sterile filtered water and aggregated by incubation at 37 °C for 4 days. Poly (ε-caprolactone) (MW: 80KDa), Aβ peptide, curcumin and sorbitan monostearate (Span 60®) were obtained from Sigma-Aldrich (St. Louis, MO). All other chemicals were of analytical grade and obtained from standard commercial suppliers.

Lipid Core Nanocapsules Preparation and Characterization

NLC C (0.6 mg/ml) were prepared by nanoprecipitation method (Sonivico et al, 2017). Briefly, the organic phase poly-ε-caprolactone, capric caprilic triglycerides, sorbitan monoestearate, curcumin and acetone were solubilized at 40 ºC. Then, this phase was poured into aqueous solution with polysorbate 80. Unloaded nanocapsules (NLC U) were prepared without any drug. The physicochemical characteristics of formulations were evaluated by the particle size, zeta potential, pH and drug content, as described by Hoppe et al. (2013).

Animals

The experiments were performed using male Swiss mice (40 – 50g; 3 months-old) from Federal University of Santa Maria, RS, Brazil. The animals were maintained in an appropriate room under a 12h light/dark cycle (lights on at 7:00 a.m.), controlled temperature (22°C - 25°C), and with free access to water and food. All experimental procedures were carried out according to the local ethics committee of the Federal University of Pampa, RS, Brazil (CEUA/UNIPAMPA 02/2015). All efforts were made

to minimize animals suffering and to reduce the number of animals used in the experiments.

Experimental protocol

In order to evaluate the effects of NLC C in the depressive-like behavior and oxidative stress induced by $A\beta_{25-35}$, mice were randomly divided into five groups (n=7-8/group):

(I) **Control:** vehicle (intracerebroventricular – i.c.v.) and NLC U by intragastric route (i.g.);
(II) **Aβ:** $A\beta_{25-35}$ (i.c.v.) and NLC U (i.g.);
(III) **NLC C:** vehicle (i.c.v.) and NLC C 10 mg/Kg (i.g.);
(IV) **Aβ + NLC C:** $A\beta_{25-35}$ (i.c.v.) and NLC C 10mg/Kg (i.g.);
(V) **Aβ + C:** $A\beta_{25-35}$ (i.c.v.) and curcumin in canola oil 10mg/Kg) (i.g.).

The animals from groups II, IV, and V received the aggregated $A\beta_{25-35}$ (3 nmol/3 µL, i.c.v.), and those from groups I and III received only vehicle (sterile filtered water - 3 µL, i.c.v.) (Ianiski et al, 2012). For this, there was used a micro syringe with a 28-gauge stainless-steel needle 3.0 mm long (Hamilton), as described by Haley and McCormick (1957).

The NLC U (10 ml/Kg), NCL C (10 mg/mL), and curcumin (10 mg/mL) treatments were carried out by intragastric gavage (i.g.) in alternate days, once every 48 h, according to previously described by NAKAMA and previous data of group. Thus, mice received treatments at days 2, 4, 6, 8, 10, and 12.In the twelfth day after induction, open field, tail suspension (TST), and forced swimming (FST) tests were performed 30 minutes after the last treatment. In the follow day, mice were decapitated and the prefrontal cortices and hippocampi were dissected for posterior analyses.

Behavioral tests

Open Field

Spontaneous locomotors activity was measured in the open field test as described by Walsh and Cummins (1976). The floor of the open field apparatus (50

cm × 50 cm × 50 cm) was divided into equal nine squares. Each animal was placed individually in the center of the area, and the number of segments crossed (four-paw criterion) and rearing were recorded in a 5 min session.

Tail Suspension test (TST)

Mice were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. The immobility time was recorded for 6 min. The immobility behavior was defined according to the method described by Steru (1976).

Forced Swimming test (FST)

In this test, mice were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water at 25 ± 1 °C. The total time of immobility was recorded during the 6 min period. Each mouse was judged immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. A decrease in the immobility time is indicative of an antidepressant-like effect (Porsolt et al. 1977).

