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Interleucina - 4 recombinante como possível alvo para o tratamento da depressão

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Hot heads and colds hearts never solved anything.

- Billy Graham

Resumo

SMANIOTTO, Thiago Â. **Interleucina - 4 recombinante como possível alvo para o tratamento da depressão.** 2020. 74F. Dissertação (Mestrado) – Programa de Pós-Graduação em Biotecnologia. Universidade Federal de Pelotas, Pelotas.

A interleucina-4 (IL-4) é uma citocina com atividade anti-inflamatória capaz de agir na redução dos efeitos de inflamações sistêmicas e centrais no organismo. As citocinas pró e anti-inflamatórias estão presentes nas respostas inflamatórias induzidas por patógenos ou por alterações ocasionadas pelo estresse através de estímulos físicos e psicológicos. O estresse é um dos principais fatores que levam ao desencadeamento de respostas inflamatórias, através da liberação de padrões moleculares associados ao perigo (DAMPs, do inglês *damage-associated molecular pattern*). Essas alterações, são utilizadas para explicar o desenvolvimento e progressão da depressão, que afeta cerca de 322 milhões de pessoas mundialmente. Na presente dissertação foi utilizada a IL-4 para avaliar o seu efeito no comportamento do tipo-depressivo induzido pelo modelo de indução do estresse crônico imprevisível e moderado (CUMS, do inglês *chronic unpredictable mild stress*) em camundongos *swiss*. Os camundongos submetidos ao CUMS apresentaram comportamento do tipo-depressivo nos testes de suspensão da cauda (TSC) e teste do splash (TS), bem como alterações fisiológicas, como o aumento de espécies reativas (ER), peroxidação lipídica e alterações nas atividades das enzimas antioxidantes catalase (CAT) e superóxido dismutase (SOD) em córtex pré-frontal, hipocampo e plasma. A nível molecular, ocorreram alterações nas expressões de citocinas como IL-1β, IL-4 e alterações na expressão de NRF2, NF-kB, BDNF e alteração na atividade da IDO em córtex pré-frontal e hipocampo. O tratamento efetuado foi administrar uma única dose de IL-4 via intranasal na dose de 1ng/camundongo (5 µL) que apresentou efeito do tipo-antidepressivo no TSC e TS, e apresentou efeito na modulação do estresse oxidativo e no decréscimo da neuroinflamação. Essas alterações foram comprovadas pelo decréscimo de ER, peroxidação lipídica, e normalização da atividade das enzimas CAT e SOD em córtex pré-frontal, hipocampo e plasma. A diminuição da concentração de corticosterona no plasma e na normalização de expressão de IL-1β, IL-4, NRF2, NF-kB, DBNF e atividade da IDO em córtex pré-frontal e hipocampo também comprovam a atividade da IL-4 na neuroinflamação em camundongos. Esses dados sugerem que a capacidade da IL-4 em reverter o comportamento do tipo-depressivo em camundongos é promissora na utilização no tratamento de sintomas depressivos.

Palavras chave: interleucina-4, CUMS, depressão, estresse, neuroinflamação.

Abstract

Smaniotto, Thiago Â. **Recombinant interleukin-4 as a possible target for the treatment of depression**. 2020. 74F. Dissertation (Master's degree) – Postgraduate program in biotechnology Federal University of Pelotas, Pelotas.

Interleukin-4 (IL-4) is a cytokine with anti-inflammatory activity capable of reducing the effects of systemic and central inflammation in the body. Pro and anti-inflammatory cytokines are present in inflammatory responses induced by pathogens or changes caused by stress through physical and psychological stimuli. Stress is one of the main factors that lead to the onset of inflammatory responses, through the release of damage-associated molecular pattern (DAMPs). These changes are used to explain the development and progression of depression, which affects about 322 million people worldwide. In this dissertation, IL-4 was used to evaluate its effect on depressive-like behavior induced by the chronic unpredictable mild stress induction model (CUMS), in *swiss* mice. The mice submitted to CUMS showed a depressive-like behavior in the tail suspension tests (TST) and splash test (ST), as well as physiological changes, such as the increase in reactive species (RS), lipid peroxidation and changes in the activities of the antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) in prefrontal cortices, hippocampi and plasma. At the molecular level, changes occurred in the expression of cytokines such as IL-1β, IL-4 and changes in the expression of NRF2, NF-kB, BDNF and changes in IDO activity in the prefrontal cortices and hippocampi. The treatment performed was to administer a single dose of IL-4 intranasally at a dose of 1ng / mouse (5 µL), which had an antidepressant-like effect on TSC and TS and influenced the modulation of oxidative stress and decreased neuroinflammation. These changes were confirmed by the decrease in ER, lipid peroxidation, and normalization of the activity of CAT and SOD enzymes in the prefrontal cortex, hippocampus and plasma. The decrease in the concentration of corticosterone in plasma and the normalization of expression of IL-1β, IL-4, NRF2, NF-kB, DBNF and IDO activity in prefrontal cortex and hippocampus also prove the activity of IL-4 in neuroinflammation in mice. These data suggest that the ability of IL-4 to reverse the depressive-like behavior in mice is promising for use in the treatment of depressive symptoms.

Keywords: Interleukin-4, CUMS, depression, stress, neuroinflammation.

Lista de Figuras

Lista de Abreviaturas e Siglas

5-HT	Serotonina
ACTH	Hormônio adrenocorticotrófico
ADTS	Antidepressivos tricíclicos
BDNF	Fator neurotrófico derivado do cérebro
CAT	Catalase
COMT	Catecol-O-metil transferase
CRF	Fator liberador de corticotrofina
CUMS	Estresse crônico imprevisível e moderado
DAMPs	Padrões moleculares associados ao perigo
DM	Depressão maior
DP	Dopamina
DSM-5	Manual de diagnóstico e estatístico de transtornos mentais
ER	Espécies reativas
HPA	Eixo hipotalâmico hipófise e adrenal
IDO	Indoleamina 2,3-dioxigenase
IFN-γ	Interferon gama
IL-1β	Interleucina-1 beta
IL-2	Interleucina-2
IL-4	Interleucina-4
IL-6	Interleucina-6

IL-7	Interleucina-7
IL-10	Interleucina-10
IL-13	Interleucina-13
IMAO	Inibidores da monoamina oxidase
IN	Intranasal
ISRN	Inibidores seletivos da receptação de noradrenalina
ISRS	Inibidores seletivos da recaptação de serotonina
MAO	Monoamina oxidase
NA	Noradrenalina
NF-kB	Fator de transcrição nuclear kappa B
NRF2	Fator nuclear eritróide 2-relacionado ao fator 2
$OH^{\cdot}$	Radical hidroxila
RL	Radicais livres
SNC	Sistema nervoso central
SOD	Superóxido dismutase
SUS	Sistema Único de Saúde
TCA	Teste do campo aberto
TGF-β	Fator de crescimento transformante beta
TLR4	Receptores do tipo *toll-like* do tipo 4
TNF-α	Fator de necrose tumoral alfa
TNPs	Transtornos neuropsiquiátricos
TS	Teste do *splash*
TSC	Teste do campo aberto

Sumário

1. INTRODUÇÃO GERAL

As ameaças a homeostase do nosso organismo estiveram presentes constantemente durante todas as etapas da nossa evolução. Na tentativa de combater patógenos, por exemplo, contamos com um complexo sistema imunológico formado por diferentes estruturas e processos celulares especializados em prevenir ou limitar uma infecção (DE MELO CRUVINEL et al., 2010).

Para desempenhar sua função, o sistema imune conta com ajuda da sinalização de mediadores moleculares, especialmente as citocinas. As citocinas são um grupo heterogêneo de proteínas que são produzidas por células imunocompetentes com a finalidade de regular a resposta imune (HAROON et al., 2012; KIM et al., 2015).

Apesar das particularidades, as citocinas podem ser divididas em pro-inflamatórias e anti-inflamatórias. As principais citocinas pró-inflamatórias são: a interleucina 1 beta (IL-1β), interleucina-6 (IL-6), interferon gama (IFN-γ) e fator de necrose tumoral alfa (TNF-α), enquanto que as anti-inflamatórias são conhecidas como a interleucina-4 (IL-4) e interleucina-10 (IL-10) (COMMINS et al., 2010).

Além do sistema imune, nosso organismo possui uma resposta neuroendócrina que induz mudanças fisiológicas responsáveis por preparar o indivíduo para enfrentar uma situação que ameace a sua homeostase (JURUENA; CLEARE; PARIANTE, 2004). Essa resposta ao estresse é mediada principalmente pelo eixo hipotalâmico-pituitária-adrenal (HPA) e caracterizada pelo aumento nos níveis circulantes de cortisol (ou corticosterona em roedores), culminando com alterações metabólicas, imunes e neuronais (BAUMEISTER et al., 2014).

As sinalizações do eixo HPA são mediadas e reguladas pelos receptores de glicocorticoide (GR) e de mineralocorticoide (MR) (DASKALAKIS et al., 2015). Notavelmente, o mecanismo de *feedback* negativo do eixo HPA é regulado pelos GR presentes no hipocampo e córtex pré-frontal. Nesse mecanismo, o cortisol se liga aos seus receptores regulando assim a sua auto expressão, a expressão do fator neurotrófico derivado do cérebro (BDNF) e citocinas pró- e anti-

inflamatórias (GOUJON et al., 1996; MORSINK et al., 2006; PARIANTE, 2006; SCHULKIN; GOLD; MCEWEN, 1998; SCHULTE-HERBRÜGGEN et al., 2006).

Embora o sistema imune e o eixo HPA desempenhem funções cruciais na sobrevivência humana, prejuízos no seu funcionamento têm sido implicadas na patofisiologia da depressão maior (DM). A DM é uma das principais doenças psiquiátricas da atualidade, e a principal causa de incapacidade entre as pessoas (WORLD HEALTH ORGANIZATION, 2017). Atualmente, a DM afeta mais de 300 milhões de pessoas ao redor do mundo e é a maior contribuinte em mortes por suicido (800.000 casos por ano) (WORLD HEALTH ORGANIZATION, 2017).

Por ser um transtorno crônico e heterogêneo, sua etiologia ainda não foi completamente elucidada, o que dificulta o desenvolvimento de medicamentos eficientes para o seu tratamento. A terapia medicamentosa atual baseia-se no uso de moduladores de neurotransmissores, apresentando eficácia relativa e inúmeros efeitos adversos (PESAR, 1999).

Diante da relevância socioeconômica da DM, da necessidade de tratamentos mais eficientes para esse transtorno e do envolvimento do sistema imune e do estresse na patofisiologia da DM, hipotetizou-se que a modulação desses sistemas seria capaz de atenuar o comportamento tipo-depressivo em camundongos submetidos ao estresse crônico, imprevisível e moderado (CUMS).

A IL-4 é uma das principais citocinas anti-inflamatórias do nosso organismo, responsável por regular a resposta imune pro-inflamatória, vista em testes clínicos para o tratamento de esclerose múltipla (VOGELAAR, et al., 2018). No entanto, não há nenhum estudo investigando o possível efeito terapêutico da IL-4 para transtornos de humor.

Nesse sentido, o objetivo desse projeto foi investigar se a administração intranasal de IL-4 seria capaz de reverter o comportamento do tipo-depressivo que se manifesta em animais submetidos ao estresse crônico imprevisível e moderado (CUMS, do inglês *chronic unpredictable mild stress*) através da modulação do eixo HPA, sistema imune e estresse oxidativo.

2. REVISÃO BIBLIOGRÁFICA

2.1. Citocinas

As citocinas são moléculas que participam de respostas inflamatórias no organismo, tanto periféricas, como centrais. Sua liberação ocorre em inflamações agudas e crônicas, através de uma complexa cascata de sinalização exercida por padrões moleculares associados a patógenos (PAMPs, do inglês *pathogen-associated molecular pattern*) ou DAMPs (TURNER et al., 2014).

Dentre essas citocinas, existem as pró-inflamatórias e as anti-inflamatórias (JAFFER, WADE; GOURLAY, 2010). A regulação da produção de citocinas é responsável pelo sistema regulatório positivo e negativo, que determina a liberação ou inibição da produção de citocinas pró ou anti-inflamatórias (ARAI, et al. 1990; SMALE, TARAKHOVSKY; NATOLI, 2014).

Com isso, uma das características das citocinas é a sua resposta rápida aos estímulos. Embora sejam proteínas complexas, elas não são estocadas, iniciando assim a sua produção quando ocorre a sinalização dos receptores do tipo *toll-like* do tipo 4 (TLR-4), localizados na membrana das células, proporcionando assim a ativação transcricional para sua síntese (QUESENBERRY, 1995; SMALE, TARAKHOVSKY; NATOLI, 2014; WALKER et al., 1995).

Um fator essencial para a sinalização de síntese de citocinas, e a ativação do fator nuclear kappa B (NF-kB), que se encontra de forma inativa no citoplasma das células. Após ativação, ocorre a sua translocação para o núcleo da célula, que sinaliza a produção de citocinas pró-inflamatórias (FUJIHARA et al., 2003; DE OLIVEIRA et al., 2016).

