UNIVERSIDADE DO SUL DE SANTA CATARINA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE
LUCINEIA GAINSKI DANIELSKI

INVESTIGAÇÃO DO ENVOLVIMENTO DO INFLAMASSOMA NLRP3 SOBRE ALTERAÇÕES CEREBRAIS PRECOCES E TARDIAS APÓS A SEPSE

Tubarão
2020

LUCINEIA GAINSKI DANIELSKI

INVESTIGAÇÃO DO ENVOLVIMENTO DO INFLAMASSOMA NLRP3 SOBRE ALTERAÇÕES CEREBRAIS PRECOCES E TARDIAS APÓS A SEPSE

LINHA DE PESQUISA: NEUROCIÊNCIAS

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ciências da Saúde para obtenção do título de Doutora em Ciências da Saúde.

Orientadora: Profa. Fabrícia Cardoso Petronilho, Dra.

Tubarão

2020

D18 Danielski, Lucinéia Gainski, 1990 -
Investigação do envolvimento do inflamassoma NLRP3 sobre alterações cerebrais precoces e tardias após a sepse / Lucinéia Gainski Danielski. – 2020.
99 f. : il. color. ; 30 cm

Tese (Doutorado) – Universidade do Sul de Santa Catarina, Pós-graduação em Ciências da Saúde.
Orientação: Profa. Dra. Fabricia Cardoso Petronilho

1. Septicemia. I. Petronilho, Fabricia Cardoso. II. Universidade do Sul de Santa Catarina. III. Título.

CDD (21. ed.) 616.944

Ficha catalográfica elaborada por Carolini da Rocha CRB 14/1215

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÙDE - DOUTORADO

Título da Tese

INVESTIGAÇÃO DO ENVOLVIMENTO DO INFLAMASSOMA NLRP3 SOBRE ALTERAÇÕES CEREBRAIS PRECOCES E TARDIAS APÓS A SEPSE

LUCINÉIA GAINSKI DANIELSKI
AUTOR

Aprovado pela Banca Avaliadora de Defesa da Tese em 04 de dezembro de 2020.

Doutora Fabricia Cardoso Petronilho (Orientador) _____

Doutora Gabriela Delevati Colpo (Avaliador externo -University of Texas Health Science Center at Houston – EUA) – *presente por videoconferência*

Doutora Silvia Resende Terra (Avaliador externo - UFRGS)– *presente por videoconferência*

Doutora Gislaine Tezza Rezin (Avaliador interno – PPGCS) – *presente por videoconferência*

Doutora Josiane Somariva Prophiro (Avaliador interno - PPGCS) – *presente por videoconferência*

Professor Doutor Jefferson Luiz Traebert
COORDENADOR DO PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE - UNISUL

A todos os pacientes que esperam por nós.

AGRADECIMENTOS

A segunda chance que a vida e sua finitude me deram, e a consciência que de “tudo o que somos é poeira ao vento” (Kansas).

Ao Laboratório de Neurobiologia de Processos Inflamatórios e Metabólicos – Neuroimet - e a Universidade do Sul de Santa Catarina (UNISUL), minha casa desde a graduação.

À University of Texas Health Sciences Center at Houston e a Universidade do Extremo Sul Catarinense, pelo apoio em experimentos.

À professora e orientadora Fabrícia Cardoso Petronilho. Pela parceria de anos e por sempre incentivar os alunos a irem além.

A todos os colegas do Núcleo Sepse, pela parceria nos experimentos e no dia a dia do nosso lab.

A todos os animais envolvidos neste e em outros trabalhos científicos, os verdadeiros heróis de toda a pesquisa.

Ao Conselho Nacional de Pesquisa e Desenvolvimento (CNPq) e a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) por propiciar fomento para a realização desta pesquisa.

Ao programa de Pós-Graduação em Ciências da Saúde, o qual proveu os meios necessários para a conclusão do doutoramento.

A todos vocês, meu muito obrigada!

RESUMO

Introdução: Pacientes sobreviventes de sepse desenvolvem danos cognitivos agudos e em longo prazo e a ativação microglial está envolvida com a sua fisiopatologia. Recentemente, foram identificados receptores que formam complexos proteicos, chamados inflamassomas. O inflamassoma NLRP3 pode estar envolvido com a exacerbação da resposta inflamatória microglial após a sepse.

Objetivo: Investigar o envolvimento do inflamassoma NLRP3 sobre alterações cerebrais precoces e tardias na sepse experimental.

Métodos: Ratos *Wistar* machos de dois meses de vida, submetidos a de sepse por ligação e perfuração cecal (grupo CLP) ou somente laparotomia (grupo sham). Imediatamente após, receberam salina ou inibidor do inflamassoma NLRP3 (MCC950, 140 ng / kg), via icv. Vinte e quatro horas após, córtex pré-frontal e hipocampo foram isolados. Analisou-se os níveis de citocinas, níveis de NLRP3, ativação microglial e astrocitária, estresse oxidativo, formação de nitrito/nitrato e atividade da cadeia respiratória mitocondrial. Durante dez dias houve avaliação de sobrevida, e em seguida foram realizados testes comportamentais.

Resultados: MCC950 evitou a elevação dos níveis de citocinas IL-1β, TNF-α, IL-6 e IL-10 no hipocampo, 24 horas após a sepse. No mesmo tempo, houve elevação dos níveis do receptor NLRP3 no córtex pré-frontal e hipocampo, associada a ativação microglial, mas não astrocitária. MCC950 evitou dano em lipídios e proteínas, bem como preservou a atividade da enzima SOD no hipocampo. Houve variação na atividade da cadeia respiratória mitocondrial, sem padrão reconhecível. A administração de uma única dose do inibidor de formação do NLRP3, gerou melhora na sobrevida, e diminuição do dano cognitivo nos animais avaliados 10 dias após a sepse.

Conclusão: A formação do inflamassoma NLRP3 está associada a alterações bioquímicas e neuroinflamatórias agudas, elevação da mortalidade e presença de dano cognitivo em longo prazo após a sepse experimental.

Descritores: Sepse. Microglia. NLRP3.

ABSTRACT

Introduction: Surviving sepsis patients develop acute and long-term cognitive damage and microglial activation is involved in its pathophysiology. Recently, they have been identified as protein-forming receptors, called inflammasomes. The NLRP3 inflammasome may be present with the exacerbation of the microglial inflammatory response after sepsis.

Objective*:* To investigate the involvement of the NLRP3 inflammasome in early and late brain changes in experimental sepsis.

Methods: Two-month-old male Wistar rats submitted to sepsis by cecal ligation and perforation (CLP group) or only laparotomy (sham group). Immediately, they received saline or NLRP3 inflammasome inhibitor (MCC950, 140 ng / kg), via icv. Twenty-four hours later, prefrontal cortex and hippocampus were isolated for analysis of cytokine levels, NLRP3 levels, microglial and astrocyte activation, measures of oxidative stress, nitrite/nitrate formation and mitochondrial respiratory chain activity. Survival was assessed for ten days, and then behavioral tests were performed.

Results: MCC950 prevented elevated levels of cytokines IL-1β, TNF-α, IL-6 and IL-10 in the hippocampus, 24 hours after sepsis. At the same time, there was an increase in the levels of the NLRP3 receptor in the prefrontal cortex and hippocampus, associated with microglial activation, but not astrocyte. MCC950 prevented damage to lipids and proteins, as well as preserved the activity of the SOD enzyme in the hippocampus. There was variation in the activity of the mitochondrial respiratory chain, with no recognizable pattern. The administration of a single dose of the inhibitor of NLRP3 formation, improved survival, and decreased cognitive damage in animals evaluated 10 days after sepsis.

Conclusion: The NLRP3 inflammasome formation is associated with acute biochemical and neuroinflammatory changes, increased mortality and the presence of long-term cognitive damage after experimental sepsis.

Keywords: *Sepsis. Microglia. NLRP3.*

LISTAS

Lista de abreviaturas

ANOVA – Análise de Variância

ASC – Proteínas semelhantes a partícula apoptótica contendo domínio de recrutamento de caspase, do inglês "*apoptosis-associated speck-like protein containing a caspase-recruitment domain"*

ATP – Adenosina trifosfato

BHE – Barreira hematoencefálica

CAT – Catalase

CLP – Ligação e perfuração cecal, do inglês "*cecal ligation and puncture*"

cm – Centímetros

DAMPs – Padrões moleculares associados ao dano, do inglês "*damage-associated molecular patterns*"

DCIP – 2,6-diclorofenol-indofenol

EAS – Encefalopatia associada a sepse

EROs – Espécies reativas de oxigênio

EUA – Estados Unidos da América

h – Hora

icv – Intracerebroventricular

ICAM-1 – Molécula de adesão intercelular tipo 1, do inglês "*intercellular adhesion molecule 1*"

IGF-1 - Fator de crescimento símile a insulina tipo 1

IL-10 – Interleucina 10

IL-18 – Interleucina 18

IL-1β – Interleucina 1 beta

IL-6 – Interleucina 6

iNOS – Óxido nítrico sintase induzível do inglês "*inducible nitric oxide synthase*"

K^+ – Potássio

kg – Quilogramas

LPS – Lipopolissacarídeo

ng – Nanogramas

mM – Milimolar

MMPs – Metaloproteinases de matriz, do inglês *"matrix metalloproteinase*"

NADH – Nicotinamida adenina dinucleotídeo reduzido

NADPH – Nicotinamida adenina dinucleotídeo fosfato reduzida

NALP3 – Sinônimo de NLRP3

NF-kB – Fator nuclear kappa B do inglês "*nuclear factor kappa B*"

NLRP3 – Domínio de ligação e oligomerização de nucleotídeos contendo domínios ricos em leucina e domínio pirina tipo 3, do inglês "*NLR family pyrin domain containing 3"*

NLRs – Receptores do tipo domínio de ligação e oligomerização de nucleotídeos, do inglês "*nucleotide-binding oligomerization domain-like receptors*"

nm – Nanômetros

NOD – Domínio de ligação e oligomerização de nucleotídeos, do inglês "*nucleotide oligomerization domain*"

NO – Óxido nítrico do inglês "*nitric oxide"*

P2X7 – Receptor purinérgico P2X7

PAMPs – Padrões moleculares associados a patógenos, do inglês *"pathogen-associated molecular pattern*"

qSOFA – Medida sequencial rápida de falências orgânicas, do inglês "*quick Sequential Organ Failure Assessment Score"*

RRP – Receptor de reconhecimento padrão

SNC – Sistema Nervoso Central

SOD – Superóxido dismutase

SOFA – Medida sequencial de falências orgânicas, do inglês "*Sequential Organ Failure Assessment*"

TLR – Receptores semelhantes a Toll, do inglês "*toll like receptor"*

TLR-2 – Receptor semelhante a Toll tipo 2, do inglês *"toll like receptor 2"*

TLR-4 – Receptor semelhante a Toll tipo 4, do inglês "*toll like receptor 4"*

TLR-6 – Receptor semelhante a Toll tipo 6, do inglês "*toll like receptor 6"*

TNF-α – Fator de necrose tumoral alfa, do inglês "*tumor necrosis factor alpha*"

UCP2 – Proteína desacopladora 2

Lista de Símbolos

α - Alfa

β - Beta

κ - Kappa

γ - Gama

λ - Lambda

Lista de figuras

SUMÁRIO

1. INTRODUÇÃO

A sepse é uma síndrome complexa e extremamente comum nas unidades de terapia intensiva ao redor do mundo, apresenta elevados índices de mortalidade e morbidade, com prevalência de até 60% em pacientes internados[1]. Na sepse, a resposta inflamatória é amplificada de tal forma, que causa dano em órgãos diferentes daquele que acometidos pela infecção inicial, sendo que o encéfalo está entre os acometidos, o que gera alterações neurológicas agudas e em longo prazo[2,3].

Há anos, estudos tem associado o dano neurológico na sepse com a elevação da permeabilidade das barreiras encefálicas, causada pela exacerbada liberação de citocinas pró-inflamatórias e pelo estresse oxidativo periférico[4,5]. Estes mediadores ativam células imunes no encéfalo, como a microglia.

A microglia tem funções imunes no SNC, ela exibe diversos receptores de superfície celular, que podem ser ativados por diferentes ligantes, e em certos momentos, pode ocorrer até uma super ativação da microglia, gerando uma resposta inflamatória persistente, que pode ser causa de dano celular no encéfalo[6]. Todavia acredita-se que ainda existam outros mecanismos, até mesmo mecanismos fisiológicos que quando superativados podem contribuir para o dano encefálico após a sepse[7].

Quando ativada, a microglia libera citocinas, como a interleucina 1β (IL-1β), a qual é sintetizada a partir de precursores inativos e que dependem da clivagem executada pela enzima caspase-1 para se tornar ativa e exercer suas funções[6]. Para a ativação da caspase-1 é necessária a clivagem de pro-caspase 1 realizada por um complexo multiprotéico denominado inflamassoma[6]. O inflamassoma é um grupo de complexos proteicos de alto peso molecular presentes no citosol que são formados para mediar as respostas imunes do hospedeiro à infecção microbiana e ao dano celular[8]. Todavia, estudos recentes vêm demostrando que a ativação exacerbada deste complexo está envolvida na patogênese de diversas doenças neuroinflamatórias, como Alzheimer[9] e Parkinson[10] sendo responsável pela exacerbação da neuroinflamação e danos cognitivos nestas doenças.

O inflamassoma chamado domínio de ligação e oligomerização de nucleotídeos contendo domínios ricos em leucina e domínio pirina tipo 3 (NLRP3), regula a ativação da via da caspase-1 até a liberação e ativação das citocinas IL-1β e

a interleucina-18 (IL-18), podendo assim estar relacionado com a potencialização da resposta inflamatória e com isso, levando a alterações neuroquímicas e comportamentais como já verificados em estudos experimentais e possivelmente nas apresentadas pelos pacientes com sepse[7].

Utilizando um inibidor específico da via do inflamassoma NLRP3 diretamente no encéfalo, acreditamos ser possível gerarmos mais informações sobre o envolvimento desse inflamassoma sob alterações cerebrais na sepse. Tais respostas podem assim esclarecer o seu papel na resposta inflamatória potencializada, na morte celular associada a disfunção cerebral aguda e consequentemente, em sobreviventes da sepse, ao prejuízo cognitivo, resultado da extensa degeneração de neurônios cerebrais.