Oxidative stress parameters

Sample preparation

Twenty-four hours after the last drug administration, at 13th day, mice were euthanized, decapitated, and the prefrontal cortices and hippocampi were dissected. The samples were homogenized in 50 mM Tris–HCl, pH 7.4 (1/10, w/v). The homogenates were centrifuged at 2400*g* for 10 min at 4°C, and the low-speed supernatant fraction (S1) was used for the following analyses: reactive species (RS) and non-protein thiol (NPSH) levels, and superoxide dismutase (SOD) and catalase (CAT) enzymatic activities.

Reactive Species (RS)

The RS levels were determined by a spectrofluorometric method using the 2′,7′dichlorofluorescein diacetate (DCHF-DA). DCHF-DA is a non-fluorescent

compound that easily crosses cell membranes and, in the presence of RS, is rapidly oxidized to its fluorescent derivative dichlorofluorescein (DCF) (Loetchutinat et al., 2005). The RS production was determined by diluting S1 (1:10) in 50 mM Tris–HCl (pH 7.4). S1 was incubated with 10 µl of DCHF-DA 1 mM at room temperature for 60 min. The DCF fluorescence intensity emission was recorded at 520 nm (with 480 nm excitation). The RS levels are expressed as units of fluorescence of DCF (UF).

Non-protein Thiol (NPSH) levels

The NPSH levels were determined by the method of Ellman (1959). S1was mixed (1:1) with 10% trichloroacetic acid. After the centrifugation, the protein pellet was discarded and free thiols groups were determined in the clear supernatant. An aliquot of supernatant was added in 1 M potassium phosphate buffer (pH 7.4) and 10 mM 5.5´dithiobis-(2-nitrobenzoin acid). The color reaction was measured at 412nm. NPSH levels are expressed as nmol NPSH/g tissue.

Superoxide Dismutase (SOD) activity

SOD activity was measured spectrophotometrically as described by Misra and Fridovich (1972). This method is based on the capacity of SOD in inhibiting the autoxidation of epinephrine to adrenochrome. The color reaction was measured at 480 nm. For SOD activity determination, S_1 was diluted 1:10 (*v/v*). Aliquots of S_1 were added in a 50 mM sodium carbonate (Na_2CO_3) buffer, pH 10.3 and the enzymatic reaction was initiated by adding epinephrine. One unit of enzyme was defined as the amount of enzyme required to inhibit the rate of the epinephrine autoxidation by 50% at 26ºC. The enzymatic activity is expressed as Units (U) SOD/mg protein.

Catalase (CAT) activity

CAT activity in S1 was assayed spectrometrically by the method of Aebi, (1984), which monitors the disappearance of H_2O_2 in the presence of sample at 240nm. An aliquot of S1was added in 50mM potassium phosphate buffer pH 7.0 and the enzymatic reaction was initiated by adding H_2O_2. One unit of enzyme was defined as the amount of enzyme required for monitoring the disappearance of H_2O_2. The enzymatic activity is expressed as Units ((IU decomposes 1µmol H_2O_2/min at pH 7 at 25 ºC) CAT/mg protein.

Statistical Analysis

The results are represented as means ± S.E.M. The statistically significant difference between the groups was calculated by one-way analysis of variance (ANOVA) followed by the Tukey's test. Values of probability less than 0.05 ($P < 0.05$) were considered statistically significant. Statistical analysis was performed using the GraphPad Prism 6.0 (GraphPad, San Diego, CA, USA).

Results

NLC characterization

NLC were successfully prepared by the nanoprecipitation method. The parameters are in agreement with a colloidal system. The particle diameter ranged from 291 to 312 nm, with adequate poly disparity (SPAN below 2). The zeta potential was negative (22-36 mV), obtained pH was in the neutrality range (6.01 - 6.98). The content of curcumin was close to 100% in the formulations, indicating minimal losses throughout the formulation preparation process.

Behavioral assays

In order to avoid bias linked to motor impairments on the putative antidepressive-like effect of NLC C, the spontaneous locomotor activity was evaluated in the open field test. The number of crossings and rearings addressed in the open field was not altered by any treatments (Table 1). On the other hand, $A\beta_{25-35}$ infusion induced a depressive-like behavior, observed by an increase of immobility time in the TST and FST, which was reversed by the NLC C treatment (Figures 2 and 3). Additionally, it was observed a main effect of NLC C on the immobility time in the FST, indicating a *per se* antidepressive-like action (Figure 3). Importantly, the free curcumin did not reverse the depressive-like behavior induced by $A\beta_{25-35}$ infusion in the TST and FST (Figures 2 and 3).