Entre as diversas citocinas existentes, podemos citar as pró-inflamatórias (Th1) na quais temos as interleucinas (IL) 1, 2, 6, 7, e as IL com propriedades anti-inflamatórias (Th2), IL-4, 10 e 13. Em respostas a infecções e lesões, são liberadas as citocinas pró-inflamatórias, que mediam a resposta de sinalização para favorecer a resposta imune do organismo, mas em excesso podem causar instabilidade hemodinâmica ou até mesmo distúrbios metabólicos (SALOMÃO et

al., 2013). Com isso, as IL (Th2) agem de forma a minimizar esses efeitos no organismo.

Em geral, as citocinas pró-inflamatórias IL-1, 2, 6, INF-γ, TNF-α, são responsáveis pela sinalização do sistema imune para ativarem respostas anti-inflamatórias, como a liberação de BDNF, IL-4, 10, 11, 13 e fator de crescimento transformante beta (TGF-β) (KRONFOL E REMICK, 2000). Na periferia as citocinas pró-inflamatórias são ativadas por respostas imunológicas por monócitos ou macrófagos (FELGER E LOTRICH, 2013; JEON; KIM, 2016).

Em pacientes com depressão, são encontrados níveis elevados de IL-1β nas regiões de córtex, hipocampo e na corrente sanguínea (SANTOS-BARRIOPEDRO; VAQUERO, 2018). Em ambas as estruturas, esse excesso ocasiona danos nas células, podendo assim, diminuir a atividade e liberação de citocinas anti-inflamatórias (KIM; WON, 2017).

Contudo, o cérebro é um órgão que possui muitas defesas contra as ações das citocinas e uma delas é a barreira hematoencefálica, que impede que as citocinas cheguem ao sistema nervoso central (SNC). No entanto, o estresse ocasiona alterações na permeabilidade da barreira ocorrendo assim o alcance desses mediadores ao SNC (WATKINS et al., 1995; DANTZER et al., 1999; SCHIEPERS et al., 2005; SERUGA et al., 2008; KIM et al., 28 2015; SLYEPCHENKO et al., 2016).

Com isso, uma das alternativas do sistema imunológico através da micróglia é liberar citocinas anti-inflamatórias, como a IL-4, capaz de agir na reestruturação de danos axonais em neurônios ocasionados pela neuroinflamação (CHRISTINA F. VOGELAAR, SHIBAJEE MANDAL et al., 2018). A IL-4 apresenta efeito sobre macrófagos ativados, reduzindo os efeitos de citocinas como IL-1β, TNFα, IL-6 e IL-8, e pode atuar como inibidora de radicas livres de oxigênio (HELTH, 1978; SALOMÃO et al., 2013).

Sua produção ocorre por linfócitos-TCD4, mastócitos, eosinófilos e basófilos, podendo atuar sobre linfócitos T e B, células matadoras naturais (NK do inglês, *natural killer*), através da via JAK/STAT. Através dessas atividades, é utilizado em estudos para o tratamento de esclerose múltipla, câncer, asma, psoríase, osteoartrite e linfoma (CHRISTINA F. VOGELAAR, SHIBAJEE MANDAL et al., 2018; KURTZ et al., 2007; YORIMITSU et al., 2008). Sendo uma proteína em potencial para seu uso no tratamento de sintomas depressivos.

2.2. Estresse

O estresse, descrito por SELYE, et al. (1950), depois de aproximadamente 70 anos, ainda continua sendo um dos principais assuntos em questão de saúde pública (MCEWEN; AKIL, 2020). Sendo desencadeado por estímulos físicos e psicológicos, o estresse está relacionado com o surgimento e progressão de transtornos neuropsiquiátricos (TNP's). Isso acaba sendo associado à incapacitação de pessoas, com prejuízos na cognição, aumento da ansiedade, crises depressivas e perda de memória (MCEWEN; AKIL, 2020).

Um dos mecanismos do estresse é a hiperativação do eixo HPA, responsável pela liberação de cortisol na corrente sanguínea. A hiperativação do eixo HPA tem sido relatada em pacientes com depressão maior (DM), através de analises de saliva, plasma, urina e liquido espinhal, que contém um elevado nível de cortisol (JURUENA; CLEARE; PARIANTE, 2004; PARIANTE; LIGHTMAN, 2008). Inclusive, uma das características encontradas é o aumento no volume das glândulas adrenais, onde o seu peso aumenta em comparação com pacientes saudáveis (NEMEROFF; VALE, 2005).

A exposição continua ao estresse ocasiona a redução na resposta dos receptores de glicocorticoides ao cortisol. A inibição do feedback negativo é responsável pelas altas concentrações de cortisol no sangue, conhecido como hipercortisolemia (BEATO; HERRLICH; SCHÜTZ, 1995). Essa hipercortisolemia está relacionada com uma redução do volume hipocampal e decréscimo na atividade de neurogênese e sinaptogênese (BOGDAN; HYDE; HARIRI, 2013).

Em resultados relatados na literatura, o cortisol em excesso na corrente sanguínea promove o aumento da expressão de NF-KB ligado a sinalização de produção de citocinas pró-inflamatórias, que também ocasionam o aumento de espécies reativas (ER) (DONG et al., 2018). Com isso, estudos demonstram que a depressão está ligada ao aumento crônico de cortisol na corrente sanguínea (SANTANA et al., 2015; WANG et al., 2015).

2.3. Depressão maior

Os TNP’s prosseguem sendo um dos assuntos mais importantes em questão de saúde pública nas últimas décadas. Sendo a DM a mais evidenciada na população mundial (WORLD HEALTH ORGANIZATION, 2017). Na atualidade são cerca de 322 milhões de pessoas afetadas mundialmente, acarretando grandes prejuízos aos cofres públicos.

No Brasil, gera um grande impacto ao Sistema Único de Saúde (SUS), pois ele disponibiliza medicamentos para o tratamento. Esse prejuízo também se estende na vida social e pessoal do paciente (SAÚDE et al., 2017; WORLD HEALTH ORGANIZATION, 2017).

Até o final de 2020 estima-se que a DM chegue na marca de 400 milhões de pessoas incapacitadas, tornando a TNP que mais incapacita pessoas mundialmente (GALTS et al., 2019). Embora existam inúmeros tratamentos para os sintomas da depressão, cerca de apenas 60% dos pacientes respondem ao tratamento (PESAR, 1999).

Estudos clínicos têm demonstrado que a DM não é diagnosticada logo no início, corroborando para que 40% dos pacientes continuem sem um tratamento adequado. Por ser uma patologia heterogênea e multifatorial que não apresenta um sintoma especifico que a diferencie de outras TNP’s, acaba gerando grande dificuldade de um diagnóstico correto (PARANHOS; WERLANG, 2009).

Algumas alternativas encontradas no mercado geralmente são de medicamentos aliados a psicoterapia. Essa união de tratamento, podem trazer grandes benefícios em alguns casos, podendo até normalizar as alterações cerebrais ocasionados pela DM (EBMEIER; DONAGHEY; STEELE, 2006).

A DM atinge um amplo espectro de indivíduos, de diferentes idades, classes sociais e etnias, o que dificulta o seu tratamento. Para que uma pessoa seja diagnosticada com DM, ela deve apresentar os sintomas de pelo menos 2 semanas. Esses sintomas são descritos pelo Manual de Diagnóstico e Estatistico de Transtornos Mentais 5ª edição (DSM-5, do inglês *Diagnostic and Statistical Manual of Mental Disorders – Fifth Edition*).

Com base nesse manual, é descrito que os sintomas incluem, sentimento de tristeza leve ou moderado, perda de interesse e prazer em atividades antes prazerosas, insônia, perda de apetite, dificuldades em pensar e tomar decisões,

fadiga, pensamentos suicidas e até mesmo suicido, em casos mais graves. Caracterizando esse quadro, os sintomas devem apresentar recorrência de no mínimo duas semanas (AMERICAN PSYCHIATRIC ASSOCIATION, 2013).

Para compreender essas alterações de humor, são utilizadas algumas teorias, que abordam disfunções fisiológica para explicar o surgimento e progressão da DM. Em 1950, foi postulado que a DM era ocasionada pela diminuição da disponibilidade de monoaminas (dopamina (DA), noradrenalina (NA) e serotonina (5-hidroxitriptomina, 5-HT)) na fenda sináptica. De acordo com COPPEN, (1967); IVERSEN, (2008), esse decréscimo promove declínios cognitivos que culminam para o desenvolvimento e progressão da DM.

Com isso, um dos primeiros medicamentos a chegar no mercado foram os inibidores seletivos da monoamina oxidase (IMAO), enzima responsável pela degradação das monoaminas. Após alguns anos surgiram no mercado os antidepressivos tricíclicos (ADTS), inibidores seletivos da recaptação de serotonina (ISRS) e os inibidores seletivos da recaptação de noradrenalina (ISRN), que atua aumentando a disponibilidade desses neurotransmissores na fenda sináptica (HILLHOUSE; PORTER, 2015).

Com todos esses tratamentos existentes, ainda existia uma alta prevalência de pessoas depressivas, e os resultados dos tratamentos não apresentavam uma eficácia satisfatória. Apenas 50% dos pacientes apresentavam resposta e, 30% apresentavam uma remissão completa (GAYNES et al., 2009; HILLHOUSE; PORTER, 2015; THOMPSON, 2002).

Embora existissem os tratamentos expostos, os pacientes ainda apresentavam os sintomas depressivos. Com isso, aliado ao decréscimo das monoaminas, surge a teoria do estresse oxidativo para tentar explicar o desenvolvimento da DM. Esse estado é caracterizado pelo desequilíbrio entre a produção de ER e defesas antioxidantes (PERSSON; POPESCU; CEDAZO-MINGUEZ, 2014).

Em excesso, as ER interagem com proteínas, lipídios e material genético, interferindo assim na homeostase celular (NHEA et al., 2019). Em contrapartida, o organismo é capaz de produzir defesas, como as enzimas catalase (CAT) e superóxido dismutase (SOD), que regulam os níveis de ER no organismo (AGUILAR; NAVARRO; PÉREZ, 2016).

Em pacientes depressivos, são encontradas disfunções nas atividades das enzimas CAT e SOD, corroborando assim para o desenvolvimento do estado de estresse oxidativo. Como o cérebro consome muito oxigênio em seus processos fisiológicos e provem de muito lipídio em sua estrutura, acaba sendo suscetível a esses danos.

Apesar de existir essas teorias usadas até o momento para explicar o desenvolvimento e progressão da DM, ainda faltavam questionamentos a serem respondidos. Com isso, surge uma nova teoria, de que a DM teria origem através do aumento de mediadores ligados a respostas inflamatórias (BRITES; FERNDANDES, 2015).

Alguns estudos demonstram que o aumento de citocinas pró-inflamatórias promovem as mesmas alterações neuroquímicas envolvidas com a DM (MAES et al., 2011; WON et al., 2016). Com isso, foi postulado de que a DM também teria origem através da neuroinflamação.

Entretanto, não existem dados que relatam como realmente ocorre o desenvolvimento e progressão da DM. Na literatura são utilizados ambas as teorias, podendo assim ter incidência juntas, ou individual, indicando que os sintomas e alterações são diferentes em pacientes depressivos.

2.4. Estresse crônico imprevisível e moderado

Um dos modelos mais utilizados e validados na literatura vem sendo o do CUMS. O modelo consiste em promover alterações comportamentais, fisiológicas e neuroquímicas a semelhantes em humanos com DM através de diferentes estressores (PAULO; CENTRE, 2012; SONG et al., 2018; ZHANG et al., 2015).

Na literatura, existem diversas variações do modelo do CUMS, com diferentes números de dias, estressores e frequência em que os animais são submetidos a esses estresses durante o dia (KONG et al., 2019; LIU et al., 2018).

Entretanto, uma disfunção imune também pode ser considerado um fator que contribui para os efeitos do estresse na saúde (HAROON; RAISON; MILLER, 2012). Em contrapartida, uma resposta aguda ao estresse pode ser um

evento benéfico, pois ela prepara o corpo para uma possível ameaça. No entanto, uma resposta a longo prazo acaba levando a prejuízos e desencadeando doenças associadas ao estresse crônico.

Os efeitos do estresse crônico mais vistos são o desenvolvimento de crises ansiosas (BONDI et al., 2008; VENTURA-SILVA et al., 2012), comportamento depressivo (BESSA et al., 2009; STREKALOVA et al., 2004) e déficits cognitivos (BONDI et al., 2008; CERQUEIRA et al., 2007). Não se limitando apenas às alterações de humor, o estresse ocasiona alterações na transcrição de genes, alterando o funcionamento do organismo (DHABHAR et al., 2012).

O estresse crônico possui diferentes formas de ser utilizado para protocolos de estudo da depressão. Um deles é CUMS. Nesse modelo são utilizados no mínimo 4 semanas de estressores, incluindo privação de comida e água. Outra variação é o estresse crônico imprevisível (CUS, do inglês *chronic unpredictable stress*). Usado também pelo mínimo de 4 semanas, possui a diferença de não aplicar estressores que envolvam privação de água e comida (MONTEIRO et al., 2015).