1.1 REFERENCIAL TEÓRICO

1.1.1 Sepse: breve histórico e definições

A palavra sepse deriva do grego, e significa alguma coisa (animal ou vegetal) que se decompôs na presença de bactérias. Hipócrates foi um dos primeiros a identificar a sepse, onde ele descreve um “decaimento biológico” que parecia ocorrer no cólon, com a liberação de “princípios perigosos” que causavam a autointoxicação[11].

Com as diversas teorias criadas, predominando a teoria dos animalcúlos e do miasma, foi apenas no século XIX que surgiram explicações mais científicas sobre a sepse. Um achado histórico relevante, foi observado por *Ignaz Semmelweis*, na data de 1841, enquanto trabalhava em uma maternidade. *Semmelweiss* observou que a taxa de mortalidade entre as mulheres que eram atendidas por estudantes de medicina era muito superior (16%) a taxa de mortalidade entre as mulheres que eram atendidas por parteiras (2%). O pesquisador intrigado começou a fazer observações. Todavia, a explicação para tal fato, surgiu quando um de seus colegas faleceu, após se machucar durante uma necropsia. *Semmelweis* então, observou que os alunos de medicina, não lavavam as mãos após as necropsias e assim, levavam as “substâncias tóxicas” para as próximas mulheres em trabalho de parto, o que ocasionava a elevada mortalidade[12]. Estes foram os primeiros relatos da sepse.

No entanto, apenas na década dos anos 1990, foi que surgiu a primeira definição conceitual para sepse[13], e desde estão o conceito vem sendo aprimorado com o passar dos anos. O mais recente, data de 2016, onde o Jornal da Associação Médica Americana publicou uma proposta para definições e critérios de sepse, chamada de sepse-3. Neste consenso, a sepse, passou a ser definida como uma síndrome, na qual o paciente apresenta disfunção orgânica com risco de vida em decorrência da inflamação exagerada causada por uma infecção[14].

Na suspeita clínica de infecção, o diagnóstico precoce da sepse é crucial para redução da mortalidade. Para isso sugerem-se o uso de critérios rápidos do sistema de pontuação de disfunção orgânica (qSOFA; do inglês *quick Sequential Organ Failure Assessment Score*). Onde a alteração de dois dos três parâmetros indica a presença de sepse, como por exemplo, alteração do estado mental e pressão arterial sistólica de 100 mmHg (milímetros de mercúrio)[15].

Para a confirmação e seguimento do paciente com disfunção orgânica, passa-se a considerar a alteração de 2 pontos na pontuação total do sistema de pontuação de disfunção orgânica completo (SOFA; do inglês *Sequential Organ Failure Assessment Score*). O escore SOFA associa as seguintes alterações orgânicas: respiração, coagulação, hipotensão, marcadores hepáticos e renais, bem como a escala de coma Glasgow. Quanto maior a pontuação do paciente, mais grave é a sua disfunção orgânica, ou seja, mais órgãos estão lesionados pela resposta inflamatória exacerbada[15–17].

Outra fase, ainda mais grave na evolução da sepse, é chamada de choque séptico, nessa fase o paciente encontra-se em estado de hipotensão persistente após a reposição volêmica. Os critérios para essa classificação são, necessidade do uso de vasopressores para manter a pressão arterial média igual ou superior a 65mmHg, associado ao aumento dos níveis de lactato sérico acima de 18 mg/dL, independente do volume de reposição volêmica. Pacientes com esta clínica tem um acréscimo de 40% na mortalidade hospitalar[14].

Uma recente conquista, para a disseminação da importância do diagnóstico precoce da sepse, ocorreu no ano de 2017, ocasião na qual a Organização Mundial da Saúde reconheceu a sepse como uma doença de prioridade na saúde global, incluindo-a nas estatísticas globais da carga de doenças, o *Global Burden of Disease Statistics*. Sendo assim, as ações voltadas ao objetivo de melhoria, prevenção e

gerenciamento da sepse em caráter mundial, visando diminuição de morbidade e mortalidade, recebem maior importância e destaque, fortalecendo ações de saúde pública[18].

1.1.2 Epidemiologia da sepse

Devido a sua gravidade e prevalência, a sepse é uma importante preocupação em saúde pública, com estimativas globais de 31,5 milhões de casos anualmente, incluindo 19,4 milhões de casos graves, resultando potencialmente em 5,3 milhões de mortes[19]. No Brasil, os dados disponíveis, demonstram prevalência de 290 casos de sepse para cada 100.000 habitantes adultos por ano, o que gera 420.000 casos por ano, dos quais mais, da metade dos pacientes falecem ainda no hospital[20]. Embora sejam dados alarmantes, os mesmos são semelhantes aos encontrados em países desenvolvidos, onde descrevem-se 270 casos por 100.000 habitantes por ano[19].

Felizmente, na última década houve a diminuição da mortalidade de pacientes com sepse. Em 2014, 1,3 milhão de adultos norte-americanos sobreviveram a uma internação por sepse, dos quais 56% tinham 65 anos ou mais[21]. Entretanto, outros desafios surgem, principalmente os relacionados a qualidade de vida e ao manejo dos danos neurológicos agudos e persistentes apresentados pelos sobreviventes. Dentre os pacientes que sobrevivem a sepse, até 75% sofrem de alguma incapacidade funcional e/ou laboral, e cerca de 17% manifestam algum grau de comprometimento cognitivo, o que afeta diretamente a sua qualidade de vida[22].

Durante a internação por sepse os pacientes experimentam delírio e comprometimento da consciência, independente do uso de medicamentos que poderiam causar essas manifestações. Após a alta hospitalar, um estudo evidenciou comprometimentos a longo prazo, na memória, atenção, fluência verbal e funcionamento executivo[23]. Outro estudo demonstra que os sobreviventes, enfrentam alta taxa de mortalidade em um ano, quando comparados com sobreviventes de outras doenças[24]. Ainda, existem evidências, que demonstram que essas sequelas não podem ser atribuídas, apenas, ao mau estado de saúde antes da admissão com sepse, o que indica que a sepse agrava a saúde do paciente, mesmo depois da recuperação[25]. Dessa forma, conclui-se que mecanismos relacionados a fisiopatologia da sepse no encéfalo, e ainda não totalmente esclarecidos, sejam os causadores ou

potencializadores das sequelas observadas nos sobreviventes de sepse, em outras palavras, o que ocorre no SNC durante a sepse, pode resultar em danos permanentes nos pacientes sobreviventes.

1.1.3 Fisiopatologia da sepse

A resposta imunológica inata do hospedeiro contra uma infecção bacteriana é um processo complexo, que visa identificar o foco infeccioso, eliminar o agente patogênico e reparar o tecido lesionado[26]. Envolve a ativação de células fagocíticas residentes e circulantes, bem como a geração de mediadores pró-inflamatórios e oxidantes necessários para a eliminação do patógeno[27]. Na grande maioria das vezes, o sistema imunológico é capaz de controlar a infeção, sem que ocorra doença infecciosa, em outros casos, há necessidade do uso de medicamentos. Entretanto, em determinados pacientes, embora ocorra o controle dos microrganismos no foco infeccioso, por diferentes motivos a resposta imune se torna descontrolada, e os mediadores químicos que deveriam ter ação local, passam a gerar resposta sistêmica que levam a disfunção orgânica[1], o que caracteriza a sepse.

As células humanas, como os neutrófilos, macrófagos, microglia, entre outras, apresentam em sua superfície celular um receptor de reconhecimento de padrões (RRP). Esses são capazes de reconhecer, microrganismos, que apresentam diferentes padrões associados a patógenos (chamados de PAMPs). E da mesma forma, os RRP podem reconhecer padrões moleculares associados ao dano celular (DAMPs)[28]. Estes RRP estão ligados a vias de transdução de sinais intracelulares que ativam várias respostas celulares, incluindo o estímulo a inflamação[29].

São exemplos de RRP, os receptores semelhantes a *Toll* (TLR), os receptores varredores, as lectinas tipo C, os receptores semelhantes a NOD (NLR), e os receptores *N*-formil-Met-Leu-Phe[28]. Na sepse, tem se estudado majoritariamente os receptores semelhantes a *Toll* tipo 4 (TLR-4), especializados em reconhecimento de lipopolissacarídeo (LPS) de bactérias gram-negativas e o TLR-2, responsável pela sinalização da presença dos peptiodeoglicanos de bactérias gram-positivas[30–34].

Como exemplo, na sepse polimicrobiana, há liberação de LPS e peptiodeoglicanos que após reconhecidos pelos RRP, ativam vias de sinalização levando a translocação do fator nuclear kappa B (NF-kB) do citoplasma para o interior

do núcleo da célula humana. O NF- kB é responsável pela ativação de diversos genes, incluindo os codificadores de citocinas pró-inflamatórias, como a pró-interleucina IL-1β, fator de necrose tumoral alfa (TNF-α), interferon gama (IFN-y) e a interleucina-6 (IL-6)[35]. A liberação *in loco* destas citocinas é muito importante para o estímulo do recrutamento de leucócitos para o foco infecioso, onde acontecerá a fagocitose, com liberação de radicais livres, processo este importante para o controle dos patógenos. Todavia, na sepse, mesmo ocorrendo o controle dos microrganismos no local inicial da infecção, a resposta inflamatória gerada pelo hospedeiro é capaz de causar dano tecidual em órgãos diferentes do acometido pelo infecção[36].

Na fisiopatologia da sepse, há envolvimento de respostas geradas pela inflamação exacerbada, como isquemia tecidual, lesão citopática por mediadores inflamatórios e espécies reativas de oxigênio (EROs). Desse modo, ocorre lesão endotelial difusa, trombose microvascular, ruptura das junções das células endoteliais gerando dano tecidual e orgânico. A combinação desses mecanismos contribui para a disfunção orgânica, incluindo dano no SNC[37,38].

1.1.4 Disfunção do SNC na sepse

Normalmente a sepse tem início com uma infecção periférica, ou seja, externa ao encéfalo, como pneumonia ou apendicite, sendo então a resposta inflamatória disseminada por todo organismo, incluindo o SNC[6]. Certamente, são crescentes as evidências de acometimento do SNC, tanto em modelos animais[39] como em estudos clínicos[40]. Humanos sobreviventes de sepse, experimentam diferentes sequelas, entre estas as físicas, como fraqueza muscular[24], as cognitivas, como deficiências de longo prazo na memória, atenção, fluência verbal e funcionamento executivo[42]. Ainda há a possibilidade de sintomas de ansiedade, de depressão e o aparecimento de estresse pós traumático[41–43].

Em pacientes sobreviventes existem evidências de melhora na função cognitiva nos primeiros seis meses após a alta hospitalar, alguns estudos evidenciam que as alterações cognitivas podem persistir por até seis anos[44]. Nestes casos os pacientes apresentam alterações na velocidade de processamento mental, na memória, atenção e nas habilidades espaço-visuais[45,46]. Quando há comprometimento da memória e cognição, existe um indicativo de disfunção do hipocampo e córtex pré-frontal, sendo

este último ainda responsável por funções executivas e que requerem atenção[47,48], funções estas prejudicadas em pacientes que passaram por um episódio de sepse.

De fato, no encéfalo de pacientes sobreviventes de sepse foram encontradas lesões isquêmicas, leucoencefalopatia difusa, edema vasogênico grave com anormalidades de distribuição posterior predominante e eventualmente a presença de atrofia no hipocampo, por exemplo[49–52], achados estes que podem justificar, pelo menos em partes, as alterações cognitivas apresentadas.

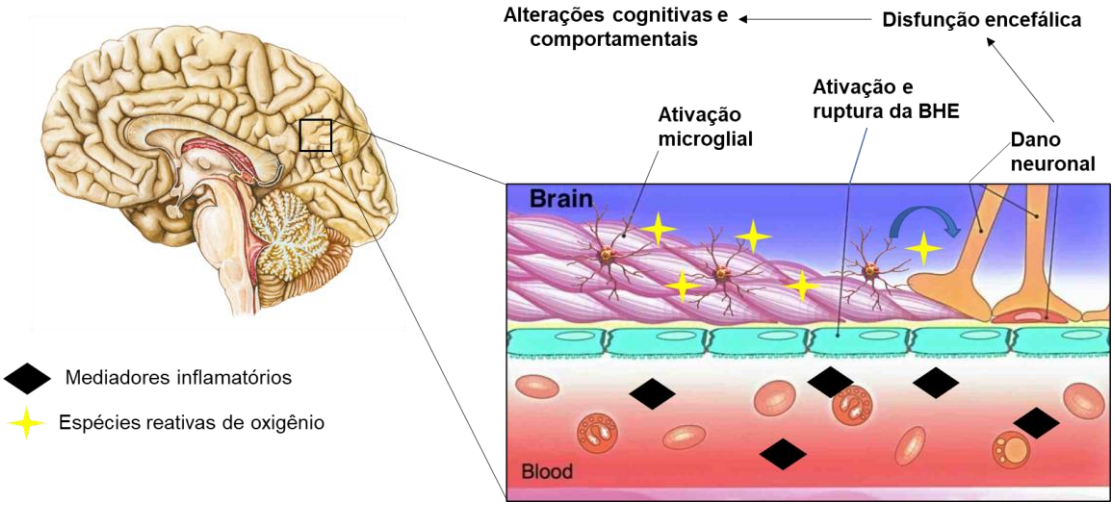
No entanto, estas manifestações neurológicas e em exames de imagem podem decorrer de alterações neuroimunes causadas pela sepse e que afetam a homeostasia do SNC. Estudos pré-clínicos tentam evidenciar possíveis mecanismos fisiopatológicos envolvidos nessa disfunção, e cada vez mais evidências sustentam o envolvimento da elevação da permeabilidade da BHE, ativação microglial com estresse oxidativo e nitrosativo que sustentam a neuroinflamação por períodos prolongados e que por sua vez causam danos aos neurônios e seu funcionamento normal[53].

1.1.4.1 Fisiopatologia da encefalopatia associada à sepse

A resposta imune exacerbada que ocorre durante a sepse, pode elevar a permeabilidade da BHE, facilitando a infiltração de células imunes da corrente sanguínea para o encéfalo, que, juntamente com a resposta imune encefálica, causam danos neuronais (Figura 1)[54].

A sinalização periférica gerada pelos mediadores inflamatórios, leva a ruptura da BHE, elevação de infiltrado de células imunes periféricas no encéfalo, causando a ativação microglial e fenômenos de estresse oxidativo e nitrosativo. A ativação microglial favorece a elevação do estresse oxidativo e da presença de substâncias pró-inflamatórias, potencializando os danos neuronais[55]. Dessa forma, dois fatores que podem ser os iniciadores das alterações encefálicas, poderiam ser a sinalização imune gerada pela reposta sistêmica da sepse associada ao aumento da permeabilidade da BHE, seguida de neuroinflamação[54].