Oxidative stress parameters

Overall, our data demonstrated that the $A\beta_{25-35}$ infusion caused an increase in the oxidative stress in the prefrontal cortex, which was reversed by the NLC

treatment. $A\beta_{25-35}$ administration induced an elevation on the RS levels, SOD and CAT activities in the prefrontal cortex when compared to the control group. Of note, the NLC C treatment was able to restore all $A\beta_{25-35}$-inducedchanges (Figures 4A, 6A and 7A). Interestingly, the free curcumin was effective in reversing only the CAT activity in the prefrontal cortex (Figure 7A). NPSH levels were no change by any treatment in the prefrontal cortex (Figure 5A).No significant differences were observed in the evaluated hippocampal parameters (Figures 4B, 5B, 6B and 7B).

Discussion

In this study, we explore the behavioral and biochemical effects of NLC C treatment against to $A\beta_{25-35}$ administration in mice. $A\beta_{25-35}$ infusioninduced a depressive-like behavior observed by an increase in the immobility time in both TST and FST, which were reversed by NLC C treatment. In addition, our data demonstrated an antioxidant action of NLC C against to oxidative stress caused by $A\beta_{25-35}$ infusion in the prefrontal cortex. It should be noted that the free curcumin was not able to restore the most changes induced by $A\beta_{25-35}$.

Although the AD etiology is inconclusive, there is a substantial body of evidence demonstrating the pivotal role of Aβ in the pathophysiology of this disease (Bateman et al., 2012; Zhao et al., 2011). At the histopathological level, the neurofibrillary tangles are formed by hyper phosphorylated tau protein, whereas the neurotic plaques are derived from Aβ, which coexists with astrogliosis and neuronal death in some brain regions, such as the cerebral cortex and hippocampus (Hampel et al., 2018). In this context, 11-amino acid synthetic peptide ($A\beta_{25-35}$) is employed as an effective alternative to the transgenic animals. Further, the intracerebral injection of Aβ fragment has contributed considerably to advances in understanding the mechanisms involved in the neurotoxicity (Van Dan and De Deyn, 2006).

AD is classically considered a cognitive disorder. However, almost all AD carriers develop neuropsychiatric symptoms at some stage during the disease (Cheng et al. al., 2012).

Depression affects a large number of elderly people and is related with poor cognitive function (Fiske et al., 2009). In fact, up to 50% of AD patients exhibits clinically significant depressive symptoms (Starkstein et al, 2005). Moreover, depressive symptoms in AD have been associated to an accelerated functional

decline and mortality (Orgeta et al, 2017). Corroborating to this, we found that the $A\beta_{25-35}$ infusion caused depressive-like behavior both TST and FST. Of note, NLC C treatment reversed these changes, indicating an antidepressant-like effect. In this sense, Fan et al. (2019) recently demonstrated the antidepressant-like action of free curcumin in the FST against a model of chronic unpredictable mild stress. However, it should be highlighted that our data revealed a more prominent antidepressant-like effect of NLC C than free curcumin form in the TST.

In addition, NLC C treatment displayed an antioxidant effect against to oxidative changes $A\beta_{25-35}$-induced in the prefrontal cortex of mice. Studies have shown that the formation of reactive oxygen species is an important mechanism in the pathogenesis of AD (Baum et al., 2002). Herein, we found that the NLC C treatment was able in decrease the RS levels, SOD and CAT activities elevated by $A\beta_{25-35}$ administration in the prefrontal cortex. According to our data, Jaques et al. (2013) demonstrated that free and nanoencapsulated curcumin reversed the smoke-caused oxidative stress. However, in our study, free curcumin restored only the CAT activity. Studies suggest that the neuroprotective effect of curcumin is linked to reduction of RS production (Ono et al., 2004; Jagatha et al., 2008; Jaques et al. 2013). Curcumin protects neurons from oxidation by restoring the mitochondrial membrane potential, enhancing the SOD function, and inhibiting the intracellular RS production (Jagatha et al., 2008). In addition, curcumin binds to Aβ and prevents the protein aggregation, reducing the progression of neuronal damage in AD brains (Ono et al., 2004).