Em ambos os modelos, a utilização de ratos ou camundongos depende da disponibilidade e dos objetivos do estudo. O uso de camundongos, apresenta maiores vantagens, visto que eles apresentam maior disponibilidade de cepas modificadas, facilidade no manuseio e menores custos de manutenção em comparação com ratos, o que confere um maior uso deles (MONTEIRO et al., 2015).

Entretanto, existem outros tipos de indutores para o desenvolvimento do comportamento do tipo-depressivo, como a injeção intracerebroventricular por estreptozotocina (STZ), desafio por lipopolissacarídeo (LPS) através de injeção intraperitoneal e estresse agudo de restrição (ARS, do inglês *acute restriction stress*). Ambos modelos são utilizados para estudo de alterações fisiológicas ocasionadas pela depressão.

Embora todos promovam alterações no organismo, o modelo do CUMS ou CUS, apresenta melhores resultados, pois mimetiza com clareza os efeitos do estresse no organismo semelhantes à de humanos.

3. Hipóteses e objetivos

3.1. Hipótese

Avaliar a atividade da IL-4 na reversão do comportamento do tipo-depressivo na modulação do estresse oxidativo e na neuroinflamação pelo modelo do CUMS em camundongos.

3.2. Objetivo geral

Investigar o efeito da IL-4 no comportamento do tipo-depressivo induzido pelo CUMS, bem como a modulação do estresse oxidativo, neuroinflamação e a hiperativação do eixo HPA.

3.3. Objetivos específicos

- Avaliar a eficácia da administração via intranasal de IL-4 em camundongos;
- Avaliar se uma única administração de IL-4 seria capaz de reverter o comportamento do tipo-depressivo induzido pelo CUMS;
- Avaliar a modulação do estresse oxidativo pela quantificação de ER, peroxidação lipídica e atividades das enzimas antioxidantes;
- Avaliar biomarcadores da neuroinflamação em córtex pré-frontal e hipocampo.

4. Manuscrito escrito nas normas da revista Brain Behavior and Immunity

Intranasal administration of interleukin-4 ameliorates depression-like behavior and biochemical alterations in mice submitted to the chronic unpredictable mild stress: modulation of neuroinflammation and oxidative stress

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Abstract

Physical and psychological stress affect the body homeostasis through modulation of the hypothalamic-pituitary-adrenal axis (HPA) and redox and inflammatory systems. Indeed, extensive data have reported that impairment in these systems is related to major depression (MD). Despite the efforts, the pharmacological therapy is not efficient to a significant subpopulation of depressed patients. Therefore, the aim of our study was to address whether the interleukin (IL)-4 would present antidepressant-like effect in mice submitted to the chronic unpredictable mild stress (CUMS) for 28 days. On the 29th day, mice received IL-4 intranasally (1 ng/mice) or vehicle (sterile saline), and after 30 min, they were submitted to behavioral tests or euthanasia for the removal of adrenal glands, axillary lymph nodes, spleen, thymus, prefrontal cortices (PFC), hippocampi (HC), and blood. We observed that a single administration of IL-4 reversed CUMS-induced depression-like behavior in the tail suspension test and splash test, without evoking locomotor changes. IL-4 administration reduced the plasma levels of corticosterone and the increased weight of adrenal glands in stressed mice. Meantime, IL-4 restored the expression of *NRF2*, *NF-kB*, *IL -1β*, *IL-4*, *BDNF* and *IDO* in the PFC and HC of stressed mice and modulated oxidative stress markers in these brain structures. Together, our results showed for the first time the antidepressant-like effect of IL-4, which involves the modulation of neuroinflammation and oxidative stress. The potential effect of IL-4 administered intranasally arises as an innovative strategy for the treatment of MD to improve the quality of life of depressive patients.

Keywords: Interleukin-4; CUMS; depression; HPA axis; immunomodulatory; oxidative stress.

1. Introduction

Regardless of the environmental context, survival is a fundamental priority for all organisms, depending largely on the ability to adapt to the most diverse threats to homeostasis or well-being. These threats (physical or psychological and real or perceived) are called stressors and initiate the so-called stress response, which is mediated mainly by the activation of the hypothalamic-pituitary-adrenal axis (HPA). However, impairment in the HPA axis activity has been implicated in several disorders, such as obesity, cardiovascular disease, and stroke (Holsboer, 2000; Rosmond and Bjorntorp, 2000). In line with that, it has been reported that HPA axis hyperactivity is the commonest alteration found in depressive patients (Holsboer, 2000; Pariante and Lightman, 2008). Major depression (MD) affects approximately 322 million people worldwide and patients may experience symptoms of sadness, uneasiness, guilt, despair, and suicidal thoughts (World Health Organization, 2017). Due to the growing number of cases, it is believed that by the end of 2020, MD will be responsible for disabling 400 million people worldwide (Galts et al., 2019).

In addition to the HPA axis, accumulating evidence has linked the etiology of MD to several types of inflammatory conditions and has indicated that targeting immune-to-brain interactions might be relevant for future treatment strategies (Brites and Fernandes, 2015; Felger, 2019). In turn, proinflammatory cytokines can induce the production of reactive species (RS) and impair the antioxidant defences, contributing to the establishment of oxidative stress seen in depressed patients (Sowa-Kućma et al., 2018). However, this is a bidirectional communication, as RS can act as important signalling molecules and promote the activation of transcriptional factors, such as the nuclear factor kappa B (NF-

kB) (Hahn et al., 2007). Indeed, proinflammatory cytokines can also induce the activity of the enzyme indoleamine 2,3-dioxigenase (IDO). IDO converts the amino acid L-tryptophan into kynurenine decreasing the concentration of serotonin (Uyttenhove et al., 2003), and its activation may be involved in inflammation-induced depression (O'Connor et al., 2009).

Interestingly, despite using high levels of oxygen and having increased lipid content, the central nervous system does not have a robust antioxidant defense, emphasizing the relevance of boosting the antioxidant system (REF). One of the main drivers of the antioxidant response is the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), which induces the expression of antioxidant enzymes, such as heme oxygenase, superoxide dismutase (SOD), and catalase (CAT). Notably, stress- and inflammation-induced reduction in Nrf2 activity may contribute to decreased brain-derived neurotrophic (BDNF) signaling, thus contributing to the depression-like phenotype (Hashimoto, 2018). BDNF is the main brain neurotrophin and participates in neurogenesis and synaptogenesis (Choi et al., 2018).

In addition to BDNF, it has been reported that IL-4, a pleiotropic cytokine, helps the maintenance of brain homeostasis (Derecki et al., 2010) and supports neurogenesis and oligodrendrogenesis (Butovsky et al., 2006). IL-4 is secreted primarily by mast cells, Th2 cells, eosinophils, basophils, microglia, and astrocytes and functions as a potent regulator of immunity (Choi and Reiser, 1998; Gadani et al., 2012). Several studies have reported the role of IL-4 in depression. IL-4 deficiency weakens the resilience against stress-induced depression, while increasing IL4 levels can alleviate depressive-like behaviours (Han et al., 2015; Park et al., 2015; Wachholz et al., 2017). Whilst several

inflammatory cytokines are able to upregulate the serotonin transporter (SERT) activity (Morikawa et al., 1998; Mössner et al., 1998), IL-4 reduces it and, thereby, increases the availability of serotonin (Mössner et al., 2001). Although IL-4 delivery in animal models of multiple sclerosis, brain ischemia and spinal cord injury is neuroprotective (Butti et al., 2008; Francos-Quijorna et al., 2016; Zhao et al., 2015), it has never been administered as a potential pharmacological agent in animal models of depression. Therefore, the present study was conducted to shed some light into the role of IL-4 as an antidepressant. Here we used the chronic unpredictable mild stress (CUMS) to induce HPA axis hyperactivation, neuroinflammation, oxidative stress, and, ultimately, depression-like behavior. To the best of our knowledge, this is the first study addressing the potential of IL-4 administration as an antidepressant.

2. Materials and methods

2.1. Animals

Swiss male mice (25 – 35g), obtained from the animal facility of the Federal University of Pelotas were used in this study. All procedures were approved by the Committee on the Care and Use of Experimental Animal Resources at the Federal University of Pelotas, Brazil (38715) and comply with The Animal Research: Reporting in Vivo Experiments (ARRIVE) guidelines, items 5 to 13 (Kilkenny et al., 2010).

2.2. Drugs

IL-4 was acquired from Peprotech under the reference number 214-14 (Pelotas-RS, Brazil). IL-4 was dissolved in sterile Milli-Q water at a concentration

of 0.2 ng/µl and administered at a volume of 5 µl via the intranasal route (i.n) to yield a dose of 1ng/mice. This dose was chosen based on preliminary results from unpublished data through a curved dose and response. They were chosen as doses of 1 and 10 ng / mice without 0:15, 0:30 and 1h, as doses of 1ng / mice without 0:30 as showing a better way to reverse the depressive-like of mice. The route is effective in delivering fat-soluble compounds and proteins, avoiding the need to be degraded when passing through the gastrointestinal tract. Described by Hason et al. (2013) this route is able to speed up the delivery of compounds to the central nervous system because it does not need to pass through the blood-brain barrier (BBB).

2.3. Experimental protocol

The chronic unpredictable mild stress (CUMS) was used to induce depression-like behavior as previously reported (Paulo and Centre, 2012; Song et al., 2018), with some modifications. Mice were randomly distributed into four experimental groups (n=8 mice/group) and subjected to one of the following stressors per day in a random order: water restriction, food restriction, movement restriction, tail pressure, and inclined box with wet bedding. The CUMS was conducted for 28 consecutive days, and on the 29th day, the animals received IL-4 or vehicle. After 30 minutes, the animals underwent the behavioral tests followed by euthanasia. A different cohort of mice underwent the same experimental protocol and were euthanized (by overdose of isoflurane inhalation) for the removal of organs for biochemical analysis. An overview of the experimental procedures is exposed in Fig. 1.

Please insert Fig. 1 here

2.4. Behavioral tests

2.4.1. Open field test (OFT)

The OFT (Walsh and Cummins, 1976) was performed to verify if IL-4 administration would cause psychomotor alterations. In this test, each mouse was individually placed in the central square of a wooden box (30 × 30 × 15) divided in nine equal squares. The total number of quadrants crossed with the four paws (crossings) and the number of elevations (rearings) were scored over a 5 min period as an indication of the locomotor and exploratory behavior, respectively.

2.4.2. Tail suspension test (TST)

The TST was used to evaluate the depressive-like behavior of mice (Steru et al., 1985). An increase in the immobility time reflects the depression-like behavior. Mice were individually suspended by the tail 50 cm from the ground and were visually isolated. The test was conducted for 6 min, but the immobility time was recorded in the last 4 min, as the first 2 min were used for mouse habituation.

2.4.3. Splash test

The splash test was performed to assess the anhedonic behavior (Ducottet et al., 2004). This test consists of spraying a 10% sucrose solution in the dorsal coat of the mice. The sucrose solution adheres to the hair and induces

the cleaning behavior due to its viscosity. The grooming time was observed during 5 min.

2.5. Biochemical determinations

2.5.1. Sample collection

Following euthanasia, prefrontal cortices (PFC), hippocampi (HC), blood (plasma), adrenal glands, axillary lymph nodes, spleen and thymus were collected. The blood was collected in heparinized tubes, centrifuged at 2500 × *g* for 10 min, and the plasma was collected for the analysis of corticosterone, reactive species (RS) and lipid peroxidation levels. The PFC and HC were homogenized in Tris-HCl 50 mM (1:4 weight/volume), centrifuged at 2500 × *g* for 10 min, and the supernatant was used for the determination of RS, nitric oxide (NO), and lipid peroxidation. The PFC and HC of another cohort of mice was kept on TRIzol until the mRNA extraction for the analysis of gene expression. Adrenal glands, axillary lymph nodes, spleen and thymus were collected and weighted.

2.5.2. Relative organs weight

During euthanasia, the adrenal glands, axillary lymph nodes, spleen and thymus were removed. The organs were cleaned with saline solution and all traces of fat were removed. Next, they were weighed to assess the effect of stress on the relative weight of each organ. The relative organ weight was calculated based on the body weight of each mouse (organ weight/animal weight x 1000). Organ weight was represented as mg.

2.5.3. Corticosterone levels

To determine the corticosterone levels (Zenker, 1957), aliquots of plasma were incubated with chloroform and centrifuged for 5 min at 2500 rpm, followed by addition of 0.1 M NaOH and another round of centrifugation. After the addition of the fluorescence reagent (H_2SO_4 and ethanol 50%), samples were centrifuged (5 min at 10000 × *g*) and incubated at room temperature for 2 h. Fluorescence intensity emission, corresponding to plasma corticosterone levels, was recorded at 540 nm (with 247-nm excitation) and corticosterone levels were expressed as ng/ml.