Figura 1- Fisiopatologia da encefalopatia associada à sepse



Fonte: Adaptado de Danielski LG., et al, 2018[54]

Durante a sepse há liberação de mediadores inflamatórios, capazes de ativar o endotélio da BHE e elevar sua permeabilidade[56]. Com elevação da permeabilidade, tais mediadores e moléculas potencialmente tóxicas acessam mais facilmente o SNC[54]. Em resposta a estes eventos agressores, ocorre ativação microglial, na tentativa de recuperação da homeostase[57]. As microglias ativadas liberam citocinas e espécies reativas de oxigênio, por exemplo, que podem causar dano a BHE e além disso, aos próprios neurônios e outras células gliais[58]. Estas alterações originam danos teciduais importantes, que podem causar alterações agudas e/ou persistentes na cognição e comportamento do paciente sobrevivente de sepse[53].

1.1.4.2 Ativação do SNC e disfunção da BHE após a sepse

Os mediadores inflamatórios liberados durante a sepse podem causar ativação do SNC por diferentes vias. Uma delas, seria por meio da ativação das fibras nervosas do sistema nervoso autônomo, mais especificamente através do nervo vago, que se comunica diretamente com as estruturas encefálias[59]. As outras duas compreendem a permeação através dos órgãos circunventriculares[60], ou a difusão através da BHE ativada ou danificada[61,62].

A BHE é componente da maioria dos microvasos do SNC sendo formada por células endoteliais especializadas, cercadas por astrócitos e microglias[63]. No entanto, em alguns casos a presença desta barreira não é suficiente para proteger o SNC, justamente o que ocorre nos pacientes que desenvolvem sepse[54,64]. A sinalização

imune através da ativação das células endoteliais que compõem a BHE, é bastante estudada, uma vez que pode causar o aumento da permeabilidade da mesma e nesse sentido gerar sua disfunção[65], aumentando a infiltração de substâncias tóxicas dos tecidos periféricos para o encéfalo.

As citocinas pró-inflamatórias liberadas perifericamente desempenham papel importante na ruptura da BHE, seja por ativação endotelial direta ou relacionada a capacidade que as mesmas têm de elevar a expressão de metaloproteinases de matriz extracelular (MMPs, do inglês *matrix metalloproteinase*), enzimas da classe das proteases zinco-dependentes[66]. Na sepse, se sabe que as MMP-2 e MMP-9 têm o potencial de degradar as junções entre as células endoteliais da BHE entre 12 a 48 horas após a indução da doença em modelo animal, especialmente no hipocampo[67], o que leva ao aumento de permeabilidade desta barreira encefálica.

O aumento da permeabilidade da BHE tem sido descrito como uma característica inicial de diversas doenças inflamatórias, como esclerose múltipla, convulsões e doenças neurodegenerativas[65]. Os estudos que buscaram avaliar as causas da elevação da permeabilidade da BHE na sepse, demonstram que o maior dano às células endoteliais ocorre 24 horas após o início da sepse, momento este em que também foi verificada a elevação do rolamento de leucócitos nos microvasos do encéfalo[68,69].

Ainda, há evidências de aumento de expressão de molécula de adesão do tipo 1, responsável pelo rolamento e infiltração de leucócitos na microvasculatura cerebral do hipocampo, estriado, córtex total e córtex pré-frontal por até 24 horas após a indução de sepse experimental[61,70]. Com a ruptura da BHE, citocinas, células imunes periféricas e outras moléculas imunogênicas e tóxicas tem acesso livre ao encéfalo, podendo migrar para regiões cerebrais que estão relacionadas à memória e cognição, como o córtex pré-frontal e hipocampo, causando danos neuronais, por ativação microglial e astrocitária[71]. Evidências demonstram que a presença de citocinas em regiões encefálicas mais profundas pode levar à apoptose e insuficiência bioenergética neuronal, bem como interferências no metabolismo oxidativo, elevando a produção de EROs e diminuindo a capacidade energética celular[72,73], resultando em lesão cerebral e edema do tecido axonal[74–76].

1.1.4.3 Ativação microglial e astrocitária na sepse

A microglia representa até 12% das células no SNC[77]. Ela é a principal célula imune residente no encéfalo e está relacionada com a homeostasia e defesa contra patógenos. No entanto, sua ativação constante, tem forte associação com a fisiopatologia de doenças neuroinflamatórias e neurodegenerativas[78]. Uma das primeiras evidências de ativação microglial em pacientes com sepse foi relatada em 2007[79], onde os encéfalos de 13 pacientes que faleceram de sepse tinham níveis mais elevados de CD68 (marcador de microglia ativada) quando comparados com pacientes controle. Outro estudo, mais recente demonstrou a ativação microglial (também com o uso do marcador CD68) em pacientes que não sobreviveram ao choque séptico[80].

Em resposta a um estímulo, a microglia pode iniciar uma resposta neuroinflamatória, onde exibe fenótipos capazes da produção e liberação de citocinas como o fator de necrose tumoral alfa (TNF-α) e a IL-1β[81], e possivelmente moléculas de adesão celular[82], importantes para recrutamento de células imunes adicionais com objetivo de reestabelecer a homeostasia. No entanto, a ativação microglial de forma sustentada pode induzir neurotoxicidade, levando a neurodegeneração[78].

Assim como as células imunes periféricas, a microglia expressa TLR (por exemplo, TLR-4 e TLR-6)[83]. A ativação destes receptores, gera respostas intracelulares semelhante as células periféricas, além da capacidade de causar a polarização da microglia, para seu estado dito com pró-oxidante[84]. Além dos TLR, há envolvimento de outros receptores presentes nessas células, como os receptores da via CD40-CD40 ligante. Com o bloqueio do receptor CD40, houve redução significativa na liberação de citocinas pró-inflamatórias, como IL-1β em cultura microglial. Ainda houve a melhora da memória de habituação com a inibição desta via de ativação em ratos submetidos a sepse[85].

Além da capacidade de ativação microglial através de PAMPs, acredita-se que os DAMPs também tenham um papel muito importante durante a sepse. Por exemplo, interações entre microglia e depósitos de proteína β-amilóide, podem levar à perda precoce de sinapses[86], produção de EROs e óxido nítrico (NO, do inglês *nitric oxide)* bem como a ativação do inflamassoma NLRP3 em modelo animal de Alzheimer[87]. O mesmo pode estar ocorrendo na sepse, uma vez que trabalhos recentes demonstram depósitos desta proteína no encéfalo de ratos[88,89].

Sendo a microglia, uma célula imune, as respostas geradas por sua ativação têm como objetivo inicial, o reestabelecimento da homeostase encefálica. No entanto, sua ativação excessiva e sustentada, pode causar danos as células adjacentes, como os neurônios[58]. A microglia pode causar danos aos neurônios de forma direta ou indireta. A forma indireta deve-se a mudanças geradas por substâncias liberadas pela microglia e que podem influenciar na homeostasia nos neurônios. Seja, por meio da liberação de TNF-α, IL-1β e mediadores oxidativos, ou pela redução da sua produção de fator neuroprotetor derivado do encéfalo (BDNF) e do fator de crescimento símile a insulina tipo 1 (IGF-1), fatores estes, importantes na redução da apoptose neuronal[90]. Ou ainda pela liberação de glutamato, o que favorece o mecanismo de exitotoxicidade neuronal mediado por este neurotransmissor[91].

Por outro lado, a liberação de proteínas como as catepsinas e MMP[58] é capaz de causar dano direto aos neurônios. Ainda, quando ativadas, as microglias podem reconhecer células estressadas e eliminá-las, nesse sentido, neurônios estimulados durante a sepse podem ser reconhecidos como estressados e serem fagocitados[58].

As microglias são capazes de formar radicais livres, mediante complexos enzimáticos como a NADPH oxidase, ou por escape de elétrons na cadeia respiratória mitocondrial. Há formação de radical superóxido (O^{-}_{2}), que pode ser transformado em peróxido de hidrogênio (H_2O_2) pela ação da superóxido dismutase (SOD) ou ao reagir com o óxido nítrico (NO) formar peroxinitrito ($ONOO^{-}$) importante espécie reativa de nitrogênio (ERNs), moléculas estas que são capazes de causar dano em macromoléculas, e portanto, podem lesionar as células neuronais e então, comprometem sua função[92]. Este fenômeno de ataque as macromoléculas, é chamado de dano oxidativo. Quando ocorre danos aos lipídios ou proteínas de membrana, por exemplo, há redução da fluidez, alterações no potencial de membranas e elevação da permeabilidade celular aos íons, resultando em edema das organelas, perda de despolarização da membrana e ruptura da membrana plasmática, levando à necrose, neuronal por exemplo[78].

Outra célula que faz parte da glia, e que pode estar envolvida com a fisiopatologia do dano neuronal na sepse, é o astrócito. Esta célula não tem função imune como a microglia, no entanto é muito importante para a manutenção da homeostasia no encéfalo, devido a capacidade de liberação de metabólitos, fluídos, neurotransmissores e fatores de crescimento[93]. Interessantemente os astrócitos tem

papel importante no auxílio da manutenção da integridade da BHE[94], no entanto são poucas as evidências pré-clínicas na sepse e são ainda mais raras as evidências clínicas. Alguns estudos recentes, utilizando modelo de endotoxemia, demonstraram que os canais de cálcio podem estar envolvidos na ativação astrocitária e liberação de radicais livres e portanto quando ativados poderiam contribuir para a diminuição da homeostasia encefálica[95].

Nesse sentido, a formação de radicais livres, derivados de fagossomos ou de disfunção da mitocôndria, que se unem ou não ao NO, podem atacar as células encefálicas levando ao dano tecidual e alterações encontradas em modelos animais e, provavelmente expliquem parte do dano cognitivo em sobreviventes de sepse[54].

1.1.4.4 Estresse oxidativo/nitrosativo e disfunção energética mitocondrial na sepse

O termo radical livre é empregado na química para identificar moléculas que apresentam um ou mais elétrons desemparelhados[96]. Este fenômeno ocorre também em células de organismos vivos. Estes radicais apresentam elevada instabilidade química, e nesse sentido, podem reagir de forma espontânea com macromoléculas biológicas, o que por sua vez causa danos as mesmas, o oxigênio e nitrogênio são as principais moléculas precursoras destes radicais[97]. Ainda, algumas moléculas podem ser altamente reativas e lesivas, mesmo sem elétrons desemparelhados, por esse motivo, utiliza-se o termo espécies reativas, que representa ambas as moléculas[98].

As espécies reativas podem causam danos em macromoléculas, e esse dano têm papel ambíguo. Por exemplo, quando os radicais livres reagem com macromoléculas de patógenos, os mesmos acabam sendo lesados e por vezes mortos, e este se torna um mecanismo de defesa contra doenças infeciosas[99]. Por outro lado, quando há reatividade contra as macromoléculas de células humanas, as mesmas podem ser desestabilizadas e enfim entrarem em morte celular programada, o que em grandes extensões pode ser altamente prejudicial para o ser humano[100].

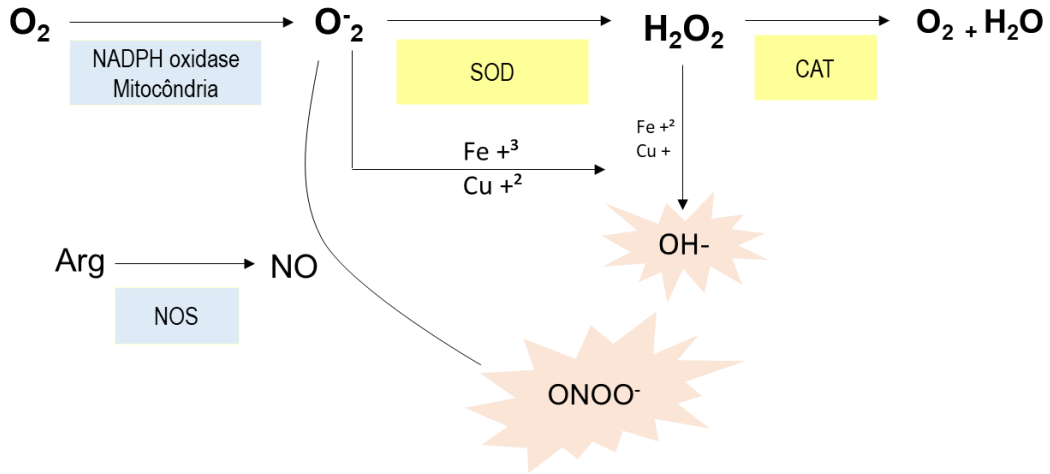
No ser humano, as principais fontes geradoras de espécies reativas são as reações que utilizam oxigênio ou nitrogênio[99]. As mitocôndrias representam a maior fonte endógena de produção de EROs nos eucariotos, onde até 2% do oxigênio utilizado na respiração celular pode se transformar em espécies reativas[101]. No entanto, as células imunes, como a própria microglia, macrófagos e neutrófilos, que

apresentam as enzimas NADPH oxidase, são capazes de produzir elevadas concentrações destes metabólitos com potencial microbicida[101].

Entretanto, devido a dualidade exibida pelas espécies reativas, e ao potencial tóxico que sua elevação oferece, nosso organismo dispõe de mecanismos enzimáticos antioxidantes que visam exercer controle negativo sobre as espécies geradas, evitando dano na células humanas[97,102]. Portanto, quando a produção de espécies reativas não é demasiadamente elevada o sistema antioxidante é capaz de manter equilíbrio entre a geração e a conversão destas moléculas em metabólitos menos reativos. Todavia, quando a produção é exacerbada ou a concentração/atividade das enzimas antioxidantes é prejudicada, ocorre o fenômeno conhecido como estresse oxidativo, e nesse processo as células humanas acabam sendo excessivamente prejudicadas[103].

Na mitocôndria, por exemplo, os complexos mitocondriais I, II e III são capazes de se auto oxidarem e produzirem semiquinona, que transfere diretamente elétrons para o oxigênio, formando o radical O^{-}_{2}[101]. O radical superóxido também pode ser formado pela enzima NADPH oxidase[104] (Figura 2).