In summary, the present study showed that Aβ infusion induced a depressive-like behavior observed in the tail suspension test and forced swimming test, which were reversed by NLC C treatment. Furthermore, NLC C was able to restore the Aβ-generated oxidative stress in the prefrontal cortex, evidenced by the increase in the reactive species levels, superoxide dismutase and catalase activities. Importantly, NLC C displayed superior effects than the free curcumin. Thus, we demonstrated the antidepressant-like and antioxidant effects of NLC C in a mice model of AD, suggesting its therapeutic potential for this disorder.

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Glossary

Alzheimer's disease (AD)

Amyloid-β peptide (Aβ)

Amyloid precursor protein (APP)

Tail Suspension test (TST)

Forced Swimming test (FST)

Reactive Species (RS)

Non-protein Thiol (NPSH)

Superoxide Dismutase (SOD)

Catalase (CAT)

Nanocapsule of curcumin (NLC C)

Intracerebroventricular (i.c.v)

Unloaded nanocapsules (NLC U)

2′,7′dichlorofluorescein diacetate (DCHF-DA)

Intragastric route (i.g.)

Figure Captions

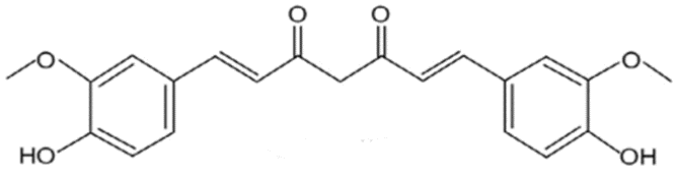

Figure 1: Chemical structure of curcumin

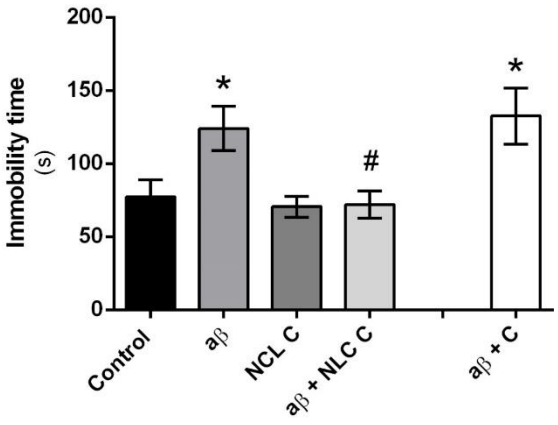

Figure 2: The NLC C and curcumin effects on immobility time in the TST in a mouse model of AD induced by Aβ.Data are shown as mean ± S.E.M. of 7-8 animals per group. *$P < 0.05$ as compared to the control group and $^{\#}P < 0.05$ as compared to the Aβ group (One-way ANOVA followed by Tukey's test).

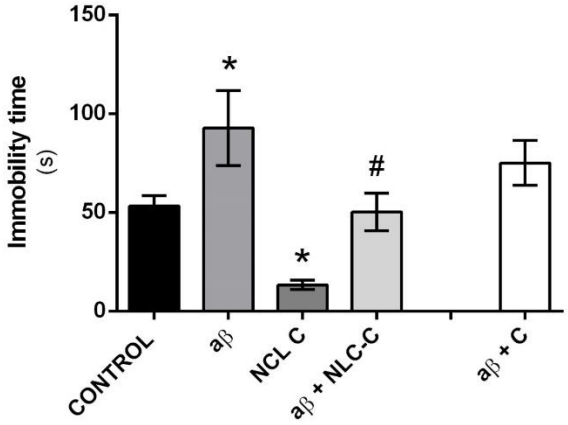

Figure 3: The NLC C and curcumin effects on immobility time in the FST in a mouse model of AD induced by Aβ. Data are shown as mean ± S.E.M. of 7-8 animals per group. *P < 0.05 as compared to the control group and $^{\#}P$ < 0.05 as compared to the Aβ group (One-way ANOVA followed by Tukey's test).