2.5.4. Gene expression

Total mRNA was extracted from the PFC and HC using TRIzol followed by DNase treatment with the DNA-free® kit and mRNA quantification. The cDNA synthesis was performed using a High Capacity cDNA Reverse Transcription kit according to the manufacturer's protocol. The amplification was made with the UltraSYBR Mix using the Stratagene Mx3005P. Gene expressions were normalized using glyceraldehyde β2-microglobuline as a reference gene, and the conditions for the reaction involved 95 °C for 15 s, 60 °C for 60 s, and 72 °C for 30 s. The 2ΔΔCT (delta-delta comparative threshold) method was used to normalize the fold change in gene expressions. The following genes were analyzed: *NF-κB* (fwd 5′-GCT TTC GCA GGA GCA TTA AC-3′, rev 5′-CCG AAG CAG GAG CTA TCA AC-3′), *IL-1β* (fwd 5′-GCT GAA AGC TCT CCA CCT CAA TG-3′, rev 5′-TGT CGT TGC TTG GTT CTC CTT G-3′), *IDO* (fwd 5′-AAT CAA AGC AAT CCC CAC TG-3′, rev 5′-AAA AAC GTG TCT GGG TCC AC-3′), *Nrf2* (fwd 5′-GTC TTC ACT GCC CCT CAT C-3′, rev 5′- TCG GGA ATG GAA AAT AGC TCC-3′), *BDNF* (fwd 5′-CCA TAA GGA CGC GGA CTT GTA C-3′, rev 5′-

AGA CAT GTT TGC GGC ATC CAG G-3′), *IL-4* (fwd 5′- CCA AGG TGC TTC GCA TAT -3′, rev 5′-ATC GAA AAG CCC GAA AGA -3′) and *β2 microglobulin* (fwd 5′- AAGTATACTCACGCCACCCA -3′, rev 5′-AAGACCAGTCCTTGCTGAAG-3′).

2.5.5. Reactive species (RS) levels

The RS levels were determined spectrophfluorimetrically in the plasma, PFC, and HC of mice (Loetchutinat et al., 2005). In this protocol, dichlorofluorescein diacetate (DCFH-DA) is oxidized to dichlorofluorescein (DCF) by intracellular RS. DCF emits a green fluorescence that can be measured with 520 nm emission and 488 nm excitation. The RS levels were expressed as fluorescence units.

2.5.6. Levels of nitric oxide (NO) metabolites (NO_x)

The NO is a highly unstable compound, which can be oxidized in nitrite and nitrate. Here, the NO_x levels were analyzed indirectly through the Griess reaction (Murphy and Noack, 1994) and the reaction was measured spectrophotometrically at 462 nm. The NO_x levels were expressed as Umol NOx/g tissue.

2.5.7. Thiobarbituric acid reactive species (TBARS) levels

The TBARS assay assessed the levels of malondialdehyde (MDA) present in the blood, PFC, and HC of mice. The MDA is a final product of lipid peroxidation and reacts with the thiobarbituric acid (Ohkawa et al., 1979). The colorimetric

reaction was measured at 532 nm and the data were expressed as nmol MDA/ g tissue.

2.5.8. Superoxide dismutase (SOD) activity

The SOD is responsible for the dismutation of the radical anion superoxide ($O_2^{\cdot -}$) in hydrogen peroxide (H_2O_2) and oxygen. SOD activity was measured spectrofluorimetrically (480 nm) through the analysis of epinephrine autooxidation (Misra, h.p., Fridovich, 1972). SOD activity was expressed as U SOD/mg prot.

2.5.9. Catalase (CAT) activity

Catalase is an enzymatic antioxidant defense responsible for converting H_2O_2 into oxygen and water. The CAT activity (Aebi, 1984) was expressed as U CAT/mg prot.

2.7. Protein determination

Protein concentration in the samples was determined as described by Lowry et al. (1951).

2.8. Molecular docking protein-protein

In this study, protein-protein docking was performed using RosettaDock package (GRAY et al., 2003). Briefly, using PyMol (DELANO, 2002) each pair of proteins (IL-KEAP1, IL-NFKB, IL-TRKB) was positioned in such a way that their binding pockets were facing each other, and within 10 Angstroms of distance. Next, the Rosetta local docking protocol was applied to each of the protein-protein

pairs. At the start of each docking simulation, the protein-protein complex was randomly perturbed by 3 Angstroms translation and 8º rotation. 15 structures were generated for each pair. The structures were then ranked by score, and further analyzed using PDBSum (LASKOWSKI et al., 1997), where the protein-protein interface residues were observed.

2.9. Statistical analysis

All experimental results are expressed as the mean ± standard error of the mean (S.E.M.). Comparisons between experimental and control groups were performed by two-way ANOVA. When ANOVA revealed a significant interaction, the Tukey post-hoc test was used for between-group comparisons. A value of $p < 0.05$ was considered to be significant. Main effects are presented only when the first order interaction was non-significant. The statistical analysis was accomplished using GraphPad Prism version 8.0 for Windows, Graph Pad Software (San Diego, CA, USA).

3. Results

3.1. Intranasal administration of IL-4 ameliorated depression-like behavior in mice

The OFT was used to evaluate whether IL-4 administration would influence exploratory activity of mice. A two-way ANOVA did not show any significant interaction for the number of crossings and rearings in the OFT (Fig. 2A), removing the possibility of IL-4 exerting psycholocomotor alteration.

The TST and splash test were used for the analysis of the antidepressant-like effect of IL-4 in mice. The animals exposed to stress demonstrated higher

immobility time in the TST when compared to the control group ($p < 0.001$), and the IL-4 administration reduced the increased immobility time induced by stress ($p < 0.001$; Fig. 2B). The two-way ANOVA analysis revealed significant CUMS × IL-4 interaction for the immobility time ($F_{(1, 28)} = 131.0$, $p < 0.001$).

CUMS-exposed mice presented decreased grooming time in the splash test when compared to control mice ($p < 0.001$) and the intranasal administration of IL-4 was able to reverse it ($p < 0.001$; Fig. 2C). The two-way ANOVA demonstrated a significant CUMS × IL-4 interaction for the grooming time ($F_{(1, 28)} = 10.1$, $p < 0.001$).

Please insert Figure 2 here

3.2. Intranasal administration of IL-4 reduced plasma levels of corticosterone

Mice exposed to the CUMS exhibited higher levels of corticosterone when compared to control mice ($p < 0.05$), while the administration of IL-4 was able to reverse it ($p < 0.001$; Fig. 3A). The two-way ANOVA indicated a significant CUMS × IL-4 interaction for the corticosterone levels in the plasma of mice ($F_{(1,28)} = 10.5$, $p < 0.003$).

Please insert Fig. 3 here

3.3. Effects of CUMS and IL-4 in weight organs of mice

The two-way ANOVA did not reveal a significant CUMS × IL-4 interaction for the relative weight of peripheral organs. CUMS decreased the relative weight

of axillary lymph nodes ($p < 0.01$; Fig. 3B), spleen ($p < 0.001$; Fig. 3C), and thymus ($p < 0.001$; Fig. 3D), but administration of IL-4 had no effect on it. However, IL-4 ($p < 0.05$) was able to reduce the increased relative weight of the adrenal glands induced by CUMS ($p < 0.05$; Fig. 3E).

3.4. Intranasal administration of IL-4 reduced markers of neuroinflammation in chronic stressed mice

The effects of IL-4 on the modulation of gene expression in the prefrontal cortices (PFC) and hippocampi (HC) are depicted in Fig. 4. The two-way ANOVA revealed a statistically significant CUMS × IL-4 interaction in PFC ($F_{(1,24)} = 56.04$, $p < 0.001$) and HC ($F_{(1,24)} = 116.5$, $p < 0.001$) for the *NF-κB* expression (Fig. 4A). The post hoc analysis showed that the CUMS protocol increased *NF-κB* expression in PFC ($p < 0.001$) and HC ($p < 0.001$), when compared to the control group, whilst IL-4 administration decreased the *NF-κB* expression in both brain structures (PFC: $p < 0.001$, HC: $p < 0.001$) of CUMS-induced mice.

Similarly, a two-way ANOVA revealed a statistically significant CUMS × IL-4 interaction in PFC ($F_{(1,24)} = 30.19$, $p < 0.001$) and HC ($F_{(1,24)} = 52.55$, $p < 0.001$) for the *IL-1β* expression (Fig. 4B). The Tukey post hoc test showed an upregulated *IL-1β* expression in the PFC ($p < 0.001$) and HC ($p < 0.001$) of CUMS-exposed mice, when compared to the control group, and a single intranasal administration of IL-4 downregulated the *IL-1β* expression in both PFC ($p < 0.001$) and HC ($p < 0.001$).

Additionally, a two-way ANOVA revealed a statistically significant CUMS × IL-4 interaction in PFC ($F_{(1, 24)} = 44.72$, $p < 0.001$) and in the HC ($F_{(1,24)} = 18.56$, $p < 0.001$) for the *IDO* expression (Fig. 4C). The post hoc analysis showed an

upregulated *IDO* expression in the PFC ($p < 0.001$) and in the HC ($p < 0.001$) in the CUMS-exposed mice, when compared to the control group, while the single intranasal administration of IL-4 restored the increased expression in both brain structures (PFC: $p < 0.001$, HC: $p < 0.001$).

To validated the involvement of IL-4 in our results, the *IL-4* gene expression was evaluated in mice CUMS-exposed and treated with recombinant IL-4. The two-way ANOVA showed a statistically significant CUMS × IL-4 interaction in PFC ($F_{(1,24)} = 24.45$, $p < 0.001$) and HC ($F_{(1,24)} = 380.0$, $p < 0.001$) for the *IL-4* expression (Fig. 4D). The post hoc analysis revealed a downregulated *IL-4* expression in the PFC ($p < 0.001$) and an upregulated *IL-4* expression in the HC ($p < 0.001$) of CUMS-exposed mice when compared to the control group, while the administration of recombinant IL-4 increased the *IL-4* gene expression in the PFC ($p < 0.001$) and decreased this expression in the HC ($p < 0.001$).

Ultimately, a two-way ANOVA revealed no significant CUMS × IL-4 interaction in the PFC and a statistically significant CUMS × IL-4 interaction in the HC ($F_{(1, 24)} = 15.76$, $p < 0.001$) for *BDNF* gene expression (Fig. 4E). Nevertheless, main effects for CUMS exposition ($F_{(1, 23)} = 25.21$, $p < 0.001$) and recombinant IL-4 administration ($F_{(1, 23)} = 4.39$, $p = 0.05$) were found in the PFC. The post hoc analysis showed that CUMS protocol reduced the *BDNF* expression in the PFC ($p \leq 0.05$) and in the HC ($p < 0.001$) in mice when compared to the control group, while the administration of IL-4 increased this expression in the HC ($p < 0.001$), however, no alterations were found in the PFC.

Please insert Fig. 4 here

3.5. Intranasal administration of IL-4 modulated markers of oxidative stress in the PFC and HC of mice

The two-way ANOVA revealed a statistically significant CUMS × IL-4 interaction in PFC ($F_{(1, 24)} = 44.72$, $p < 0.001$) and in the HC ($F_{(1,24)} = 18.56$, $p < 0.001$) for the *Nrf2* expression (Fig. 5A). The post hoc analysis showed an upregulated *Nrf2* expression in the PFC ($p < 0.001$) and in the HC ($p < 0.001$) in the CUMS-exposed mice, when compared to the control group, while the single intranasal administration of IL-4 restored the increased expression in both brain structures (PFC: $p < 0.001$, HC: $p < 0.001$).

The CUMS protocol promoted an increase in the RS levels in the PFC, HC and plasma and increased the TBARS levels in the PFC and plasma. The two-way ANOVA revealed a statistically significant CUMS × IL-4 interaction for RS levels (PFC: $F_{(1,28)} = 4.63$, $p < 0.04$; HC: $F_{(1,28)} = 63.0$, $p < 0.001$; Plasma: $F_{(1,28)} = 5.13$, $p<0.03$) (Fig. 5B, G). The post hoc analysis showed that CUMS-exposed mice exhibited increased RS levels in the PFC ($p < 0.001$), HC ($p < 0.001$) and plasma ($p < 0.01$) when compared to the control group, while a single IL-4 administration reduced the RS levels in the PFC ($p \leq 0.05$), HC ($p < 0.001$) and plasma (0. 001). Moreover, the two-way ANOVA revealed a statistically significant CUMS × IL-4 interaction for TBARS levels (PFC: $F_{(1,28)} = 11.3$, $p < 0.002$; Plasma: $F_{(1,28)} = 44.7$, $p < 0.001$) (Fig. 5D, H). The Tukey post hoc test showed that CUMS protocol increased TBARS level in the PFC ($p < 0.01$) and plasma ($p < 0.001$), when compared to the control group, whilst the IL-4 administration restored TBARS levels in the PFC ($p < 0.01$) and plasma ($p < 0.001$). Regarding to TBARS levels in the HC (Fig. 5D) and NO_x (Fig. 5C) in both brain structures, no alterations were found.