Figura 2 - Formação de espécies reativas e as enzimas antioxidantes



Fonte: Adaptado de: Poprac P., et al, 2017[105]

Imediatamente após a geração de O^{-}_{2}, a enzima antioxidante, SOD, converte o O_2 em H_2O_2 e oxigênio molecular. Outra enzima, a catalase (CAT) entra em ação e converte o H_2O_2 em água e oxigênio molecular. Quando não há efetividade da ação da catalase, ou excesso de H_2O_2 gerado, este é capaz de se difundir entre as camadas lipídicas livremente, ou ainda, na presença do íon ferro ou do O^{-}_{2} ser convertido no

radical hidroxila (OH^-). O radical OH^- é considerada a molécula com maior capacidade de causar dano tecidual, pois não existe sistema enzimático que garanta sua eliminação[102].

Além destas, são geradas a partir da ação de enzimas conhecidas como óxido nítrico sintase, presentes em células nervosas, epiteliais, endoteliais e macrófagos as ERNs. Estas enzimas óxido nítrico sintase, catalisam a oxidação do aminoácido L-arginina através da redução do oxigênio molecular, sendo gerados a citrulina e o NO[106]. Existem, três isoformas destas enzimas, as isoformas endotelial e neuronal são constitutivas, e a isoforma óxido nítrico sintase induzível (iNOS)[107], como o próprio nome sugere, pode ser induzida por processos inflamatórios, sendo inclusive a responsável pela elevação das concentrações de NO na disfunção neurológica na sepse[108], bem como em mecanismos de neurodegeneração[109].

Quando presente em altas concentrações, o NO reage diretamente com oxigênio molecular produzindo $ONOO^-$, um produto altamente oxidante, instável e com tempo de meia-vida curto[107]. E existem evidências de elevação dos níveis de enzima iNOS 24 horas após a administração de LPS via intraperitoneal, em diferentes regiões do encéfalo de ratos, como hipocampo e córtex[110]. Em outros estudos, foi evidenciado que a microglia é capaz de liberar NO através da ativação da iNOS, quando ativada[111,112].

Diversas doenças neuroinflamatórias estão associadas com o estrese oxidativo, inclusive a sepse[113]. Dados pré-clínicos demonstram que os níveis de marcadores de dano lipídico e proteico encontra-se elevado no encéfalo após sepse. Além disso, os níveis de enzimas antioxidantes como SOD e CAT encontram-se diminuídos, gerando condições para dano celular. Igualmente, há evidências de estresse oxidativo nas horas iniciais após a sepse (6, 24 e 48 horas), e que associada à disfunção mitocondrial pode contribuir para o aparecimento da disfunção neurológica aguda[114].

A disfunção energética mitocondrial, ou dano direto causado pelas EROs e ERNs as mitocôndrias, podem afetar diretamente a geração de ATP. No encéfalo, evidências de estudos pré-clínicos utilizando modelo animal de sepse, demonstram que o hipocampo apresenta alterações na atividade dos complexos I e II entre 12 e 24 horas após a sepse, persistindo por até 96 horas[39]. No córtex pré-frontal também foi demonstrado aumento da atividade dos complexos III e IV 10 dias após a sepse[115].

Assim, as alterações na atividade dos complexos da cadeia respiratória mitocondrial podem levar a produção de níveis inadequados de ATP, comprometendo a homeostasia das células encefálicas[116].

Dessa forma, por todos os aspectos mencionados até o momento, a perpetuação das respostas neuroinflamatórias na sepse parece ser um ponto crítico no desenvolvimento das alterações estruturais celulares e alterações cerebrais agudas e a longo prazo. No entanto, provavelmente outras vias de sinalização presentes na microglia, devem estar relacionadas com a produção exacerbada e sustentada de citocinas pró-inflamatórias e dos mediadores pró-oxidantes. Nesse sentido, outra classe de receptores de reconhecimento de padrões, a dos receptores semelhantes a NOD, tem sido relacionada com doenças neuroinflamatórias como doença de Parkinson[117] e doença de Alzheimer[118], que também tem na sua fisiopatologia a ativação microglial.

1.1.5 Receptores do tipo NOD e formação do inflamassoma NLRP3

Os receptores semelhantes a NOD (NLRs, *do inglês NOD-like receptors)* estão presentes no citoplasma de diferentes células imunes e atuam como receptores de reconhecimento de padrões, PAMPs ou DAMPs, assim como os TLR. A ativação destes receptores gera respostas imunes importantes para a defesa do hospedeiro e reparo tecidual[119].

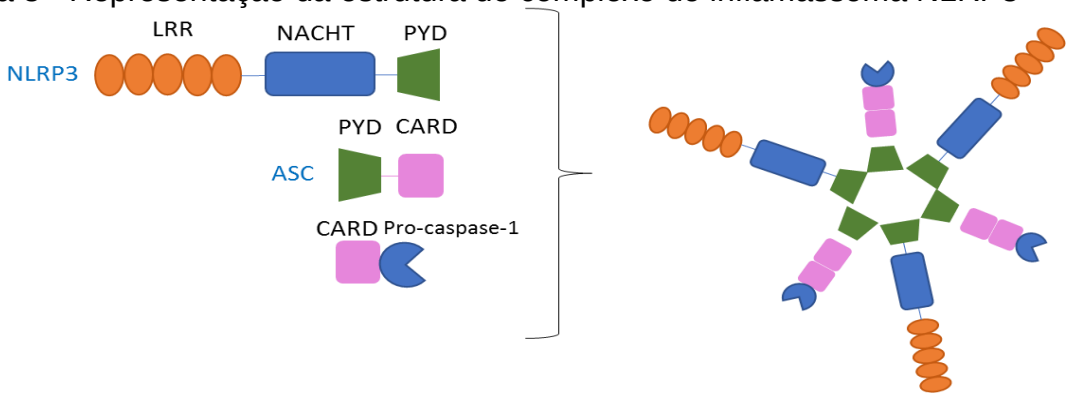
Nos seres humanos, já foram descritos, 22 membros desta família. Dentre os diferentes membros da família NLRs, os receptores NLRP1, NLRP2, NLRP3, NLRC4, NLRP6, NLRP7 e NLRP12 quando recebem estímulos específicos, são capazes de se ligarem a outras moléculas e formar estruturas chamadas de inflamassomas [120,121]. Os inflamassomas são um grupo de complexos proteicos de alto peso molecular, presentes no citosol, que são formados para mediar as respostas imunes do hospedeiro frente à infecção microbiana e danos celulares, através da ativação de proteases chamadas de caspases. A caspase -1 ativa é capaz de clivar pró-IL-1β e pro-IL-18, promovendo a maturação destas citocinas. A formação deste inflamassoma também é responsável pela ativação de gasdermina D, molécula capaz de formar poros na membrana celular e causa morte celular por piroptose[122].

O receptor NLRP3, também conhecido como criopirina ou NALP3, é um receptor expresso na maioria das células da linhagem mieloide, inclusive na microglia.

Sua capacidade de formar inflamassomas foi inicialmente associado a doenças autoimunes, mas atualmente sabe-se que está relacionado a diversas doenças humanas, devido a sua sensibilidade para componentes de bactérias gram negativas[123].

Os inflamassomas são constituídos por três partes: (1) por uma proteína sensora/receptora de localização citosólica que serve como uma plataforma para a formação do complexo, como por exemplo, o receptor NLRP3; (2) uma proteína adaptadora ASC com domínio N-terminal efetor de piridina e uma porção responsável pelo recrutamento de caspase (CARD); (3) e por uma proteína efetora, a pró-caspase-1 que será clivada em caspase-1 (Figura 3)[124,125].

Figura 3 - Representação da estrutura do complexo do inflamassoma NLRP3



Adaptado de Jo et al., 2016 [126].

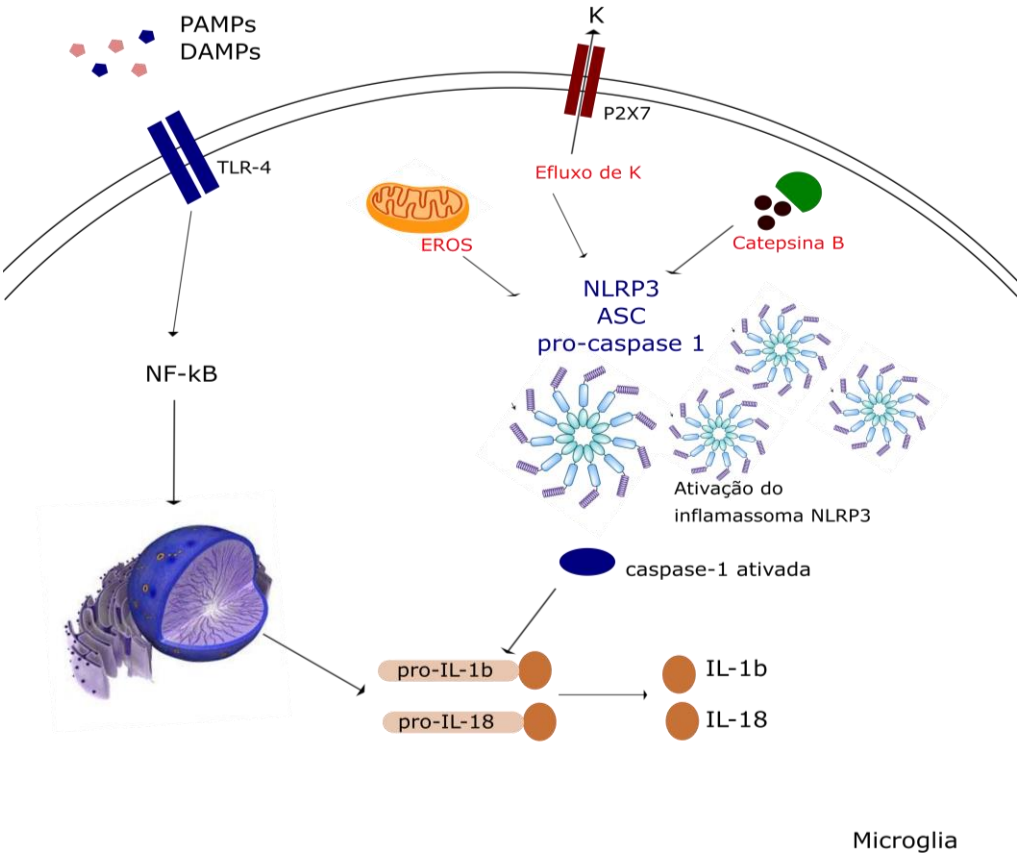
Todavia para que ocorra a ativação do inflamassoma são necessários diferentes estímulos, e estes ocorrem em duas etapas complementares (Figura 4). O primeiro estímulo consiste na sinalização de PAMPs e DAMPs através dos receptores TLR ou citocinas endógenas através dos receptores TNF-α, elevando a expressão de NF-κB, e dos níveis do receptor NLRP3, da molécula adaptadora ASC, de pro-caspase-1, pró-IL-1β e pró-IL-8[127].

Após essa etapa, para que ocorra a formação do complexo inflamassoma NLRP3 propriamente dito, são necessários, pelo menos um de três diferentes estímulos. 1-estímulo de EROs derivados da mitocôndria e que iniciam a formação do inflamassoma; 2 - ATP extracelular ou toxinas bacterianas induzem influxo de K+ através do receptor P2X7 e, 3 - a fagocitose de substâncias cristalinas específicas causam ruptura de lisossomos e leva a liberação de catepsina B que contribui para a ativação e formação do inflamassoma NLRP3. Após a formação do complexo NLRP3,

há clivagem de pro-caspase-1 em caspase-1, que por sua vez cliva as pró-interleucinas nas formas ativas de IL-1β e IL-18[128].

Trata-se, portanto de um mecanismo fisiológico de maturação das citocinas citadas, no entanto, quando se trata de doenças inflamatórias, com exacerbação da resposta imune, eventualmente o inflamassoma pode estar super estimulado, propiciando a elevação dos níveis de citocinas acima dos níveis aceitáveis e propiciando um ambiente favorável ao dano celular por exacerbação da resposta inflamatória. Nesse sentido, o inflamassoma NLRP3 pode ser um alvo promissor para terapias anti-inflamatórias em doenças como a sepse.

Figura 4 - Mecanismo de ativação do inflamassoma NLRP3



Adaptado de: Babelova et al., 2009 [129].

1.1.5.1 Inflamassoma na sepse

Atualmente são poucos os estudos envolvendo inflamassoma NLRP3 na sepse e principalmente os relacionados a disfunção neurológica. Na sepse existem

evidências da ativação da via TLR/ NF-kB como primeiro sinal, capaz de elevar a expressão das moléculas necessárias para a formação do inflamassoma[130].

Embora a formação do inflamassoma seja um processo fisiológico, tem-se mostrado efeitos negativos da ativação do inflamassoma NLRP3 nos tecidos periféricos durante a sepse[130,131]. A depleção de níveis de ATP intracelular nos macrófagos, estimula a proteína desacopladora 2 na mitocôndria (UCP2), e por consequência causa a ativação do NLRP3[132].

Ainda existem evidências do envolvimento do NLRP3 sobre o dano no sistema cardiovascular [133–135], na presença de piroptose no tecido hepático[136,137], na elevação dos níveis de IL-1β e apoptose no tecido renal[138] e tecido pulmonar[139]. Como observado, diferentes trabalhos apontam para os efeitos deletérios da ativação do inflamassoma NLRP3 na sepse em diferentes tecidos. Nesse sentindo, alguns estudos, utilizaram com sucesso substâncias inibidoras da formação do inflamassoma NLRP3, onde observou-se principalmente a redução de IL-1β[140,141]. Todavia ainda não existem estudos suficientes sobre a sua influência no encéfalo após a sepse.

1.1.5.2 Inflamassoma no sistema nervoso central

As evidências sobre o envolvimento do estresse oxidativo nas doenças neurodegenerativas são crescentes[142]. De fato, o SNC está predisposto aos efeitos tóxicos das EROs e ERNs. O tecido nervoso apresenta elevada concentração de lipídios e oxigênio, e geralmente baixas concentrações de enzimas antioxidantes, o que o torna um dos órgãos mais vulneráveis ao estresse oxidativo[143]. Especialmente cada vez mais há associação entre o SNC e sistema imune[90].

Níveis basais de citocinas são essenciais para as funções fisiológicas, mesmo no encefálo[144], todavia quando sua liberação extrapola a homeostasia, ocorre lesão tecidual. Além disso, as citocinas, como a IL-1β e IL-18 participam ativamente de mecanismos celulares e moleculares ligados a funções de aprendizado, memória e funções sensoriais[145].