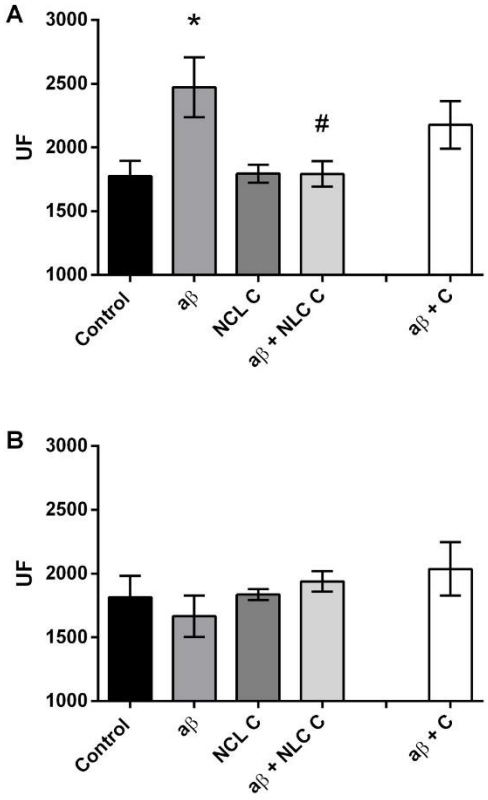

Figure 4: The NLC C and curcumin effects on the RS levels in the prefrontal cortex (A) and hippocampus (B) of mice subjected to a mouse model of AD induced by Aβ. Data are shown as mean ± S.E.M. of 7-8 animals per group. *P < 0.05 as compared

to the control group and $^{\#}P < 0.05$ as compared to the Aβ group (One-way ANOVA followed by Tukey's test).

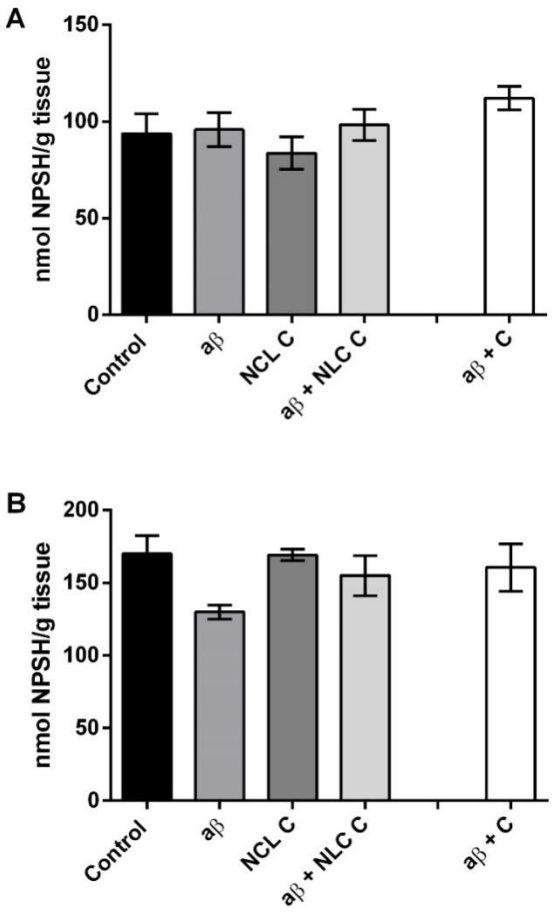

Figure 5: The NLC C and curcumin effects on the NPSH levels in the prefrontal cortex (A) and hippocampus (B) of mice subjected to a mouse model of AD induced by Aβ. Data are shown as mean ± S.E.M. of 7-8 animals per group (One-way ANOVA followed by Tukey's test).