The CAT and SOD, enzymes from the antioxidant system, are involved in the reduction of RS and, consequently, TBARS levels. Regarding to SOD, a two-way ANOVA revealed a statistically significant CUMS × IL-4 interaction in PFC ($F_{(1,36)} = 19.4$, $p < 0.001$) and in the HC ($F_{(1,28)} = 8.85$, $p < 0.006$) for the SOD activity (Fig. 5E). The Tukey post hoc test showed that the CUMS protocol increased SOD activity in the PFC ($p < 0.001$) and in the HC ($p < 0.001$) of mice, when compared to the control group, while the single intranasal administration of recombinant IL-4 restored the increased activity in both brain structures (PFC: $p < 0.001$, HC: $p \leq 0.05$). The two-way ANOVA revealed a statistically significant CUMS × IL-4 interaction in PFC ($F_{(1,27)} = 10.26$, $p < 0.003$) and in the HC ($F_{(1,28)} = 7.56$, $p < 0.01$) for the CAT activity (Fig. 5F). The post hoc analysis showed an increased CAT activity in the PFC ($p \leq 0.05$) and in the HC ($p < 0.01$) of the CUMS-exposed mice, when compared to the control group, while the single intranasal administration of IL-4 restored the increased activity in both brain structures (PFC: $p \leq 0.05$, HC: $p < 0.001$).

Please insert Fig. 5 here

3.6. IL-4 interactions with KEAP1, NF-kB and TrkB/BDNF target protein

The interactions between the amino acid residues ARG64, LEU27, HIS58, THR63, LYS61, GLU26 and GLN106 of IL-4 with amino acid residues SER365, GLY364, GLU434, SER410, ILE556, ARG368 and ARG432 of KEAP1 protein showed (Fig. 6A and 6B) the ability to interact between two protein. KEAP is a protein present in the response cascade to oxidative stress, caused by the excess of RS.

Please insert Fig. 6 here

In addition to this, can also observe (Fig. 7A and 7B) an interaction of the amino acid residues ARG64, ASP62, HIS59, GLU60, LYS61, HIS58, LEU23 and LYS84 of IL-4, which are able to interact with amino acid residues PHE564, ASP600, ALA597, ALA564, THR729, HIS728, MET730, SER714, SER594 and GLY727 of the NF-kB protein present in the cascade to inflammatory stimuli in the body, in the release of pro and anti-inflammatory cytokines.

Please insert Fig. 7 here

Among these pathways, can also see in the (Fig. 8A and 8B) the interaction capacity of amino acid residues ALA104, LYS61, THR63, HIS59, ASP62, LEU27, ARG64, BAL101 and PRO100 with amino acid residues ASN161, SER62, PRO63, ASN66, CYS113 and GLN207 of TrkB/BDNF protein, present in cascade activity of neurotrophic factors, responsible for synaptic maintenance.

Please insert Fig. 8 here

4. Discussion

Depression is undoubtedly one of the most widespread neuropsychiatric diseases among people accompanied by serious neurobiological damage (Abumaria et al., 2014), and stress is one of the most important causes of the development and progress of depression, increasingly looking for new methods to treat symptoms. To our knowledge, this is one of the first studies to involve a cytokine with anti-inflammatory activity for the treatment of depression.

In depressive patients, one of the visible symptoms is the inability to make decisions in situations that cause discomfort or even danger to their well-being, as demonstrated in tests on mice in the TST (Casaril et al., 2019). The depressed mice showed a longer immobility time compared to the control group and with the IL-4 treatment, the mice showed an improvement, decreasing the immobility time. In ST, depressed mice have a shorter grooming time than the control group as demonstrated by, and with the administration of drugs with antidepressant effect, there is an increase in grooming time (Sasibhushana et al., 2019). In our test, the results were similar, showing an increase in groomings time of mice treated with IL-4 compared to the control group.

For the results of the behavioral tests to be validated, the OFT is used to verify a possible activity of the molecules in acting on the locomotor activity of the mice (Walsh and Cummins, 1976). Based on the results, IL-4 has no effect on the locomotor activity of the animals, eliminating false positive and negative results.

Furthermore, the depressive-like behavior, in our study, the mice had an increase RS and lipid peroxidation in cortex, hippocampus and blood, results

similar to those of Domingues et al (2019). These results are validated by the increased expression of the NRF2 protein, a factor linked to the signaling of the expression of antioxidant enzymes (Liebert, 2005).

Corroborating with results the activity tests of the SOD and CAT enzymes, stress increased the activity compared to the control group, results also found by (Tsai and Huang, 2015). The possible modulation in the expression of NRF2 by treatment with IL-4 demonstrates that its use was able to regulate the activity of the enzymes CAT and SOD, demonstrating a decrease in RS and, culminating in the decrease in lipid peroxidation in the prefrontal cortex, hippocampus and plasma. Too as revealed by the molecular docking protein-protein, IL-4 interacts directly with KEAP1/NRF2, making it plausible that IL-4 improved the depressive-like behavior by oxidative stress pathway. The imbalance of the body's antioxidant defense system and signaling of immune to produce pro-inflammatory cytokines such as IL-1β, are found at high levels in patients with depression (Corwin et al., 2008; Venkatesh et al., 2019).

One of the mechanisms of the central nervous system in the face of inflammation is to activate the NF-kB protein complex, responsible for stimulating the synthesis of pro-inflammatory cytokines (Camargo et al., 2015). In our study, NF-kB expression levels increased compared to the control group in both the cortex and the hippocampus. This increase, already reported in the literature, is promoted by the stress stimulus (Kuebler et al., 2015).

These data, there was also an increase in IL-1β expression and hyperactivation of IDO enzyme, indicating neuroinflammation. This increase is correlated with the signaling of immune responses to the production of pro-inflammatory cytokines such as IL-1β. IDO is activated by several cytokines, such

as TNF-α, IL-1 and IL-6 by stimulating signaling pathways, including the MAPK p38 and NFκB pathway (Fujigaki et al., 2006). Animals exposed to IL-4 treatment showed normalization of NF-kB expression and -1β and regulation too the activity of IDO enzyme. Through the analysis of molecular docking, we can also conclude that the interaction between IL-4 and the NF-kB protein complex was effective in improving the depressive-like behavior of mice

The symptoms of depression are not fully understood, but it is known that depression can affect the concentration of BDNF in both the cortex and the hippocampus. *BDNF* is important protein responsive by manutention and restructuration of neurons, and has established that depressive patients and animal models, their expression is a decreasing, but the administration of drugs that have antidepressant-like effect leads to the restoration of their expression, antidepressant drugs (Rossetti et al., 2019).

One of the findings of this study was that the BDNF concentrations in both brain structures were decreased, thus affecting the processes of neurogenesis and synaptogenesis. Thus, our analyzes demonstrate that the ability of IL-4 to restore BDNF expression is effective in the hippocampus, in the prefrontal cortices, IL-4 had no effect in restore expression. With the molecular docking technique, we can see that the interaction between IL-4 and TrkB / BDNF presents many hydrogen bonds in their bonds between the residues of both proteins, making these interactions more consistent.

Studies have shown that one of the hypotheses of depression, with decreased neurotrophins, leads to loss of synaptic plasticity and inflammatory processes causing neuronal degeneration that can cause apoptosis (Christina F.

Vogelaar, Shibajee Mandal et al., 2018). This is due to the decrease in anti-inflammatory interleukins, such as IL-4.

In our study, the effect of stress causes a decrease in expression of IL-4 in the prefrontal structures and an increase in the hippocampus. One of the hypotheses to explain this difference is the fact that the increase in cytokines pro and anti-dependent on the type of signaling that occurs in stimulating stress (Cavaillon, 2014).

Both structures play different roles in response to stress, and according to McEwen et al., (2016), as prefrontal cortex and hippocampal structures can undergo reversible, as well as irreversible, intensity-dependent changes. Leff Gelman et al., (2019), found similar results in patients with depressive symptoms and anxiety disorders during pregnancy, where stressed patients showed an increase in the concentration of IL-4 in serum.

Thus, we suggest that the hippocampus is more susceptible to the stress response (Bremner, 2007), or increased the expression of IL-4 in response to the release of Il-1β among other pro-inflammatory cytokines. This difference between structures and their regulation can also be explained by their function of automatic application and the activation of their receptors, thus allowing them to stabilize their own concentration at regular levels (Luzina et al., 2012).

Both changes are directly linked to HPA axis hyperactivity, responsible for liberation of glucocorticoid. An example that also leads to hyperactivity of the HPA axis is a high expression of IL-1β, can decrease the function of GR by reducing the affinity of GR for cortisol (Pariante et al., 1999; Maddock and Pariante, 2001) or blocking the translation of GR from the cytoplasm to the nucleus (Pariante et al., 1999). This impairment in the signaling of GR results in the reduction of

negative feedback on the HPA axis, culminates in the increase in circulating levels of corticosterone. In this study, an interesting finding was found, that a single dose of IL-4 was able to decrease circulating levels of corticosterone and reduce the weight of the adrenal gland. One explanation is the increased weight of the adrenal glands and the high release of corticosterone in stressful situations (O'Connor et al., 2000).

These data opened a new hypothesis to analyze the correlation between stress and the relative weight of the organs responsible for mediating immune responses. Through these data mentioned above, the organs such as axillary lymph nodes, spleen and thymus also served as a parameter to analyze the adverse effects caused by stress in its structure. In the weighing of these organs, a significant difference was found between control mice and stressed mice, showing stunting and loss of mass, results also found by (Sangomla et al., 2018; Sarjan and Yajurvedi, 2019; Shah et al., 2018). Treatment with a single administration of IL-4 was not able to reverse these changes, which opens a new hypothesis to verify the effects of a chronic administration of IL-4 in the reversal of damage.

This study also served as a basis to demonstrate the importance of HPA homeostasis in the regulation of mouse organism processes, through changes found at peripheral and central levels. To the best of our knowledge, this is the first study addressing the potential of IL-4 to be used as an antidepressant. Its effects in modulating oxidative stress and reducing neuroinflammation become a potential future molecule for use as an antidepressant (Fig. 9).

Please insert Fig. 9 here

LIMITATION – USE IL-4 KO MICE TO DISSECT THE EFFECT OF EXOGENOUS IL-4 ON DEPRESSION

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Conflicts of interest

The authors declare that they have no conflict of interests.

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Legends of figures

Fig. 1. Representation of the experimental protocol. The CUMS protocol lasted for 27 days and control mice were kept undisturbed. Twenty-four hours after the last stressor the mice received the intranasal administration of IL-4 or vehicle (day 28). After further thirty minutes, mice were submitted to the behavioral tests or euthanasia.

Fig. 2. Effect of IL-4 treatment on depression-like behaviors in mice. Number of crossings and rearings in the open field test (OFT) (A). Immobility time in the tail suspension test (TST) (B). Grooming time in the splash test (C). Data are expressed as mean S.E.M of eight independent animals/group. $^{###}p < 0.001$ when compared to the control group. $^{***}p < 0.001$ when compared to mice submitted to the CUMS.

Fig. 3. Peripheral effects of IL-4 treatment. Plasma levels of corticosterone (A) and relative weight of axillary lymph nodes (B), spleen (C), thymus (D), and adrenal glands (E). Data are expressed as mean S.E.M of eight independent animals/group. $^{#}p < 0.05$, $^{##}p < 0.01$, and $^{###}p < 0.001$ when compared to the control group. $^{*}p < 0.05$ and $^{***}p < 0.001$ when compared to mice submitted to the CUMS.

Fig. 4. Effect of IL-4 treatment on markers of neuroinflammation in the prefrontal cortices (PFC) and hippocampi (HC) of mice. Relative expression of NF-κB (A), IL-1β (B), IDO (C), IL-4 (D), and BDNF (E). Data are expressed as mean S.E.M of six to eight independent animals/group. $^{#}p < 0.05$, $^{##}p < 0.01$, and $^{###}p < 0.001$ when compared to the control group. $^{***}p<0.001$ when compared to the CUMS group.

Fig. 5. Effect of IL-4 on markers of oxidative stress in the prefrontal cortices (PFC), hippocampi (HC), and blood of mice. Relative expression of Nrf2 (A), central levels of reactive species (RS) (B), nitric oxide metabolites (NO_X) (C), and thiobarbituric acid reactive species (TBARS) (D), enzymatic activity of superoxide dismutase (SOD) (E) and catalase (CAT) (F), and plasma levels of RS (G) and TBARS (H). Data are expressed as mean S.E.M of six to eight independent animals/group. $^{\#}p < 0.05$, $^{\#\#} p < 0.01$, and $^{\#\#\#}p < 0.001$ when compared to the control group. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ when compared to mice submitted to the CUMS.

Fig. 6. Protein-protein docking target (green) KEAP1 (6QMC) with ligand (red) IL-4 (4YDY). (A) view of interaction between KEAP1 and IL-4. (B) interaction list between protein residues.

Fig. 7. Protein-protein docking target (yellow) NF-kB (3VNG) with ligand (red) IL-4 (4YDY). (A) view of interaction between NF-kB and IL-4. (B) interaction list between protein residues.

Fig. 8. Protein-protein docking target (green) TrkB/BDNF (4AT4) with ligand (red) IL-4 (4YDY). (A) view of interaction between TrkB/BDNF and IL-4. (B) interaction list between protein residues.

Fig. 9. Schematic representation of the effects of IL-4 in prefrontal cortices and hippocampi evaluated in this study.

Figures

Figure 1.