O inflamassoma NLRP3 é o mais abundante inflamassoma presente no SNC, sendo uma molécula chave em processos de neuroinflamação[119]. Em modelo animal que mimetiza a depressão, a microglia foi a primeira célula a elevar a sua expressão do receptor NLRP3, associada com a elevação dos níveis de IL-1β[146].

Assim como ocorre em tecidos periféricos, a liberação de DAMPs no SNC é capaz de ativar receptores na microglia que contribuem para formação de inflamassomas[147]. Embora a ativação do inflamassoma faça parte da resposta imunológica fisiológica[126], doenças que cursam com neuroinflamação podem ter níveis elevados do receptor NLRP3 contribuindo para a sustentação da inflamação no SNC.

Recentemente, Fu e colegas demonstraram pela primeira vez que a inibição da formação do inflamassoma NLRP3 pode contribuir positivamente para a redução do comprometimento neurológico dos animais submetidos à sepse[148]. No entanto, novos estudos serão muito importantes para elucidar os efeitos potenciais do uso de inibidores do inflamassoma no dano cognitivo após a sepse.

Tendo em vista o exposto até o momento, nossa hipótese é de que a reação inflamatória encefálica gerada durante a sepse possa ser potencializada pela formação do inflamassoma NLRP3/ IL-1β. Esse processo levaria a exacerbação da liberação da citocina IL-1β. Os efeitos dessa citocina associados aos danos diretos e indiretos causados pela microglia e astrócitos ativos durante a sepse, poderiam estar relacionadas com as alterações neuroquímicas e comportamentais encontradas durante a após a sepse. Com a inibição do inflamassoma NLRP3, acreditamos ser possível encontrarmos dados mais concretos sobre o envolvimento deste mecanismo na disfunção cerebral aguda e consequentemente, no prejuízo cognitivo em longo prazo na sepse.

2. OBJETIVOS

2.1 OBJETIVO GERAL

Avaliar o envolvimento da via regulada pelo inflamassoma NRLP3 sobre alterações cerebrais precoces e tardias após a sepse experimental.

2.2 OBJETIVOS ESPECÍFICOS

- Determinar os níveis de IL-1β, TNF-α, IL-6 e IL-10 no hipocampo e córtex pré-frontal de ratos 24 horas após indução de sepse por CLP seguida pela administração do inibidor específico de NLRP3;
- Determinar os níveis de NLRP3, ativação microglial e astrocitária no hipocampo e córtex pré-frontal de ratos 24 horas após a indução de sepse por CLP seguida pela administração do inibidor específico de NLRP3;
- Avaliar o dano oxidativo em lipídios e proteínas, e a atividade antioxidante das enzimas catalase e superóxido dismutase no hipocampo e córtex pré-frontal de ratos 24 horas após a indução de sepse por CLP seguida pela administração do inibidor específico de NLRP3;
- Verificar a formação de nitrito/nitrato no hipocampo e córtex pré-frontal de ratos 24 horas após a indução de sepse por CLP seguida pela administração do inibidor específico de NLRP3;
- Avaliar a atividade dos complexos da cadeia respiratória mitocondrial I, II, II-III e IV no hipocampo e córtex pré-frontal de ratos 24 horas após a indução de sepse por CLP seguida pela administração do inibidor específico de NLRP3;
- Determinar a sobrevida em 10 dias após a indução de sepse por CLP seguida pela administração do inibidor específico de NLRP3;
- Realizar os testes de esquiva inibitória passiva e reconhecimento de novo objeto 10 dias após a indução de sepse por CLP seguida pela administração do inibidor específico de NLRP3.

3. MÉTODOS

3.1 TIPO DE ESTUDO

Estudo experimental com modelo animal de sepse polimicrobiana induzida pelo modelo de ligação e perfuração cecal.

3.2 MATERIAIS E EQUIPAMENTOS

O inibidor de formação do NLRP3, MCC950 foi obtido de Sigma, St. Louis, USA. Os reagentes utilizados para as análises bioquímicas foram: ácido tiobarbitúrico, 1,1,3,3 -tetrametoxipropano, brometo de hexadeciltrimetilamónio, dinitrofenilhidrazina, epinefrina, catalase de fígado bovino, albumina bovina, sulfanilamina, azida sódica, naftil etilenodiaminadicloridrato e reagente de Griess (Sigma, St. Louis, USA), ácido clorídrico, álcool etílico, acetato de etila, hidróxido de sódio, carbonato de sódio, ácido tricloroacético e peróxido de hidrogênio, ácido aminoacético (Labsynth, São Paulo, Brasil). Foram ainda utilizados kits para citocinas da marca R&D Systems®.

Para a realização das técnicas laboratoriais foram utilizados os seguintes equipamentos: agitador magnético (752A, Fisatom), balança analítica (M214A, BEL engeening), banho-maria (DeLeo equipamentos laboratoriais), espectrofotômetro (NOVA1103, Novainstruments), espectrofotômetro (U2010, Hitachi), estufa de secagem (DeLeo), Freezer (F21, Eletrolux), homogeneizador potter (Marconi), leitor de microplaca (TP reader, Thermo Plate), medidor de pH (pHB500, ION), microcentrífuga refrigerada (NT805, Novatécnica), osmose reversa (RO/0520, Permution), refrigerador (R270,Eletrolux), Vórtex (Biomixer).

As análises nível de NLRP3, ativação microglial (IBA-1) e ativação de astrócitos (GFAP) foram realizadas em parceria com a University of Texas Health Science Center at Houston, no Texas, Estados Unidos da América, (EUA). Enquanto que as análises bioenergéticas mitocondriais em parceria com a Universidade do Extremo Sul Catarinense (Criciúma, SC). As demais análises foram realizadas no Laboratório de Neurobiologia de Processos Inflamatórios e Metabólicos - UNISUL.

3.3 ANIMAIS

Foram utilizados um total de 194 ratos machos da linhagem *Wistar* (*Rattus norvegicus*) com 60 dias de vida (250-300g), adquiridos do Biotério da Universidade Federal de Santa Catarina (UFSC-SC) e mantidos no Biotério da Universidade do Sul de Santa Catarina (UNISUL-SC). Os animais tiveram livre acesso à água e ao alimento (ração comercial específica para a espécie). Foram alocados 4 animais por gaiola, alojadas em ambiente que possui sistema de exaustão de ar e controle de temperatura de acordo com as condições ideais para a espécie em questão. Sendo que a temperatura foi mantida a 22°±1 e sistema de iluminação que garante 12 horas de ambiente claro e 12 horas de ambiente escuro.

Com base em estudos prévios em modelos animais[149], para uma diferença de até 20% nos parâmetros a serem analisados entre os grupos, com uma variância de no máximo 10% entre as médias, calculou-se o seguinte número de animais por grupo, no mínimo 6 animais para as análises bioquímicas e entre 10 a 12 animais para cada grupo para os testes comportamentais, considerando um erro alfa de 0,05 e poder de 80%. Ainda, tendo em vista que a sepse causa elevada mortalidade, espera-se em torno de 40% de perda de animais submetidos a sepse.

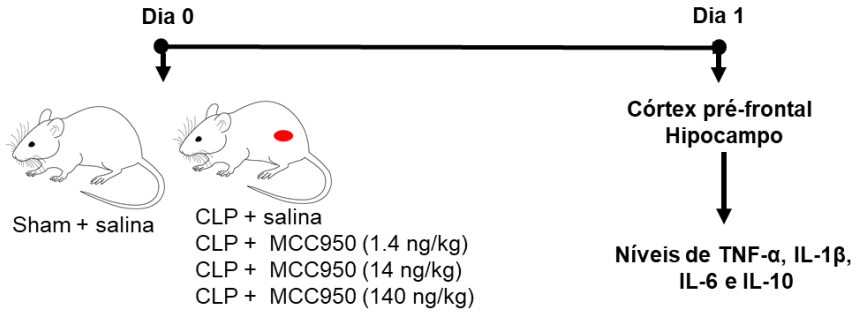
3.4 DELINEAMENTO DO ESTUDO

3.4.1 Desenho Experimental

Devido ao elevado número de análises, a necessidade de realização de procedimentos exclusivos para avaliação de determinados parâmetros e o bem-estar animal, o estudo foi desenvolvido em 4 experimentos. No experimento 1 os animais foram randomizados em cinco grupos experimentais: sham + salina (n=10), CLP + salina (n=12), CLP + MCC950 1,4 ng/kg (n=12), CLP + MCC950 14 ng/kg (n=12) e CLP + MCC950 140 ng/kg (n=12)[150]. No tempo zero após o protocolo de indução de sepse, os animais receberam salina ou MCC950 icv, na cisterna magna com o auxílio de um aparato estereotáxico, de acordo com o grupo experimental a qual pertenciam. Decorridas 24 horas, hipocampo e córtex pré-frontal foram isolados para avaliação dos níveis de citocinas, IL-1β, TNF-α, IL-6 e IL-10, com o objetivo de determinar qual a melhor dose de inibidor para prosseguir com o estudo (Figura 5). Após a avaliação

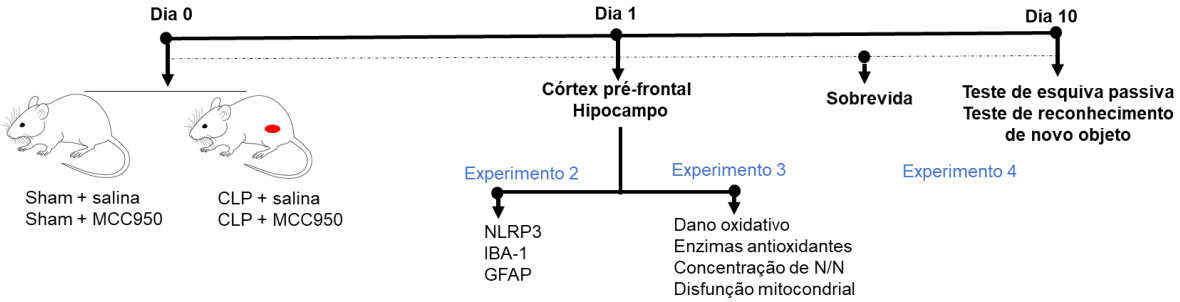
da melhor dose de inibidor, os outros experimentos foram realizados. As amostras coletadas foram armazenadas em freezer com temperatura de -80ºC até o momento da realização das análises de citocinas.

Figura 5 - Esquema representativo do experimento 1



Após o experimento 1 e a dosagem de citocinas pró-inflamatórias, foram realizados os próximos experimentos (Figura 6). Em todas as etapas os animais foram randomizados em 4 grupos, a dose de inibidor utilizada foi a de 140 ng/kg.

Figura 6 - Esquema representativo do experimento 2, 3 e 4



No experimento 2**,** vinte e quatro horas após a indução de sepse, o encéfalo total foi extraído e conservado em formol a 10% para posterior fixação em lâmina e realização das análises de níveis do receptor NLRP3, GFAP (marcador de ativação astrocitária) e IBA-1 (marcador de ativação microglial) no córtex pré-frontal e no hipocampo. No experimento 3, o córtex pré-frontal e hipocampo foram isoladas e armazenadas em freezer com temperatura de -80ºC até o momento da realização das análises bioquímicas de estresse oxidativo, dosagem de nitrito/nitrato, e atividade da cadeia respiratória mitocondrial. Para os experimentos 2 e 3, os animais foram randomizados em quatro grupos, sendo estes o grupo sham + salina (n=8), sham + MCC950 (n=8), CLP + salina (n=12), CLP + MCC950 (n=12).

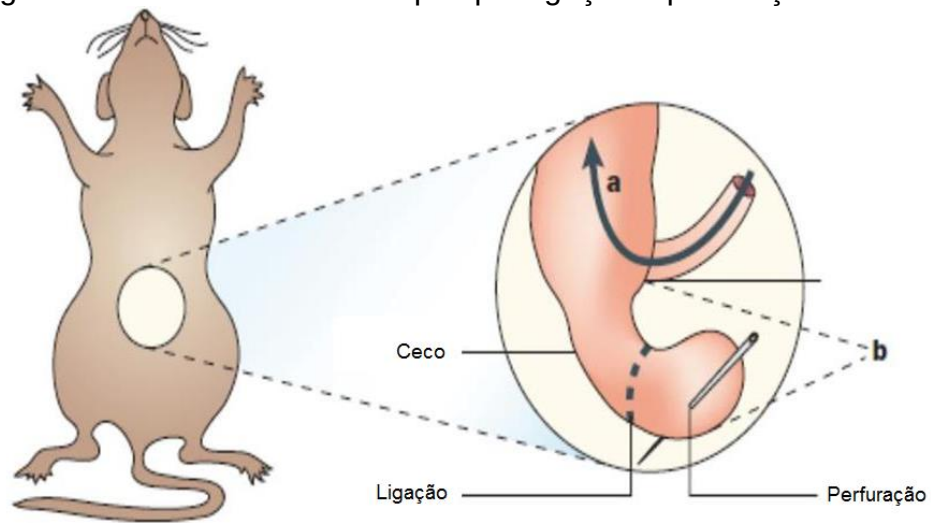
No quarto experimento, os animais foram seguidos diariamente durante 10 dias para análise da sobrevida e ao final deste tempo foram realizados os testes comportamentais para avaliação do dano neurológico. Os grupos experimentais foram os seguintes: grupo sham + salina (n=10), sham + MCC950 (n=10), CLP + salina (n=18), CLP + MCC950 (n=18).

3.4.2 Modelo animal de sepse

A sepse intra-abdominal (Figura 7) foi produzida usando a técnica de ligação e perfuração cecal (CLP)[151]. Para isso, os ratos foram anestesiados com 80mg/kg de cetamina (Cloridrato de Cetamina 10%, Vetnil) e 10mg/kg de xilazina (Calmium 2%, Agener União Saúde Animal) por via intraperitoneal, após certificação da indução anestésica, os mesmos foram submetidos à laparotomia com incisão média abdominal. O ceco foi identificado e ligado abaixo da junção íleo-cecal com fio seda 3-0 e perfurado com uma agulha número 14, gentilmente comprimido até a extrusão de conteúdo fecal. Os planos cirúrgicos foram fechados e os animais mantidos em ambiente aquecido e observados por duas horas. A reposição volêmica foi realizada através da administração de 50mL/kg de NaCl 0,9% estéril (Eurofarma) por via subcutânea. Além disso, a administração de 30mg/kg de ceftriaxona (Triaxin, Momenta) por via subcutânea foi realizada em todos animais logo após CLP e após 12 horas.