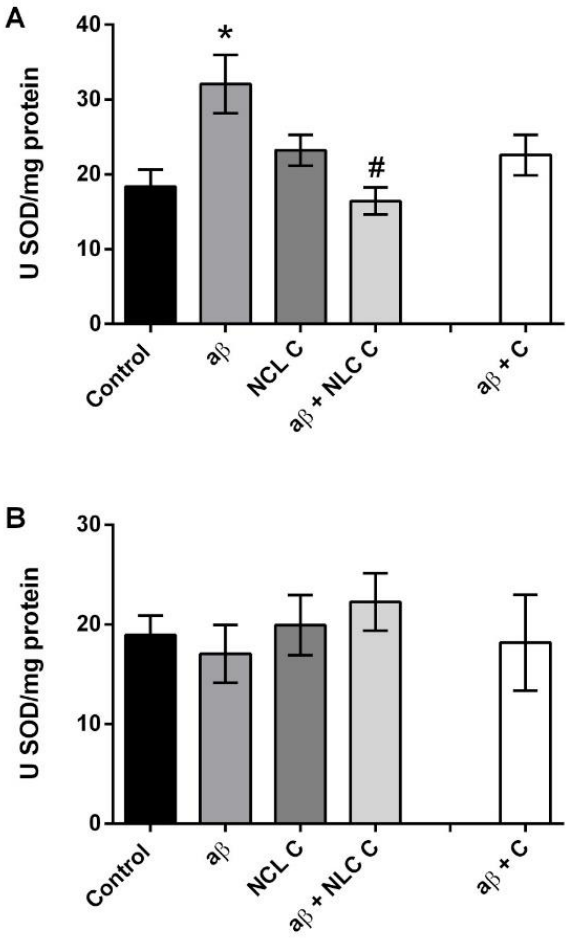

Figure 6: The NLC C and curcumin effects on the SOD activity in the prefrontal cortex (A) and hippocampus (B) of mice subjected to a mouse model of AD induced by Aβ. Data are shown as mean ± S.E.M. of 7-8 animals per group. *P < 0.05 as compared to the control group and $^{\#}P$ < 0.05 as compared to the Aβ group (One-way ANOVA followed by Tukey's test).

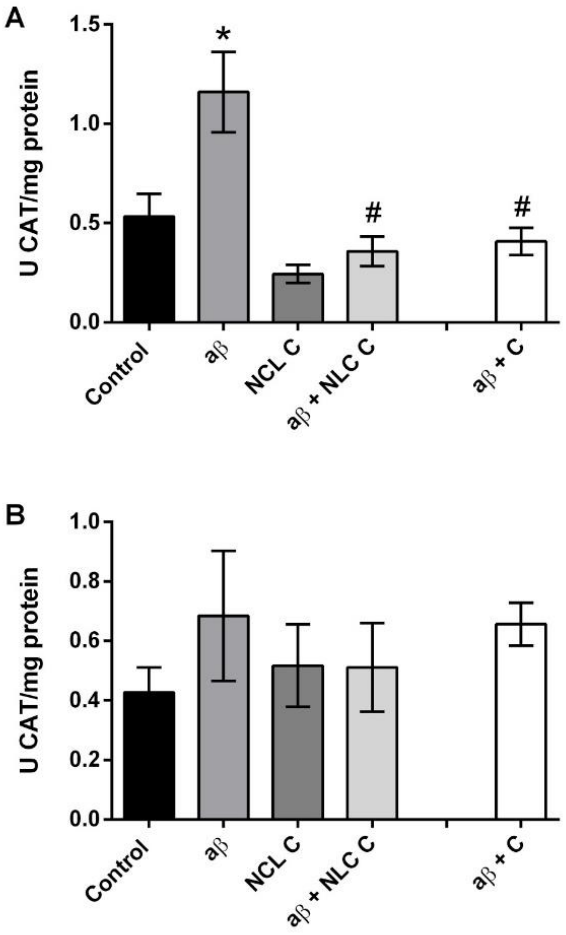

Figure 7: The NLC C and curcumin effects on the CAT activity in the prefrontal cortex (A) and hippocampus (B) of mice subjected to a mouse model of AD induced by Aβ. Data are shown as mean ± S.E.M. of 7-8 animals per group. **P* < 0.05 as compared to the control group and $^{\#}P$ < 0.05 as compared to the Aβ group (One-way ANOVA followed by Tukey's test).

Table 1: Aβ infusion, curcurmin and curcumin nanocapsules treatment did not alter the spontaneous locomotion in mice in the Open Field Test.