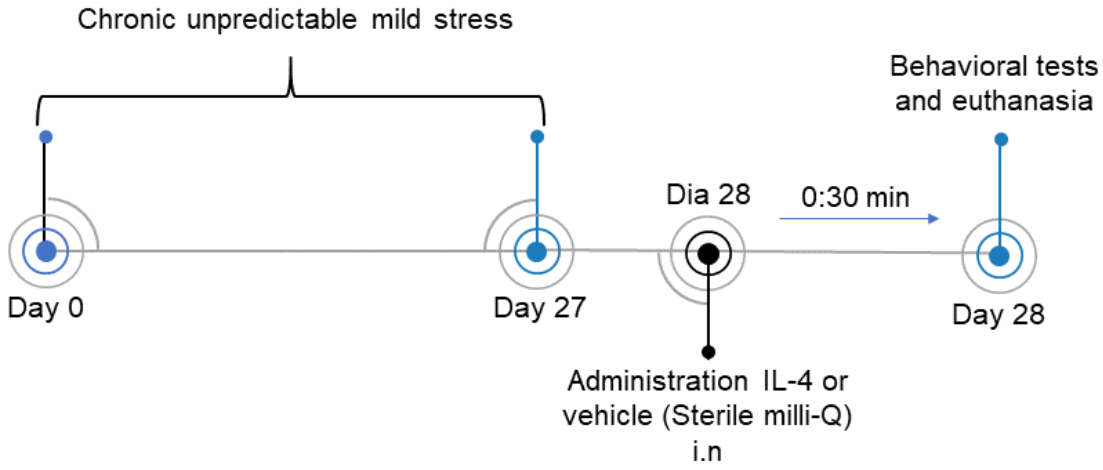


Figure 2.

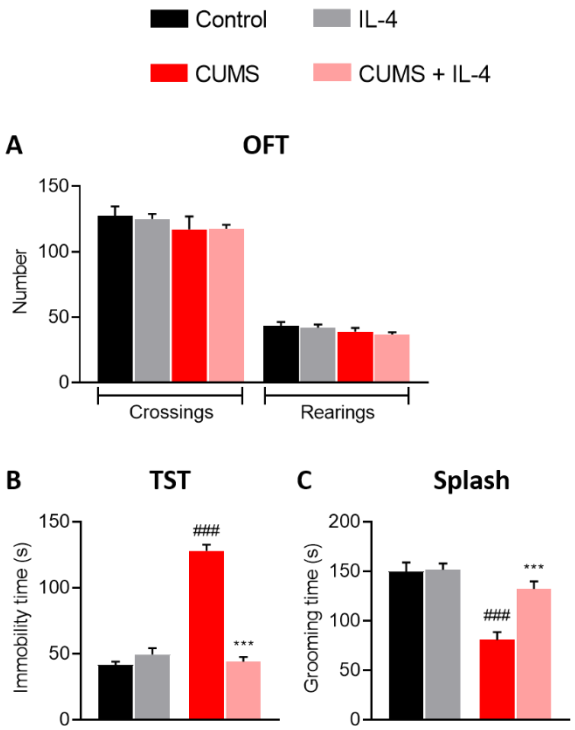

Control
IL-4
CUMS
CUMS + IL-4
A
OFT
Number
150
100
50
0
Crossings
Rearings
B
TST
Immobility time (s)
150
100
50
0
###
***
C
Splash
Grooming time (s)
200
150
100
50
0
###
***

Figure 3.

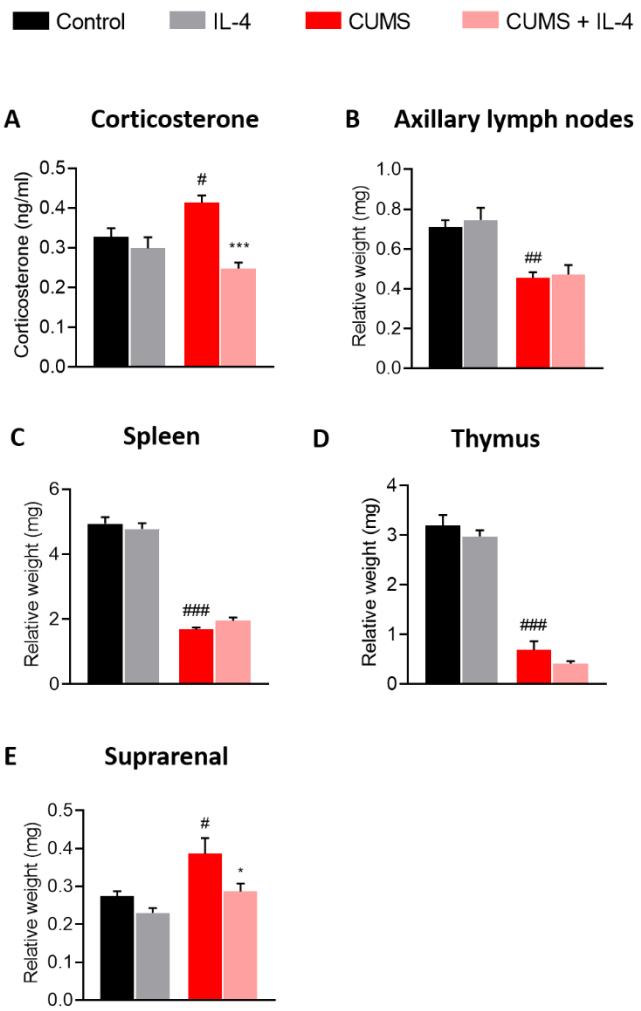

Control
IL-4
CUMS
CUMS + IL-4
A
Corticosterone
Corticosterone (ng/ml)
0.5
0.4
0.3
0.2
0.1
0.0
#
***
B
Axillary lymph nodes
Relative weight (mg)
1.0
0.8
0.6
0.4
0.2
0.0
##
C
Spleen
Relative weight (mg)
6
4
2
0
###
D
Thymus
Relative weight (mg)
4
3
2
1
0
###
E
Suprarenal
Relative weight (mg)
0.5
0.4
0.3
0.2
0.1
0.0
#
*

Figure 4.

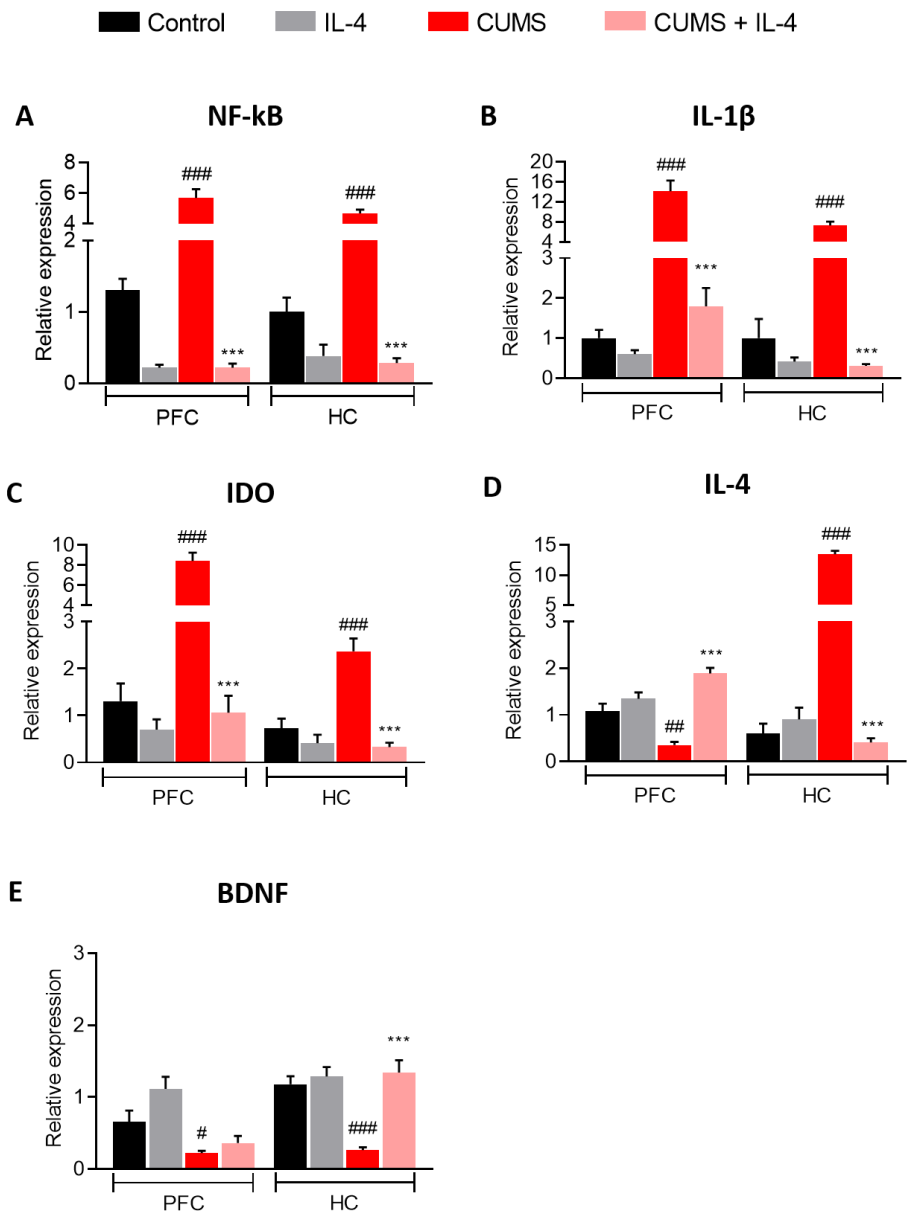

Control
IL-4
CUMS
CUMS + IL-4
A
NF-kB
B
IL-1β
C
IDO
D
IL-4
E
BDNF
Relative expression
PFC
HC
###
***
##
#

Figure 5.