Como controle, denominado grupo sham, foram utilizados animais submetidos a laparotomia, sem ligação ou perfuração cecal, recebendo também a reposição volêmica e o antibiótico. Para minimizar a variabilidade entre os diferentes experimentos, o procedimento cirúrgico para indução de sepse foi realizado sempre pelo mesmo pesquisador[152].

Figura 7 - Modelo animal de sepse por ligação e perfuração cecal



Adaptado de Buras, JA., Holzmann,B., Sitkovsky, M., 2005 [153].

3.4.3 Coleta de amostras biológicas

Após os experimentos todos os animais foram eutanasiados obedecendo as disposições da Resolução nº. 1000 do Conselho Federal de Medicina Veterinária (CFMV), de 20/06/2002, e ocorreram de forma indolor e assistida, por anestesia de uma mistura de xilazina e cetamina seguida de decapitação. O córtex pré-frontal e o hipocampo foram rapidamente dissecados e armazenados em freezer -80ºC para análises bioquímicas subsequentes. Para os testes de imuno-histoquímica, o encéfalo total foi dissecado e conservado em formol na concentração de 10%, até o momento da preparação e análise das amostras.

3.5 ENSAIOS/TESTES/TÉCNICAS

3.5.1 Dosagem de citocinas

A quantificação das citocinas (IL-1β, TNF-α, IL-6 e IL-10) foi realizada por ensaio de imunoabsorção enzimática (ELISA, do inglês; *Enzyme Linked ImmunonoSorbent Assay*), seguindo as instruções das bulas dos kits comercias. Inicialmente em uma placa com 96 poços, foram adicionados 100 µl/poço do branco, padrão ou das amostras, procedendo-se à incubação da placa por 2 horas à temperatura ambiente em agitador tipo Kline com baixa rotação. Após esse período

foi adicionado 100 µl de reagente de detecção A, e incubado por 1 hora a 37ºC. Lavou-se três vezes a placa com a solução de lavagem. Depois desse procedimento, adicionou-se 100µl do reagente B em cada poço, seguindo uma incubação de 30 minutos. Repete-se o procedimento de lavagem por cinco vezes. Então adiciona-se 90 µl de 3,3′,5,5′-tetrametilbenzidina em cada poço, incubando a placa por 20 minutos a 37ºC, esse procedimento gera uma coloração azul. Ao final da incubação deve ser adicionado, rapidamente, uma solução de parada de reação, e a leitura pontual foi realizada em leitora de microplaca, a 450 nm. A unidade de medida utilizada foi pg/ml.

3.5.2 Imuno-histoquímica

A imunocoloração foi realizada em amostras de tecido cerebral embebidas em parafina e fixadas em formalina. A desparafinização e reidratação foram realizadas usando uma série de xilenos, álcoois graduados e água de qualidade reagente. A recuperação de antígeno à base de calor foi realizada usando uma solução de citrato de sódio para recuperação de antígeno 1 × a pH 9 (Agilent Technologies, Santa Clara, CA) por 30 minutos a 950 °C, seguidos por 30 minutos em gelo. As etapas de lavagem subsequentes foram realizadas usando uma solução salina tamponada com fosfato 1x (PBS) (Bio-rad, EUA). A solução de peróxido de hidrogênio a 3% (VWR International, Radnor, PA) usada para bloquear a peroxidase endógena por 10 minutos. O anticorpo primário NLRP3 (Novus, NBP2-12446, 1: 100), IBA-1 (Abcam, ab178846, 1: 1000), GFAP (Abcam, ab7260, 1: 1000) foi aplicado e mantido a 40 ° C durante a noite após uma hora etapa de bloqueio à temperatura ambiente com soro de cavalo a 2,5% (Vector Laboratories, Burlingame, CA). As lâminas foram lavadas com PBS e o anticorpo secundário biotinilado anti-coelho de cabra (Millipore, MA, EUA) foi aplicado por 1 hora à temperatura ambiente. Em seguida, o complexo Avidin-Biotin (ABC) foi adicionado por 1 hora. Seguindo etapas de lavagem adicionais, o antígeno alvo foi visualizado usando o cromogênio DAB em tampão (Vector, Burlingame, CA). Para a contra-coloração, foi aplicada hematoxilina e as lâminas foram levadas ao xileno e montadas com Permount ™ (Fischer Chemicals). A coloração foi visualizada usando Nikon ECLIPSE Ci-S (Nikon Instruments, Tóquio, Japão) e as imagens foram capturadas (ampliação × 20)[154].

3.5.3 Avaliação do dano oxidativo

A técnica de formação de substâncias reativas ao ácido tiobarbitúrico (TBARS) é usada como índice de dano oxidativo em lipídios[155]. Nesse procedimento, 250µl de amostra homogeneizada foi precipitada com ácido tricloroacético a 10%, ao sobrenadante foi adicionado ácido tiobarbitúrico (0,67%). Na sequência há uma incubação protegida da luz, em banho–maria a 100ºC durante 30 min. A leitura da absorbância foi realizada em 535 nm usando 1,1,3,3-tetrametoxipropano como padrão externo. Os resultados foram expressos em equivalentes de malondialdeído (nmol/mg de proteína).

O dano oxidativo em proteínas foi determinado pela reação dos grupamentos carbonilados com dinitrofenilhidrazina. As proteínas foram precipitadas por adição de ácido tricloroacético a 20%, o precipitado foi dissolvido e então adicionado dinitrofenilhidrazina. Incuba-se por uma hora em temperatura ambiente, após esse período redissolve-se as amostras com 1 mL de NaOH 3% e incuba-se em banho-maria por 30 min a 60ºC. A leitura é realizada a 370 nm, com resultados expressos como carbonilação proteica por nmol/mg de proteína[156].

3.5.4 Atividade das enzimas antioxidantes

A atividade da SOD foi medida com base em sua capacidade de inibir espontaneamente a oxidação da adrenalina em adrenocromo[157]. A SOD presente na amostra compete pelo radical hidroxila diminuindo a oxidação da adrenalina. Dessa forma a velocidade de formação do adrenocromo em um meio de reação contendo glicina-NaOH (50 mM em pH 10,2) e adrenalina (60 mM), indica a atividade da SOD. A variação de absorbância foi medida em 480nm e os resultados foram expressos como U/mg de proteína.

A atividade da CAT é diretamente proporcional à taxa de decomposição do peróxido de hidrogênio. A reação utiliza o método que emprega peróxido de hidrogénio (H_2O_2) de deve ser convertido pela CAT em H_2O e O_2[158]. O tecido cerebral foi homogeneizado em tampão de fosfato 50 mmol/ L (pH 7,0), e a suspensão resultante foi centrifugada a 3000 g durante 10 min. Uma alíquota de 100 µl da amostra (20 µl) foi adicionada a 1000 µl da mistura de substrato. A mistura de substrato continha 0,3 ml de peróxido de hidrogênio em 50 ml de tampão ao fosfato 0,05 M (pH 7,0). As absorbâncias foram registadas em 240 nm nos tempos 0, 30 e 60 segundos

após o início da reação. Uma curva padrão foi estabelecida utilizando catalase purificada (Sigma, MO) em condições idênticas. Os resultados foram expressos em U/mg de proteína.

3.5.5 Análise da concentração de nitrito e nitrato

A concentração de nitrito/nitrato foi medida usando-se o reagente de Griess (1% sulfanilamida em 5% ácido fosfórico e 0.1% N-1- N-dicloridrato de (1-naftil)-etilenodiamina em água purificada) e cloreto de vanádio (III) como previamente descrito[159]. Uma curva padrão foi medida simultaneamente com as amostras e a absorbância para a leitura foi de 550 nm. Os resultados da concentração de nitrito e nitrato foram expressos em nmol/mg de proteína.

3.5.6 Atividade da cadeia respiratória mitocondrial

Determinação da atividade do complexo I: A atividade do complexo I foi avaliada pela taxa de Nicotinamida adenina dinucleotídeo reduzido (NADH) dependente da redução do ferrocianeto de potássio, este último sendo utilizado como aceptor de elétrons[160]. No meio de reação foi adicionado tampão fosfato de potássio 100 mM, ferricianeto 10 mM, NADH 14 mM, rotenona 2 mM e amostra. A leitura foi realizada em espectrofotômetro a cada 60 segundos, durante 3 min, em 420 nm.

Determinação da atividade do complexo II: foi mensurada pela diminuição da absorbância do 2,6-dicloroindofenol (DCIP)[161]. A amostra foi adicionada a um meio de incubação contendo tampão fosfato de potássio 62,5 mM, succinato de sódio 250 mM e 2,6-diclorofenol-indofenol (DCIP) 0,5 mM, seguindo de incubação por 20 minutos à 30ºC em banho-maria. Após a incubação adicionou-se azida sódica na concentração de 100 mM, rotenona a 2 mM e novamente DCIP 0,5 mM, para então realizar leitura em espectrofotômetro em 600 nm por 5 min, sendo registrados a absorbância a cada minuto.

Determinação da atividade do complexo II-III o meio de reação, constituído de tampão fosfato de potássio 40 mM (pH 7,4) contendo 16 mM de succinato de sódio, foi pré-incubado com 40-8 µg de proteínas do homogeneizado a 30 °C por 30 minutos. Em seguida, foram adicionados 4 mM de azida sódica e 7 µM de rotenona e a reação se iniciou pela adição de 0,6 µg/mL de citocromo c e as absorbâncias foram

registradas por 5 minutos a 550 nm. A atividade do complexo II-III foi medida pelo aumento da absorbância causado pela redução do citocromo C[162].

Determinação da atividade do complexo IV: foi determinada de acordo com a técnica descrita por Rustin et al[162] . O meio de incubação continha tampão fosfato de potássio 10 mM (pH 7,0), 0,6 mM de n-dodecil-D-maltosídeo e 10-2 µg de proteínas (homogeneizado). A reação foi iniciada pela adição de 0,7 µg de citocromo c previamente reduzido. A atividade do complexo IV foi medida a 25 °C por 10 minutos pelo decréscimo da absorbância a 550 nm devido à oxidação do citocromo c reduzido.

3.5.7 Determinação de proteínas totais

Todos os resultados das análises bioquímicas foram normalizados com a quantidade de proteínas avaliada de acordo com o método descrito por Lowry e colaboradores[163].

3.5.8 Análise da Sobrevida

Para a avaliação da sobrevida, os animais foram observados diariamente, durante 10 dias após indução de sepse para verificar a taxa de mortalidade[149].

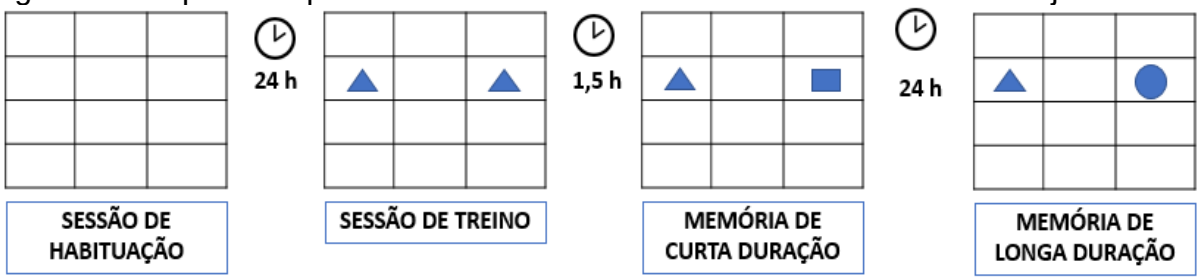
3.5.9 Teste de esquiva inibitória passiva

Para este teste foi utilizada uma caixa retangular acrílica de 30x33x54 centímetros (cm) (Insight), dividida em duas salas (uma clara e outra escura) onde no chão existem barras paralelas de ferro (1 mm de diâmetro) com espaçamento de 1 cm. Na seção de treino os animais são colocados na sala clara e ao entrar na sala escura o animal recebia um choque pelas barras de ferro de 0,4 mA até que o animal retornasse à sala clara. Após 1,5h os animais retornaram ao aparato, e foi verificado o tempo que o animal demorou para entrar na sala escura, chamado de latência, como indicativo da memória aversiva de curto prazo. E após 24h, foi realizado o mesmo procedimento, para verificar a memória em longo prazo. Cada seção teve a duração máxima de cinco minutos[164].

3.5.10 Teste de reconhecimento do objeto novo

O teste de reconhecimento do novo objeto foi realizado no aparato de campo aberto, medindo 40 x 60 cm e delimitado por 4 paredes com 50 cm de altura, sendo três de madeira e uma de vidro transparente, com o piso dividido em 12 quadrados iguais marcados por linhas pretas conforme a figura 8.

Figura 8 - Esquema representativo do teste de reconhecimento do novo objeto



No dia 1 foi realizado o treino para habituação do animal ao ambiente, onde ele foi colocado em um quadrante do aparato e pode explorar o ambiente por cinco minutos, sem a presença de objetos. No segundo dia o animal foi recolocado no aparato, no qual estavam dois objetos iguais (A1 e A2, de mesma forma, tamanho e cor), foi cronometrado o tempo que o animal explorou cada objeto. Decorridas uma hora e trinta minutos, o objeto A2 foi substituído por um objeto B, com outro formato, mas mantendo tamanho e cor, então, realizou-se a cronometragem de tempo de exploração em cada objeto. Este teste avalia a memória de curta duração.

Vinte e quatro horas após este teste, foi realizado o mesmo procedimento, apenas trocando o objeto B pelo objeto C (diferente do objeto A e B), novamente o tempo de exploração de cada objeto era cronometrado. Este último teste avalia a memória de longa duração. Neste teste aplicou-se o índice de reconhecimento, a fim de se calcular o tempo gasto para cada animal para explorar o objeto, expresso como uma razão (TB/(TA+TB), onde TA = tempo gasto para explorar o objeto familiar; e TB = tempo gasto para explorar o novo objeto[165].

3.6 PROCESSAMENTO E ANÁLISE DOS DADOS

A análise estatística dos dados e a elaboração dos gráficos foi realizada no programa estatístico GraphPad Prism® versão 6.0. Os dados foram analisados quanto

à normalidade utilizando o teste de Shapiro-Wilk e para homogeneidade usando o teste de Levene. Para dados normais e homogêneos, foi utilizado teste paramétrico; caso contrário, foi utilizado testes não-paramétricos. Para as análises bioquímicas, os dados foram apresentados como média ± desvio padrão e analisados por ANOVA de uma via, seguido de teste post hoc *Tukey*. Dados da tarefa de esquiva inibitória passiva e reconhecimento do novo objeto foram avaliados pelo teste U de *Mann-Whitney*. As comparações dentro dos grupos foram realizadas pelo teste de *Wilcoxon*. A sobrevida foi avaliada por *Kaplan-Meier*. Para todas as análises, foi adotado um nível de significância de 95% ($p<0,05$).