	Crossing	Rearing
Control	67.87 ± 4.42	23.71 ± 2.94
aβ	64.37 ± 6.98	24.14 ± 3.74
NLC C	58.72 ± 3.60	22.16 ± 3.06
aβ + NLC C	71.29 ± 1.87	28.28 ± 2.90
aβ + C	72.00 ± 4.25	25.75 ± 2.11

6 Discussão

Na DA o acúmulo da proteína βA no cérebro leva à processos inflamatórios e estresse oxidativo o que resulta na degeneração de sinapses e neurônios particularmente no hipocampo e córtex (ROJAS et al., 2015; CHEIGNON et al., 2017). Os sintomas comportamentais predominantes na DA incluem, declínio no desempenho cognitivo, como prejuízo na memória e na linguagem, depressão e ansiedade (BERGER et al., 1999; CASTILLA-PUENTES e HABEYCH, 2010; CHENG et al., 2012; CHUI et al., 2017).

Nesta dissertação exploramos os efeitos comportamentais e bioquímicos do tratamento com NLC C contra a administração i.c.v. de βA_{25-35} em camundongos. A infusão de βA_{25-35} induziu um comportamento do tipo depressivo observado por um aumento no tempo de imobilidade tanto no TST quanto no FST. Esses testes comportamentais são utilizados na literatura para caracterizar um estado tipo depressivo, onde o tempo de imobilidade, que se assemelha a falta de persistência em escapar, é percebida como uma desistência e associada à um comportamento tipo depressivo (CRYAN et al., 2005).

As substâncias antidepressivas têm função de reverter esse quadro diminuindo o tempo de imobilidade fazendo com que o animal não desista das situações impostas a ele, a NLC C exerceu efeito antidepressivo revertendo no TST e FST. Resultado similar foi descrito por FAN e colaboradores (2019), onde foi observado o efeito protetor da curcumina encapsulada nos comportamentos semelhantes à depressão causada pelo estresse crônico em ratos. A curcumina tem mostrado possuir forte atividade antidepressiva em modelos animais de depressão (LEE e LEE, 2018).

A depressão na DA é altamente proeminente (KESSLER et al., 2003) e acarreta impacto na funcionalidade e qualidade de vida do paciente (BIJL e RAVELLI, 2000), assim como na saúde somática. A DA é a forma mais comum de demência no mundo e é responsável por 60-70% do comprometimento cognitivo em idosos (CUMMINGS e COLE, 2002; PRASAD, 2019). A característica neuropatológica dos pacientes com DA é a depósitos de βA e EN nas regiões neocorticais e límbicas (VERMA et al., 2015). O acúmulo gradual de βA durante vários anos leva a apoptose de células neuronais e atrofia (JIN et al., 2011).

O estresse oxidativo é um mecanismo envolvido no envelhecimento e como doenças neurodegenerativas, o estresse é uma condição fisiológica induzida por estímulos adversos que desequilibram a função fisiológica do corpo (SAHIN e GÜÜMÜSLÜMÜSLÜ, 2007). Essas alterações levam à superprodução de radicais livres e espécies altamente reativas que em excesso causam dano oxidativo a biomoléculas celulares incluindo proteínas, lipídios e ácidos nucleicos em vários tecidos, contribuindo assim para a perda neuronal (SAMARGHANDIAN et al., 2017).

Nos últimos anos tem se buscado agentes naturais como alternativas terapêuticas no tratamento de doenças neurodegenerativas, antioxidantes e drogas anti-inflamatórias geralmente são recomendadas para pacientes com DA (OGHABIAN e MEHRPOUR, 2016).

A curcumina tem sido alvo de atenção por apresentar atividade anti-inflamatório, antioxidante e atividade antimicrobiana (MERRELL et al.,2009), apesar dessas propriedades farmacológicas, o uso da curcumina é limitada por questões relacionadas à absorção, distribuição, biodisponibilidade e metabolismo, além de sua propensão e degradação fotoquímica (LIU et al., 2016), como estratégia para melhor desempenho a utilização de nanocápsulas foram propostas para melhorar a biodisponibilidade e eficiência da curcumina.

Nossos dados demonstraram uma ação antioxidante do NLC C contra o estresse oxidativo causado pela infusão de $A\beta_{25-35}$ no córtex pré-frontal. Estudos demonstraram que o comprometimento da função do mecanismo colinérgico é de importância crítica na DA, especialmente nas áreas cerebrais de aprendizado, memória e respostas emocionais, como o córtex cerebral (GUTIERRES et al.,2014). Estudos mostram que a curcumina foi capaz de inibir a toxicidade de βA no e radicais de oxigênio (LIN et al., 2008; SHIMMYO et al.,2008). Deve-se salientar que a curcumina livre em nosso estudo não foi capaz de restaurar a maioria das alterações induzidas por βA_{25-35}.