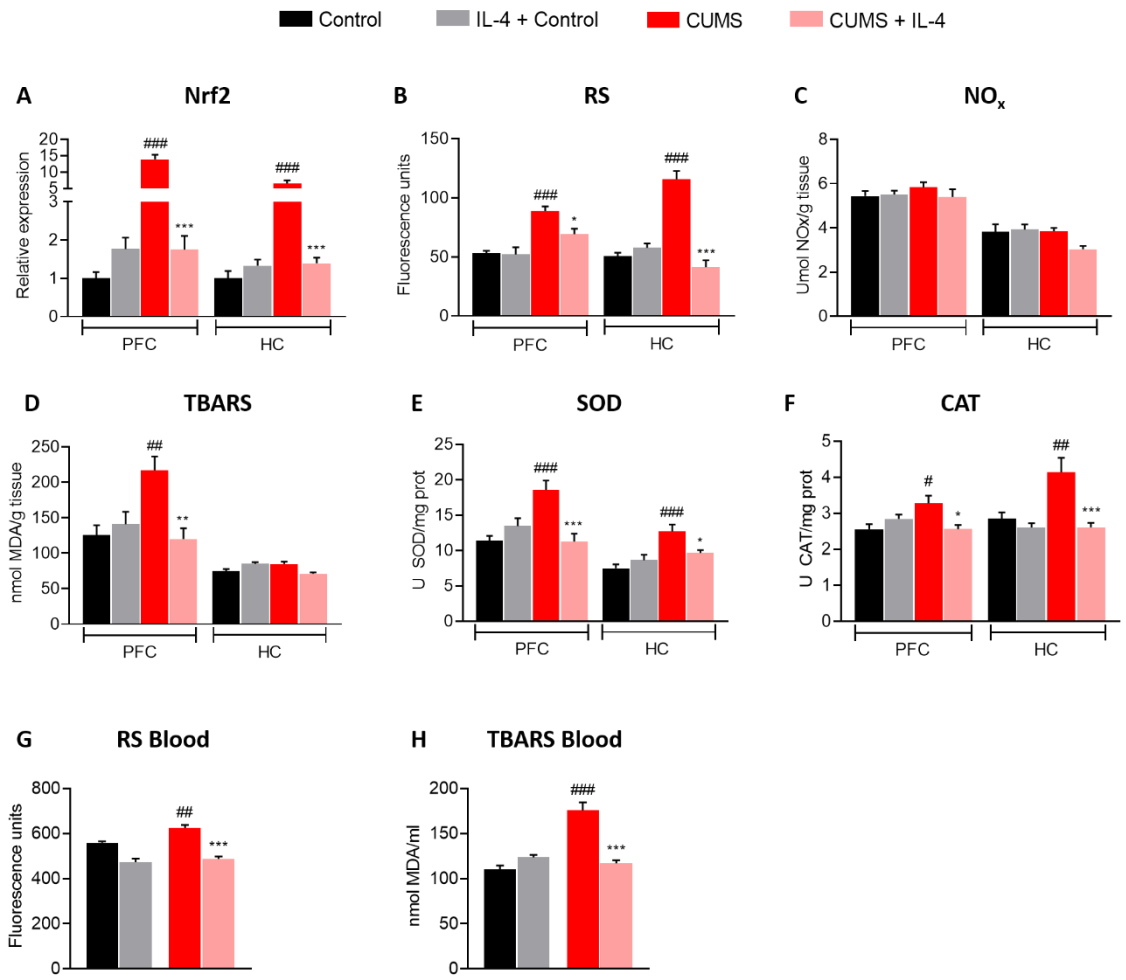
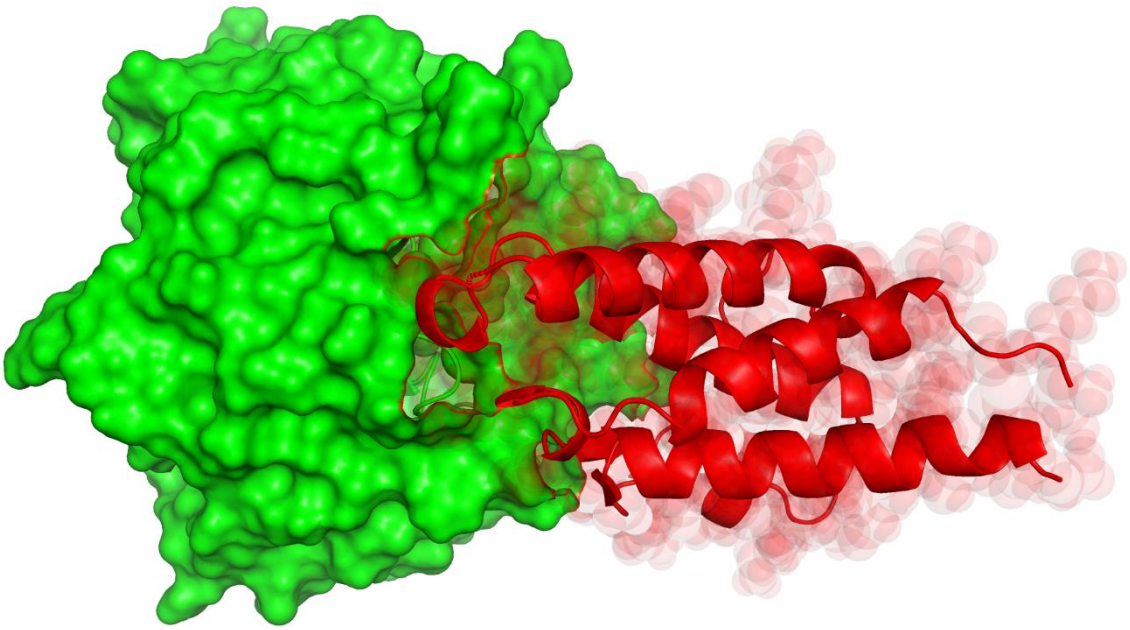
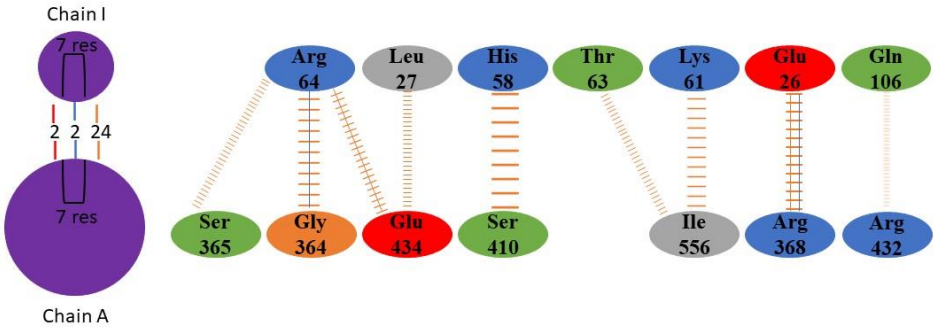

Control
IL-4 + Control
CUMS
CUMS + IL-4
A
Nrf2
Relative expression
B
RS
Fluorescence units
C
NOx
Umol NOx/g tissue
D
TBARS
nmol MDA/g tissue
E
SOD
U SOD/mg prot
F
CAT
U CAT/mg prot
G
RS Blood
Fluorescence units
H
TBARS Blood
nmol MDA/ml
PFC
HC

Figure 6.

A



B



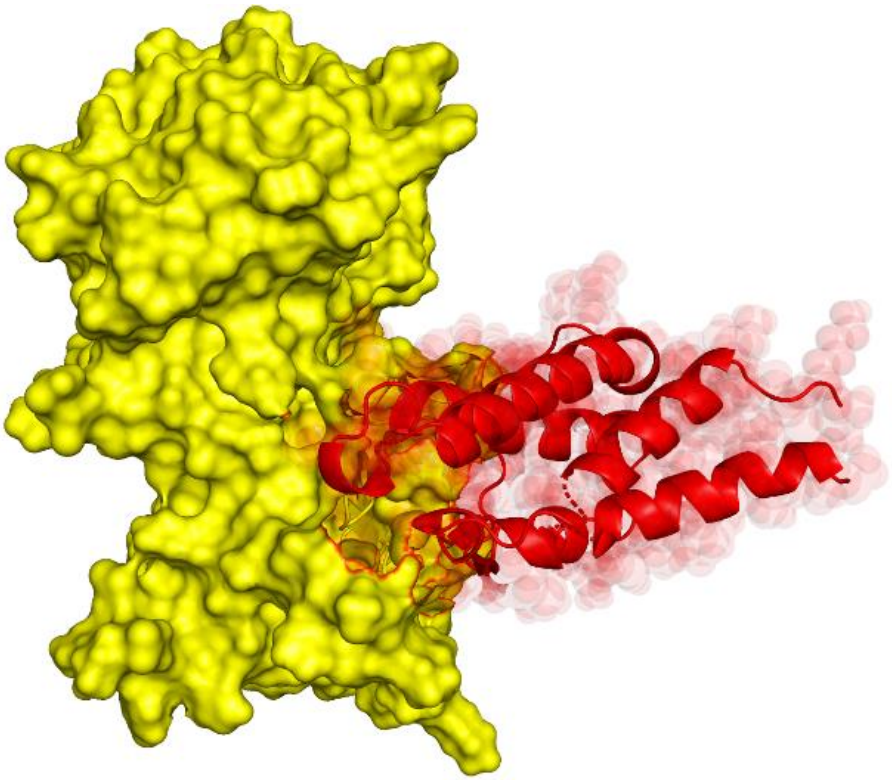
Key: Salt bridges Disulphide bonds Hydrogens bonds Non-bonded contacts

Residue colours: Positive (H, K, R); negative (D,E); S,T,N, Q = neutral; A, V, L, I, M = aliphatic; F, Y, W = aromatic; P, G = Pro&Gly; C = cysteine.

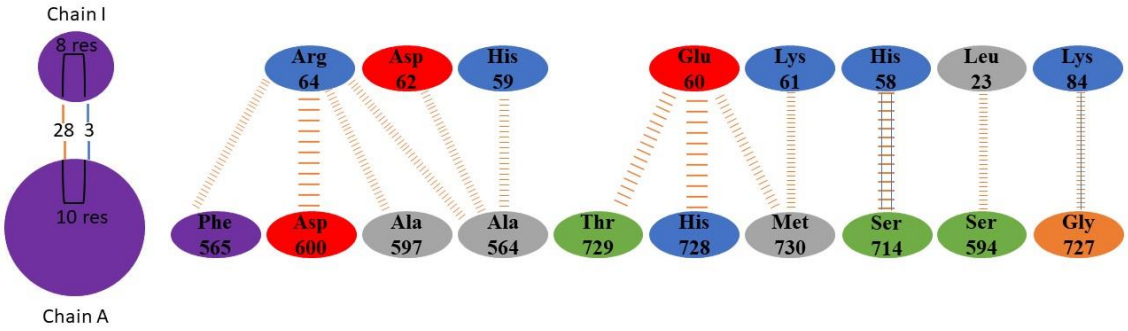
The number of H-bond lines between any two residues indicates the number of potential hydrogen bonds between them. For non-bonded contacts, which can be plentiful, the width of the striped line is proportional to the number of atomic contacts.

Figure 7.

A



B



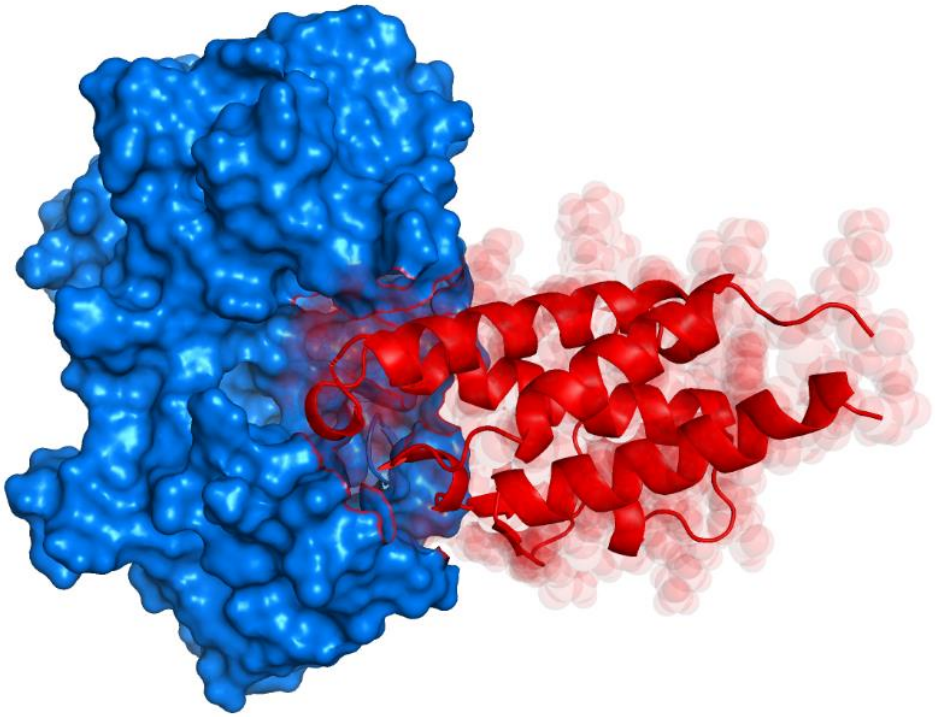
Key: Salt bridges Disulphide bonds Hydrogens bonds Non-bonded contacts

Residue colours: Positive (H, K, R); negative (D,E); S,T,N, Q = neutral; A, V, L, I, M = aliphatic; F, Y, W = aromatic; P, G = Pro&Gly; C = cysteine.

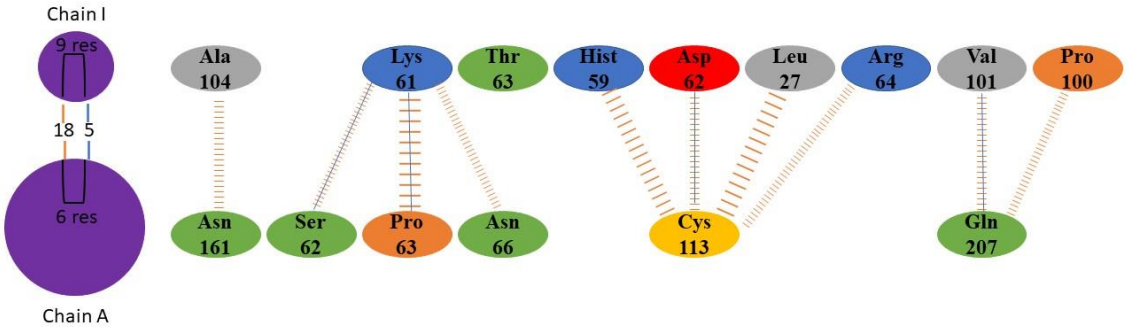
The number of H-bond lines between any two residues indicates the number of potential hydrogen bonds between them. For non-bonded contacts, which can be plentiful, the width of the striped line is proportional to the number of atomic contacts.

Figure 8.