3.7 ASPECTOS ÉTICOS DA PESQUISA

Os experimentos foram realizados após a aprovação do protocolo pela Comissão de Ética no Uso de Animais (CEUA) sob o número 16.032.5.01.IV e foram realizados de acordo com as diretrizes brasileiras atuais para o cuidado de animais de laboratório e as diretrizes éticas para investigações de dor experimental em animais conscientes[166.]

4. ARTIGO

Nesta sessão será apresentado o artigo intitulado: “NLRP3 activation contributes to acute brain damage leading to memory impairment in sepsis-surviving rats”, submetido em abril de 2020 para a revista *Molecular Neurobiology,* com fator de impacto de 4.500 no ano de 2019, e classificação A1 no Qualis CAPES na área de Medicina II. O manuscrito foi aceito para publicação em agosto e publicado em setembro do ano corrente.

O mesmo está disponível no seguinte DOI: https://doi.org/10.1007/s12035-020-02089-9 e pode ser citado como: Danielski, L.G., Giustina, A.D., Bonfante, S. et al. NLRP3 Activation Contributes to Acute Brain Damage Leading to Memory Impairment in Sepsis-Surviving Rats. Mol Neurobiol (2020). https://doi.org/10.1007/s12035-020-02089-9.

Molecular Neurobiology
https://doi.org/10.1007/s12035-020-02089-9

NLRP3 Activation Contributes to Acute Brain Damage Leading to Memory Impairment in Sepsis-Surviving Rats

Lucineia Gainski Danielski[1] · Amanda Della Giustina[1] · Sandra Bonfante[1] · Mariana Pereira de Souza Goldim[1] · Larissa Joaquim[1] · Kiuanne Lobo Metzker[1] · Erica Bernardo Biehl[1] · Thaynan Vieira[1] · Fabiana Durante de Medeiros[1] · Naiana da Rosa[1] · Jaqueline Generoso[2] · Lutiana Simoes[2] · Hémelin Resende Farias[3] · Isabela da Silva Lemos[3] · Vijayasree Giridharan[4] · Gislaine Tezza Rezin[1] · Jucelia Jeremias Fortunato[1] · Rafael Mariano Bitencourt[1] · Emilio Luiz Streck[3] · Felipe Dal-Pizzol[2] · Tatiana Barichello[2,4] · Fabricia Petronilho[1]

Received: 2 April 2020 / Accepted: 24 August 2020

Abstract
Sepsis survivors present acute and long-term cognitive impairment and the pathophysiology of neurological dysfunction in sepsis involves microglial activation. Recently, the involvement of cytosolic receptors capable of forming protein complexes called inflammasomes have been demonstrated to perpetuate neuroinflammation. Thus, we investigated the involvement of the NLRP3 inflammasome activation on early and late brain changes in experimental sepsis. Two-month-old male Wistar rats were submitted to the sepsis model by cecal ligation and perforation (CLP group) or laparotomy only (sham group). Immediately after surgery, the animals received saline or NLRP3 inflammasome formation inhibitor (MCC950, 140 ng/kg) intracerebroventricularly. Prefrontal cortex and hippocampus were isolated for cytokine analysis, microglial and astrocyte activation, oxidative stress measurements, nitric oxide formation, and mitochondrial respiratory chain activity at 24 h after CLP. A subset of animals was followed for 10 days for survival assessment, and then behavioral tests were performed. The administration of MCC950 restored the elevation of IL-1β, TNF-α, IL-6, and IL-10 cytokine levels in the hippocampus. NLRP3 receptor levels increased in the prefrontal cortex and hippocampus at 24 h after sepsis, associated with microglial, but not astrocyte, activation. MCC950 reduced oxidative damage to lipids and proteins as well as preserved the activity of the enzyme SOD in the hippocampus. Mitochondrial respiratory chain activity presented variations in both structures studied. MCC950 reduced microglial activation, decreased acute neurochemical and behavioral alteration, and increased survival after experimental sepsis.

Keywords Sepsis · NLRP3 · Inflammasome · MCC950 · Neuroinflammation · Cognitive impairment

Introduction

In the last decade, there has been a decrease in mortality in patients with sepsis. Age-standardized sepsis incidence fell by 37% and mortality decreased by 52.8% from 1990 to 2017 [1]. In this sense, other challenges arise, especially those related to quality of life and acute and persistent neurological damage presented by survivors. Surviving an episode of sepsis represents a major risk for the development of long-term neurocognitive dysfunction affecting a patient's ability to live independently, as well as reducing his work ability [2]. Several studies have attributed these behavioral changes to neuronal damage during sepsis and the microglial activation is involved in this process [3–6]. When activated, microglial cells are capable of indirectly causing neuronal damage by releasing inflammatory mediators such as IL-1β and TNF-α, or by reducing their production of neurotrophins. Directly, microglial cells cause neuronal damage by activation of

✉ Fabricia Petronilho
fabricia.petronilho@unisul.br

1 Laboratory of Neurobiology of Inflammatory and Metabolic Processes, Graduate Program in Health Sciences, Health Sciences Unit, University of South Santa Catarina, Tubarão, SC, Brazil

2 Laboratory of Experimental Pathophysiology, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina, Criciuma, SC, Brazil

3 Laboratory of Experimental Neurology, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina, Criciuma, SC, Brazil

4 Translational Psychiatry Program, Faillace Department of Psychiatry and Behavioral Sciences, McGovern Medical School, The University of Texas Health Science Center at Houston (UTHealth), Houston, TX 77054, USA

Published online: 01 September 2020

matrix metalloproteases, reactive oxygen species (ROS) production, overexpression of iNOS, and by mechanisms of excitotoxicity [7–10].

Microglia express on its surface different constitutive receptors that can be activated in the presence or absence of pathogens in the CNS [11–13]. Recently, the presence of nucleotide-binding domain leucine-rich repeat–containing receptors (NOD-like receptors or NODs) in microglial cells was verified [14]. These receptors when activated are able to form complexes called inflammasomes [15]. Inflammasomes are a group of high molecular weight protein complexes present in the cytosol that are formed to mediate host immune responses to microbial infection and cell damage [16].

The inflammasome NLRP3 is the most abundant inflammasome in the CNS, being a key molecule in neuroinflammation processes [17]. In an animal model of depression, microglia is the first cell to present elevate expression of NLRP3 with elevation of IL-1β levels [18]. In addition, recent studies show that activation of this complex is involved with the pathogenesis of several neurodegenerative diseases such as Alzheimer [19] and Parkinson [20].

In light of these findings, we hypothesize that brain inflammatory reaction generated during sepsis can be exacerbated by NLRP3 inflammasome activation in microglial cells and perpetuate the brain damage leading to long-term cognitive impairment.

Materials and Methods

Animals

Adult male Wistar rats (weighing 250–350 g) from the Universidade Federal de Santa Catarina and maintained at the Universidade do Sul de Santa Catarina were used. The animals were housed five per cage under controlled conditions of temperature (22 ± 1 °C), relative humidity (45–55%), and day/light cycle (12:12 h, lights on at 06:00). Rat chow (standard diet for laboratory animals—NUVILAB CR-1®, Brazil) and tap water were available ad libitum. The present study was approved by the Animal Research Ethic Committee of the Universidade do Sul de Santa Catarina (protocol #16.032.5.01.IV).

Sepsis Induction—CLP Model

Rats were subjected to CLP as previously described with minor modifications [13] to induce mid-grade sepsis [21]. Briefly, animals were anesthetized intraperitoneally (i.p.) with a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg). A 3-cm midline laparotomy was performed to expose the cecum and adjoining intestine. The cecum was tightly ligated with a 3-0 silk suture in the middle of its length (below the ileocecal valve), perforated once with a 14-gauge needle, squeezed gently to extrude a small amount of feces through the perforation site, and returned to the peritoneal cavity, and the laparotomy was closed with 4-0 silk sutures. Animals were resuscitated with regular saline (50 mL/kg) once immediately after CLP. All rats received ceftriaxone (30 mg/kg) and dipyrone (80 mg/kg) subcutaneously (s.c.) immediately after CLP and at 12 h after. All animals were returned to their cages with free access to food and water. In the sham-operated group (control), the rats were submitted to all surgical procedures, but the cecum was neither ligated nor perforated. To minimize variability between different experiments, the CLP procedure was always performed by the same investigators.

Treatment and Sample Obtention

NLRP3 formation inhibitor, MCC950 (Sigma, St. Louis, USA), was dissolved in saline immediately before use and protected from the light during the experiments. In the first experiment (Fig. 1a), the following doses were immediately used after surgery: rats were placed in a stereotactic apparatus and received 1.4, 14, or 140 ng/kg intracerebroventricular (i.c.v.) administration or the same volume of saline [22]. To i.c.v. injection, under anesthesia of the CLP procedure, a 27-gauge needle attached to a 10-μL Hamilton syringe was inserted perpendicularly 3 mm deep through the skull, into the left ventricle, and 2 mm laterally from the midline on the line drawn through the anterior base of the ears.

In the following experiments (Fig. 1b), the dose of 140 ng/kg was selected to reduce proinflammatory cytokine levels. Four groups (n = 10 or 12) were randomly divided into (1) sham + saline; (2) sham + MCC950, (3) CLP + saline, and (4) CLP + MCC950.

Cytokine Measurements

The levels of tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and interleukin-10 (IL-10) in the brain structures were determined by a standard sandwich ELISA [23], with commercially available kits (R&D Systems, Minneapolis, MN) in accordance with the manufacturer's instructions and the analysis protocol. The results were expressed as picograms/milliliter.

Immunohistochemistry

Immunostaining was performed on formalin-fixed, paraffin-embedded brain tissue samples. The deparaffinization and rehydration were carried out using a series of xylenes, graded alcohols, and reagent-grade water. Heat-based antigen retrieval was performed using a 1× antigen retrieval sodium citrate solution at pH 9 (Agilent Technologies, Santa Clara, CA) for 30 min at 95 °C, followed by 30 min on ice. Subsequent

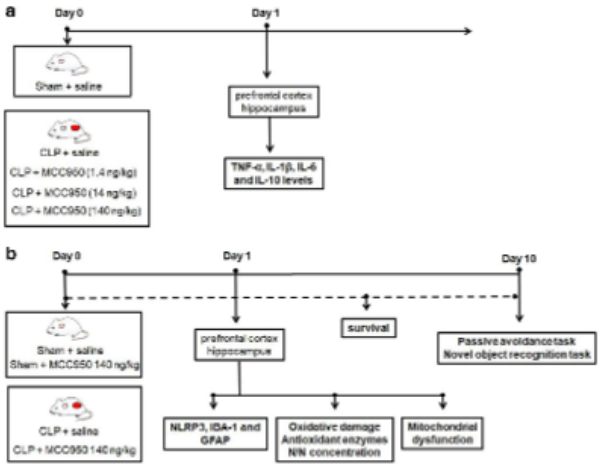


Fig. 1 Rats were submitted to CLP or sham operation and immediately after surgery received 1.4, 14, or 140 ng/kg i.c.v. of MCC950 or saline (**a**). Twenty-four hours after sepsis induction, the prefrontal cortex and hippocampus were collected for cytokine analysis. In another experiment, rats were submitted to CLP or sham and immediately after surgery, received 140 ng/kg i.c.v. of MCC950 or saline (**b**). Twenty-four hours after sepsis induction, the prefrontal cortex and hippocampus were collected for NLRP3, IBA-1, GFAP, oxidative damage, antioxidant enzymes, nitrite/nitrate concentration, and mitochondrial dysfunction determination. In addition, cognitive impairment (passive avoidance task and novel object recognition) were carried out 10 days after CLP. The survival was verified from the induction of sepsis (day 0) until the tenth day

washing steps were carried out using a 1× PBS solution (Bio-Rad, USA). The 3% hydrogen peroxide solution (VWR International, Radnor, PA) was used to block endogenous peroxidase for 10 min. Primary antibody NLRP3 (Novus, NBP2-12446, 1:100), IBA-1 (Abcam, ab178846, 1:1000), GFAP (Abcam, ab7260, 1:1000) was applied and kept at 4 °C overnight following a 1-h blocking step at room temperature with 2.5% horse serum (Vector Laboratories, Burlingame, CA). Slides were washed with PBS and goat anti-rabbit biotinylated secondary antibody (Millipore, MA, USA) was applied for 1 h at room temperature. Then avidin–biotin complex (ABC) was added for 1 h. Following additional washing steps, target antigen was visualized using 3,3′-diaminobenzidine (DAB) chromogen in substrate buffer (Vector, Burlingame, CA). For counterstaining, hematoxylin was applied and slides were taken to xylene and mounted with Permount™ (Fischer Chemicals). The staining was visualized using Nikon ECLIPSE Ci-S (Nikon Instruments, Tokyo, Japan) and the images were captured (magnification, ×20). We used the ImageJ software for the quantification of the captured images (https://imagej.nih.gov/ij/).

Oxidative Damage Parameters

The formation of thiobarbituric acid reactive species (TBARS) during an acid-heating reaction was verified as an index of oxidative damage on lipids, as previously described [24]. Briefly, the samples were mixed with 1 mL of 10% trichloroacetic acid and 1 mL of 0.67% thiobarbituric acid, and then heated in a boiling water bath for 15 min. TBARS was determined by the absorbance at $\lambda = 535$ nm using 1,1,3,3-tetramethoxypropane as an external standard. Results were expressed as malondialdehyde equivalents nanomoles/milligram protein. The protein oxidation was assessed by the determination of carbonyl groups based on the reaction with dinitrophenylhydrazine, as previously described [25]. Briefly, proteins were precipitated by the addition of 20% trichloroacetic acid and dissolved in dinitrophenylhydrazine, and the absorbance was read at $\lambda = 370$ nm. The results were expressed as protein carbonylation nanomoles/milligram of protein.