A modulação do sistema oxidante-antioxidante tem sido considerada como outra abordagem no tratamento e prevenção de DA (GHADERI et al., 2015; MEHRPOUR et al., 2012). O uso de antidepressivos na DA está associado a efeitos colaterais significativos, como cardiotoxicidade (FARKHONDEH et al.,2016) e aumento da tendência ao sangramento (SAMARGHANDIAN et al., 2014; YUAN et al., 2013).

A curcumina exerce atividade antioxidante afetando as RS e indutores de regulação positiva de proteínas antioxidantes (TRUJILLO et al., 2013). Dados na literatura mostram que a curcumina aumenta as atividades das enzimas antioxidantes SOD e CAT e os níveis de GSH em roedores (AK e GÜLÇIN, 2008; DKHAR e SHARMA, 2010). Em nosso estudo descobrimos que o tratamento com NLC C foi capaz de diminuir os níveis de RS, a atividade de SOD e CAT foram elevados pela administração de $A\beta_{25-35}$ no córtex pré-frontal exercendo assim efeito antioxidante. No entanto, em nosso estudo, a curcumina livre restaurou apenas a atividade da CAT. Corroborando com esses resultados, ananocápsula de curcumina foi capaz de minimizar o efeito antioxidante contra a lipoperoxidação em ratos infectados com *L. monocytogenes* (JAGUEZESKI et al.,2019).

De fato, a curcumina livre e encapsulada tem sido reconhecida por seu efeito antioxidante, direta ou indiretamente, aumentando a expressão gênica de antioxidantes endógenos (NIÑO e CHAVERRI et al.,2014), além de protege os neurônios da oxidação, restaurando o potencial de membrana mitocondrial inibindo a produção de RS intracelular (JAGATHA et al., 2008). Vale salientar que, possivelmente, diferentes mecanismos estão envolvidos no tratamento da DA pela curcumina, incluindo a inibição da formação de Aβ, agregação de Aβ, inibição da inflamação, estresse oxidativo e apoptose (FARKHONDEH et al., 2019).

CONCLUSÃO

Portanto está pesquisa mostrou que a infusãoi.c.v. de Aβ induziu um comportamento do tipo depressivo em camundongos, observado no teste de suspensão da cauda e no teste de natação forçada, que foram revertidos pelo tratamento com NLC C. Além disso, o NLC C foi capaz de restaurar o estresse oxidativo gerado pelo Aβ no córtex pré-frontal, evidenciado pelo aumento nos níveis de espécies reativas, atividades de superóxido dismutase e catalase. É importante ressaltar que o NLC C apresentou efeitos superiores aos da curcumina livre. Assim, demonstramos os efeitos antidepressivos e antioxidantes da NLC C em um modelo de camundongo da DA, sugerindo seu potencial terapêutico para essa desordem.

PERSPECTIVAS

Os resultados desta dissertação permitem responder alguns questionamentos e confirmar hipóteses levantadas na literatura contemplando nossos objetivos. No entanto, ajudou a produzir mais questionamentos.

Nesse contexto, considerando os resultados obtidos nesse estudo, fica evidente a perspectiva de continuar a investigação da utilização de NLC C, a qual já tem capacidade de ser explorada em trabalhos posteriores direcionados para se avaliar outras doenças neurogenerativas.

Também é uma perspectiva trabalhar com essa estratégia de nanoenpasulamento em um novo modelo de doença, isquemia e reperfusão. Esse modelo apresenta grande geração de estresse oxidativo devido a oclusão da carótida acarretando em uma reação em cascata.

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ANEXO A

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AUTHOR INFORMATION PACK

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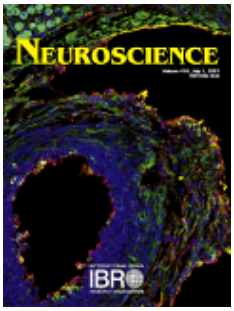

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