A



B

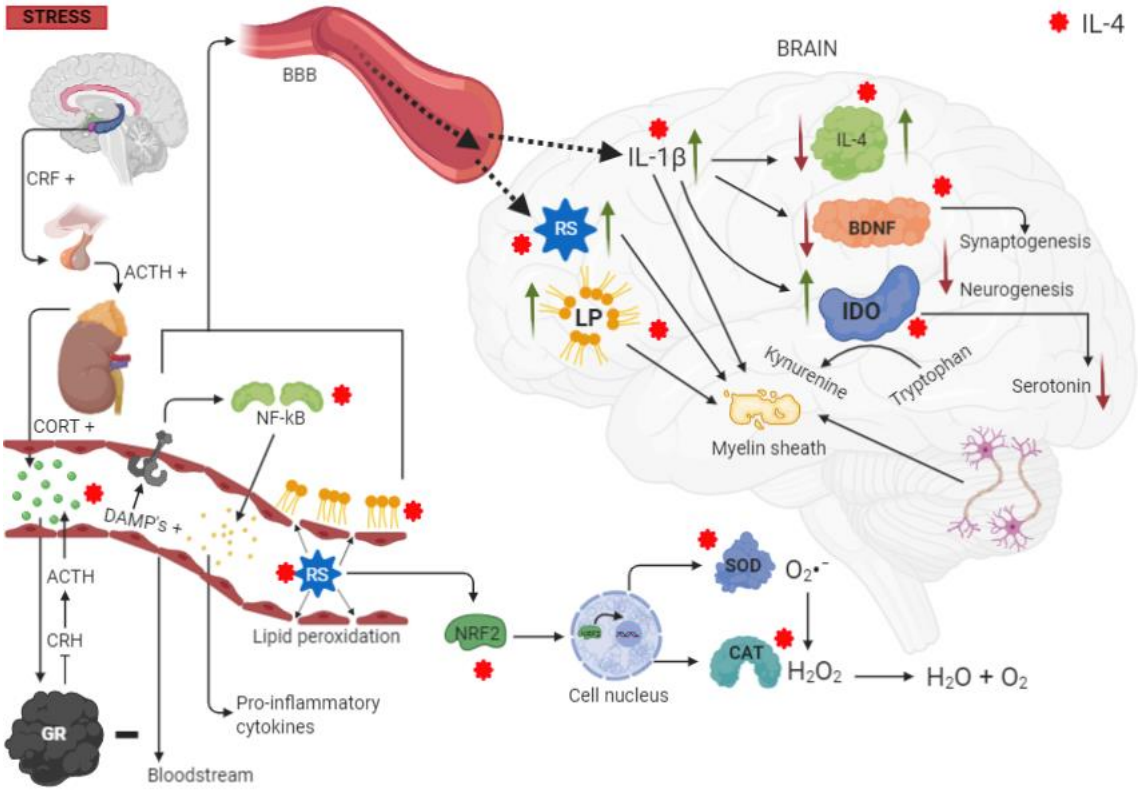


Key: Salt bridges Disulphide bonds Hydrogens bonds Non-bonded contacts

Residue colours: Positive (H, K, R); negative (D,E); S,T,N, Q = neutral; A, V, L, I, M = aliphatic; F, Y, W = aromatic; P, G = Pro&Gly; C = cysteine.

The number of H-bond lines between any two residues indicates the number of potential hydrogen bonds between them. For non-bonded contacts, which can be plentiful, the width of the striped line is proportional to the number of atomic contacts.

Figure 9.


STRESS
IL-4
BRAIN
BBB
CRF +
ACTH +
CORT +
IL-1β
IL-4
BDNF
Synaptogenesis
Neurogenesis
RS
LP
IDO
Kynurenine
Tryptophan
Serotonin
Myelin sheath
NF-kB
DAMP's +
ACTH
CRH
RS
Lipid peroxidation
NRF2
Cell nucleus
SOD
$O_2^{\bullet -}$
CAT
H_2O_2
$H_2O + O_2$
GR
Pro-inflammatory cytokines
Bloodstream

Highlights

- Intranasal administration of IL-4 ameliorated depression-like behavior in mice.
- IL-4 modulated neuroinflammation in the prefrontal cortices and hippocampi of mice.
- IL-4 modulated markers of oxidative stress in the periphery and in the brain of mice.

5. Discussão

A presente dissertação foi planejada para investigar e caracterizar a utilização de uma proteína endógena do organismo como um possível tratamento para os sintomas do tipo-depressivo em camundongos. Utilizando o modelo de indução do CUMS, os camundongos apresentaram as principais alterações existentes em pessoas com DM, como anedonia, perda de resposta a situações de perigo/desconforto, aumento de ER, peroxidação lipídica, liberação exacerbada de citocinas pró-inflamatórias, hiperativação do eixo HPA e danos a órgãos responsáveis por respostas imunes.

O trabalho demonstra a capacidade promissora de uma proteína endógena para o tratamento da DM, visto que diminuiu o tempo de imobilidade dos camundongos no TSC e aumentou o tempo de grooming no TS. Em ambos os testes, camundongos que foram submetidos ao CUMS apresentaram alterações no tempo de imobilidade e grooming quando comparados com o grupo controle, estado que foi revertido pela administração aguda de IL-4.

Embora inúmeros tratamentos com medicamentos antidepressivos apresentem um aumento no tempo de imobilidade no TSC e aumento no tempo de grooming, alguns apresentam efeito na atividade exploratória e locomotora, ocasionado assim eventuais resultados falsos positivos e negativos. A IL-4 possivelmente por ser uma proteína do mecanismo endógeno e diversas células apresentarem receptores para ela, não ocorreram alterações na atividade exploratória e locomotora em nenhum dos tratamentos dos camundongos no TCA.

Sabe-se que o estresse também é capaz de promover alterações no eixo HPA, aumentando assim a liberação de corticosterona na corrente sanguínea (JURUENA, 2004). Esse aumento é considerado um dos principais fatores no desencadeamento de TNP, como a DM e tornando os órgãos responsáveis por respostas inflamatórias mais suscetíveis a danos (SANGOMLA et al., 2018; SARJAN; YAJURVEDI, 2019; SHAH et al., 2018)

Com isso, vale ressaltar que o aumento de glicocorticoides na corrente sanguínea corrobora para a resistência dos seus receptores, contribuindo com o

desenvolvimento e progressão da neuroinflamação e do estresse oxidativo (MUNOZ-FERNANDEZ et al., 1998; LIU et al., 2015; PISOSCHI et al., 2015). Um dos possíveis fatores responsáveis pela atenuação da neuroinflamação é a capacidade de interação da corticosterona em reduzir a expressão do BDNF nas estruturas cerebrais de córtex e hipocampo (EYRE E BAUNE, 2012; KOHMAN et al., 2014), uma importante proteína do grupo de neurotrofinas responsável pela manutenção e sobrevivência de células neuronais (ROSSETI et al., 2019).

Embora seja encontrada uma diminuição nos níveis de BDNF, existem outros fatores que participam da atenuação da neuroinflamação, como por exemplo o aumento de expressão de citocinas pro-inflamatórias, como a IL-1β, e aumento da atividade da enzima IDO estimuladas pela via de sinalização do NF-kB através dos receptores TLR-4 pelos DAMPs e diminuição de citocinas anti-inflamatórias, como a IL-4, padrões alterados que são encontrados em pacientes com depressão (SANTOSBARRIOPEDRO; VAQUERO, 2018).

Corroborando com esse estado, temos o aumento de ER, que participam também como mediadores da sinalização na ativação do NF-kB (ISABEL FILIPPIN et al., 2008), que por sua vez também ativa a translocação do NRF2 para o núcleo da célula, para que ocorra a síntese de enzimas antioxidantes, como a CAT e SOD capazes de combater esse excesso de ER na regulação do sistema REDOX (HAYES et al., 2005; ITOH et al., 1997; VENUGOPAL; JAISWAL, 1996).

Nesse estudo, a IL-4 demonstrou possível efeito na modulação do eixo HPA, visto pela diminuição de níveis circulantes de corticosterona na corrente sanguínea e normalização dos pesos das glândulas adrenais. Com isso, também podemos ressaltar que a IL-4 participou do decréscimo da neuroinflamação visto pela restauração da expressão de BDNF, IL-4, NF-kB, normalização da atividade da enzima IDO e diminuição da expressão de IL-1β no córtex pré-frontal e hipocampo, bem como moduladora do estresse oxidativo, possivelmente agindo na regulação da expressão do NRF2 também em ambas estruturas cerebrais, que por sua vez atuou na regulação das atividades das enzimas CAT e SOD que promoveram a neutralização do excesso de ER e promovendo um decréscimo na peroxidação lipídica nas estruturas de córtex pré-frontal, hipocampo e plasma. Embora a IL-4 tenha apresentado efeito na restauração dos danos

ocasionados pelo estresse na glândula adrenal, uma administração aguda não foi capaz de reverter os danos no baço, linfonodos axiais e timo.Com isso, abre-se uma hipótese de que uma administração crônica de IL-4 seja capaz de reverter esses danos.

A partir disso, podemos sugerir que o efeito do tipo-antidepressivo da IL-4 está relacionado com a sua capacidade de diminuir a neuroinflamação, modular o estresse oxidativo e promover a regulação do eixo HPA de camundongos, fatores que estão intimamente ligados com a DM. Os possíveis mecanismo de ação da IL-4 estão detalhados na (Figura 1).

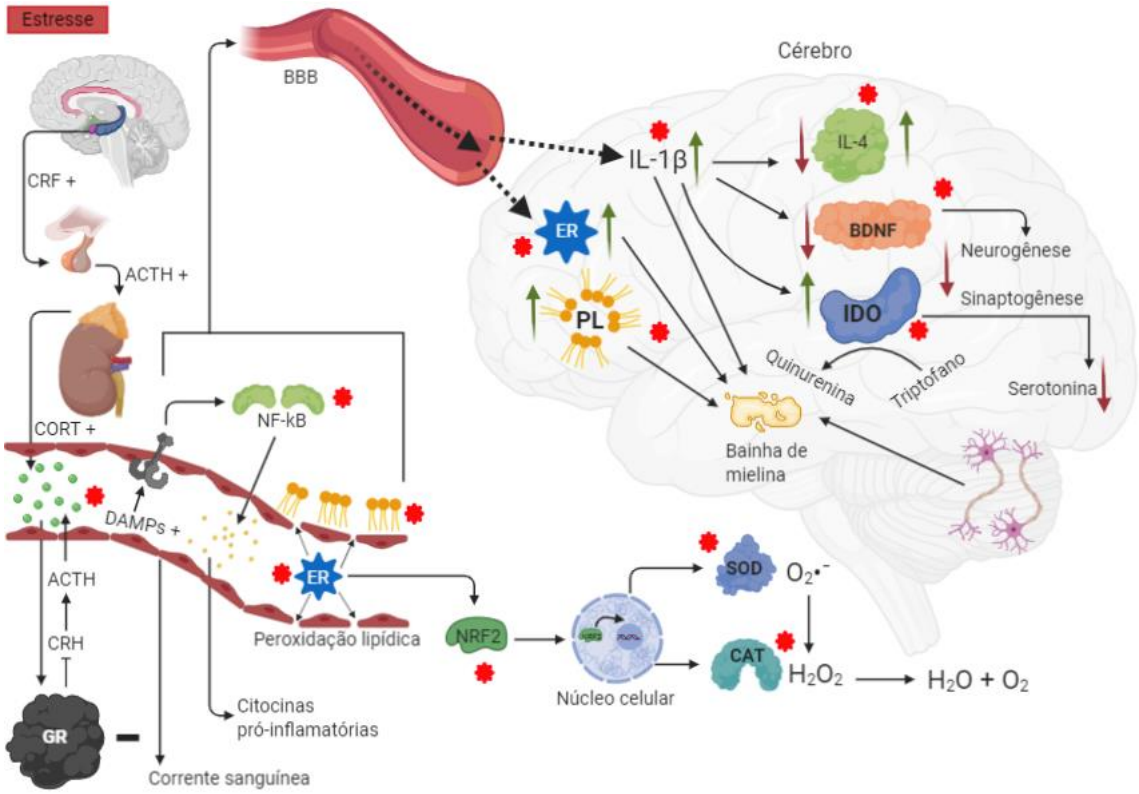


Figura 1. Mecanismo de ação responsável pelo efeito do tipo-antidepressivo da IL-4 avaliado na dissertação. Abreviações utilizadas para hormônio liberador de corticotrofina (CRF), hormônio adrenocorticotrófico (ACTH,) corticosterona (CORT), receptores de glicocorticoides (GR), padrões moleculares associados ao perigo (DAMPs), fator nuclear kappa B (NF-kB), espécies reativas (ER), peroxidação lipídica (PL), Fator nuclear eritróide 2-relacionado ao fator 2 (NRF2), superóxido dismutase (SOD), catalase (CAT), barreira hematoencefálica (BBB), interleucina 1-beta (IL-1β), interleucina-4 (IL-4), fator neurotrófico derivado do cérebro (BDNF) e Indoleamina-2,3 dioxigenase IDO. Fonte: Biorender.

Embora a depressão não tenha sido completamente elucidada, podemos observar que tratamentos que possuem atividade multialvo, ou seja, que podem agir em diferentes vias vem sendo uma das alternativas para os tratamentos dos sintomas depressivos. Nesse sentido, os estudos pré-clínicos se fazem de grande valia para avaliar os efeitos de novas moléculas para o tratamento desse TNP.

6. Conclusão

Na presente dissertação, validou-se a nossa hipótese inicial de que a IL-4 seria capaz de reverter o comportamento do tipo-depressivo induzidos pelo CUMS. Essas hipóteses foram validadas através da modulação da via do estresse oxidativo e decréscimo de marcadores neuroinflamatórias. Com isso, enfatiza-se a importância da utilização dessa proteína em novos modelos para elucidarmos melhor as vias de sinalização e ação da IL-4.

Conclui-se que se obteve resultados para encontrar uma nova alternativa para o tratamento da depressão, uma proteína do mecanismo endógeno, a fim de diminuir os efeitos adversos ocasionados pelos medicamentos atuais no tratamento da depressão.

7. Perspectivas futuras

- Avaliar a atividade da IL-4 em camundongos fêmeas frente ao modelo do CUMS;
- Avaliar a atividade da IL-4 frente a indução de LPS;
- Avaliar a atividade da IL-4 no estresse agudo de restrição;
- Avaliar as cascatas envolvidas na sinalização e ação da IL-4.

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