Antioxidant Enzyme Activity

Brain structures was sonicated with glycine buffer and the resulting suspension was centrifuged at 3000 × *g* for 10 min. Superoxide dismutase (SOD) (EC 1.15.1.1) activity was determined on the samples using a spectrophotometric assay based on superoxide-dependent oxidation of epinephrine to adrenochrome at 32 °C [26]. Absorption was measured at $\lambda = 480$ nm. SOD specific activity was represented as milliunits/milligram protein. For catalase (CAT) (EC 1.11.1.6) activity

determination, brain tissue was sonicated in phosphate buffer (pH 7.0), and the resulting suspension was centrifuged at 3000 × *g* for 10 min. The sample aliquot was added to a reaction medium containing 20 mM H_2O_2, 0.1% Triton X-100, and 10 mM potassium phosphate buffer, pH 7.0 [27]. Initial and final absorbance were recorded at 240 nm after 1 and 6 min, respectively. A standard curve was established using purified catalase (Sigma, MO, USA) under identical conditions. The specific activity was expressed as milliunits/milligram of protein.

Measurement of Nitrite/Nitrate Concentration

Brain tissue was sonicated in phosphate buffer (pH 7.0), and nitrite/nitrate concentration was assayed in the resulting suspension spectrophotometrically using Griess reagents (1% sulfanilamide in 5% phosphoric acid and 0.1% N-1-naphthylethylenediamine dihydrochloride in bidistilled H_2O (NEED solution)) and vanadium(III) chloride as described previously [28]. A standard curve was run simultaneously with each set of samples and the optical density at λ = 550 nm was measured using ELISA microplate reader. Data were expressed as nanomoles/milligram of protein.

Mitochondrial Electron Transport Chain Enzyme Activity

NADH dehydrogenase (complex I) was evaluated according to Cassina and Radi by the determination of the rate of NADH-dependent ferricyanide reduction at λ = 420 nm [29]. The activities of succinate-2,6-dichloroindophenol (DCIP)-oxidoreductase (complex II) and succinate: cytochrome *c* oxidoreductase (complex III) were determined by the method described by Fischer and colleagues [30]. Complex II activity was measured by following the decrease in absorbance due to the reduction of 2,6-DCIP at λ = 600 nm. Complex II–III activity was measured by cytochrome *c* reduction using succinate as substrate at λ = 550 nm. The activity of cytochrome *c* oxidase (complex IV) was assayed according to the method described by Rustin and colleagues [31], measured by following the decrease in absorbance due to the oxidation of previously reduced cytochrome *c* (prepared by reduction of cytochrome with $NaBH_4$ and HCl) at λ = 550 nm with 580 nm as the reference wavelength. The activities of the mitochondrial respiratory chain complexes were calculated as nanomoles per minute per milligram of protein.

Protein Determination

All results are normalized with proteins were measured by the Lowry method at λ = 700 nm [32].

Survival Curve

To assess survival, the animals were observed daily for 10 days after sepsis induction to verify mortality rates [33].

Passive Avoidance Task

The active inhibitory avoidance test was used to assess aversive memory. A 30 × 33 × 54 cm rectangular acrylic box (Insight) was used. The device is divided into two rooms (one bright and one dark) and contains a parallel iron bar (1 mm in diameter, with 1 cm spacing) floor. Individually, in the training test the animal was put in the bright room and when entering the dark room, the animal suffered a shock of 0.4 mA by the iron bars, so the animal was stimulated to escape and return to the bright room. The short-term aversive memory evaluation was performed at 1.5 h after the training session, while the long-term memory evaluation occurred at 24 h after the training session. In the two test sessions, the animal was again placed inside the device and the time taken for the animal to leave the bright field and enter the dark field was measured. Memory impairment was indicated by a shorter latency time between training and test sessions. Each session had a maximum duration of 5 min [34].

Novel Object Recognition Task

This task evaluates non-aversive and non-spatial memory. The apparatus and procedures for the object recognition task have been described elsewhere [35, 36]. This test was performed in the open field device, measuring 40 × 60 cm and bounded by four 50-cm-high walls, three of wood and one of transparent glass, or the floor divided into 12 squares marked by black lines. On day 1, the animal habituation was performed, where it was placed in the quadrant of the device and can explore the environment for 5 min without the presence of objects. On the second day, the animal was relocated to the device, neither were two items equal (A1 and A2, same size and color), timed or time the animal explored each object, and is characterized as training section. After 1.5 h, object A2 was replaced by object B, of another shape, but with the same size and color, then executed an exploration time timer on each object. This test evaluated short-term memory. Twenty-four hours after this test, the same procedure was performed, just exchanging object B for object C (different from objects A and B), again or the holding time of each object that was timed. This last test evaluated long-term memory. In this test, the recognition index is applied, an end of calculation of the time spent for each animal to explore the object, expressed as a ratio (TB/(TA + TB), where TA = time spent exploring the familiar object and TB = time taken to explore new object. Exploration was defined as sniffing (exploring the object 3–5

cm away from it) or touching the object with the nose and/or forepaws [37].

Data analysis

Statistical analysis of the data and graphing were performed using GraphPad Prism® version 6.0 statistical program. Data were analyzed for normality using the Shapiro–Wilk test and for homogeneity using the Levene test. For normal and homogeneous data, a parametric test was used; otherwise, nonparametric tests were used. For biochemical analyses, data were presented as mean ± SD and analyzed by one-way ANOVA, followed by Tukey post hoc test. Data from the passive avoidance task and novel object recognition were evaluated by the Mann–Whitney *U* test. Comparisons within groups were performed by the Wilcoxon test. Survival was assessed by Kaplan–Meier. For all analyses, a significance level of 95% ($p < 0.05$) was adopted.

Results

NLRP3 Inhibitor Reduces Brain Inflammatory Cytokine Levels

To determine the better dose of MCC950 to inhibit elevation of cytokine levels at 24 h after sepsis induction, the levels of proinflammatory and anti-inflammatory cytokines, such as IL-1β, TNF-α, IL-6, and IL-10, were evaluated (Fig. 2). Sepsis was capable of elevating IL-1β levels in both the prefrontal cortex (2a) and hippocampus (2b), and the inhibition of NLRP3 inflammasome formation generated by the administration of MCC950 was effective in inhibiting cytokine formation at doses of 14 and 140 ng/kg in the prefrontal cortex and at the dose of 140 ng/kg in the hippocampus. For TNF-α levels (2c and 2d), only the highest concentration of MCC950 was effective in inhibiting this cytokine just in the hippocampus at 24 h after sepsis induction. Although sepsis elevated IL-6 levels in the prefrontal cortex (2e) and hippocampus (2f), the 1.4 ng/kg dose of MCC950 was not sufficient to inhibit the formation of this cytokine in both structures. However, the administration of 140 ng/kg of MCC950 was effective in the hippocampus at 24 h after sepsis induction. The levels of IL-10 were also evaluated and the use of MCC950 was effective in the prefrontal cortex at both the intermediate (14 ng/kg) and the maximum dose (140 ng kg), while in the hippocampus only the higher dose was effective in reducing the release of this cytokine.

Sepsis Induces NLRP3 Expression in the Brain

We used immunohistochemistry to evaluate the brain levels of NLRP3. Sepsis elevated NLRP3 levels at 24 h after CLP in the prefrontal cortex and hippocampus, which was decreased by inhibiting NLRP3 inflammasome formation (Fig. 3).

NLRP3 Activates Microglia But Not Astrocytes

Figure 4 shows immunohistochemical results for microglial (IBA-1) and astrocyte (GFAP) activation markers in the prefrontal cortex (4a and 4b) and hippocampus (4c and 4d), respectively. The elevation of microglial and astrocyte activation was observed in both structures after sepsis induction. However, the use of MCC950 inhibitor only decreased microglial activation.

NLRP3 Inhibition Reduces Brain Oxidative and Nitrosative Stress after Sepsis

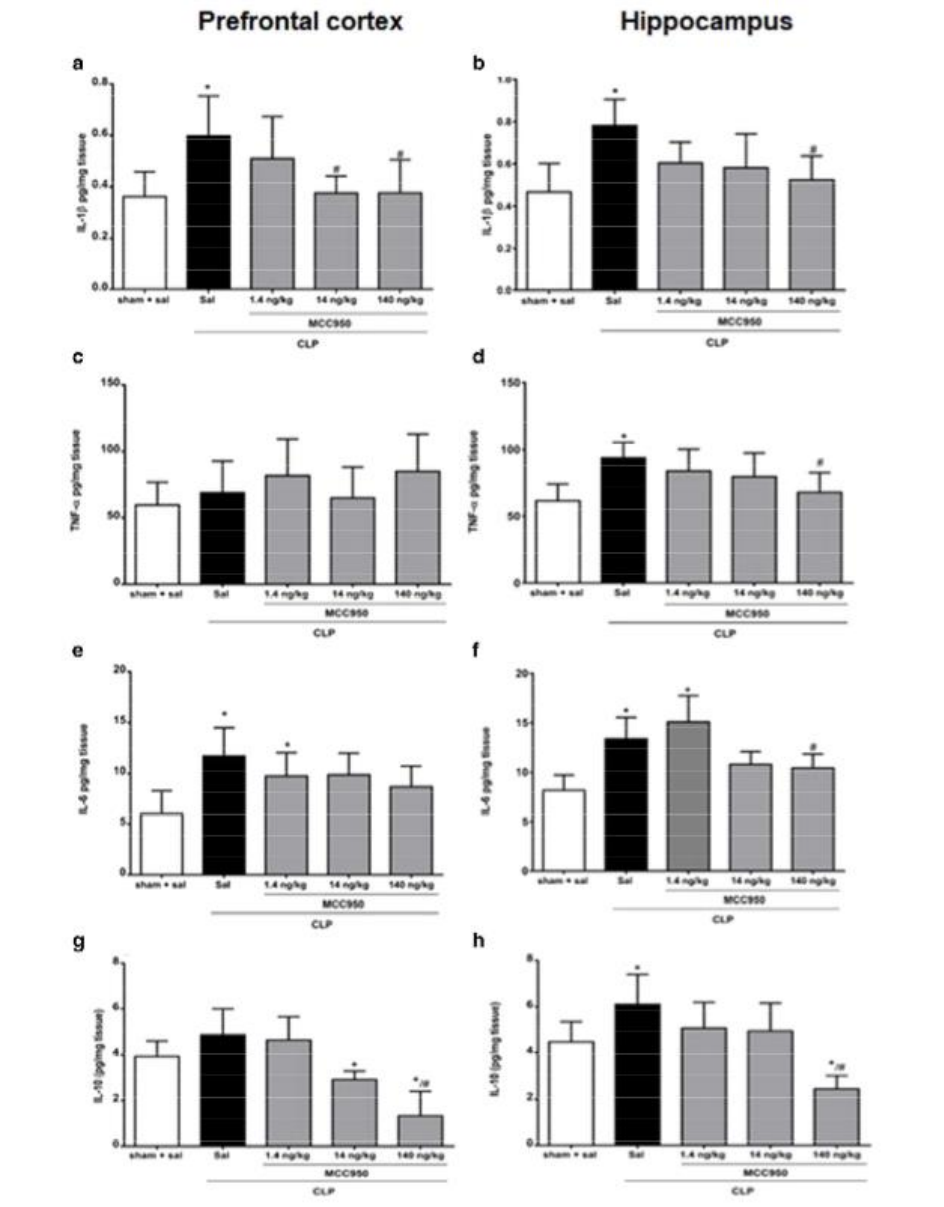
Oxidative and nitrosative stress analyzes occurred at 24 h after sepsis induction (Figs. 5 and 6). A single dose administration of MCC950 after CLP effectively protected the hippocampus against oxidative damage to lipids (5e) and proteins in both structures (5b, 5f). However, in the hippocampus, only SOD activity was preserved (5g). The administration of MCC950 inhibitor had no effect on the activity of the antioxidant enzyme CAT. However, MCC950 inhibitor was effective in decreasing the levels of nitric oxide formation in the hippocampus (Fig. 6).

NLRP3 Inhibition Restores Mitochondrial Complex Activity

The activity of mitochondrial respiratory chain complexes was assessed and shown in Fig. 7. Sepsis decreased the activity of complex I in both structures (7a and 7e), which was avoided by the administration of MCC950 inhibitor only in the prefrontal cortex. On the other hand, there was an increase in complex II activity in the hippocampus (7f) rather than in the prefrontal cortex (7b) after the use of MCC950 inhibitor. There was no change in the activity of complex II–III in both structures after sepsis and after administration of MCC950 inhibitor (7c and 7g). Sepsis induced decreased complex IV activity in both structures (7d and 7h), but the inhibition of the NLRP3 inflammasome restored this decrease in the hippocampus.

NLRP3 Inhibition Increases Survival after Sepsis

The Kaplan–Meier curve for survival analysis of rats subjected to polymicrobial sepsis and treated with MCC950 inhibitor (140 ng/kg) showed lower mortality after sepsis when compared with non-treated septic rats (Fig. 8).


Prefrontal cortex
Hippocampus
a
b
c
d
e
f
g
h
IL-1β pg/mg tissue
TNF-α pg/mg tissue
IL-6 pg/mg tissue
IL-10 (pg/mg tissue)
sham + sal
Sal
1.4 ng/kg
14 ng/kg
140 ng/kg
MCC950
CLP

◀ **Fig. 2** Levels of proinflammatory cytokines IL-1β, TNF-α, IL-6 in the prefrontal cortex (**a**, **c**, **e**), and hippocampus (**b**, **d**, **f**), and levels of anti-inflammatory cytokine IL-10 in prefrontal cortex (**g**) and hippocampus (**h**) at 24 h after induction of polymicrobial sepsis and treatment with MCC950 (1.4, 14, or 140 ng/kg). Data expressed as mean ± SD, analyzed by one-way ANOVA and Tukey post hoc tests. *$p < 0.05$ in relation to sham + saline group and #$p < 0.05$ in relation to CLP + saline group

NLRP3 Inhibition Protects Short-Term and Long-Term Memory after Sepsis

Figure 9 shows the latency of the passive inhibitory avoidance task. The comparison between the test and training sessions indicates an increased latency (9a) for the short-term memory (STM) in sham + vehicle group, with no significant effect caused by the administration of MCC950 inhibitor as verified in sham + MCC950 group, while in CLP + vehicle and CLP + MCC950 groups there is a decrease in latency. At the same time, long-term memory (LTM) (9b) MCC950 decreased the latency after CLP induction. Regarding the new object recognition task results, we can observe an increase in the recognition index in the short-term evaluation only to sham + vehicle and CLP + MCC950 groups (9c), thus indicating the inhibition of NLRP3 inflammasome was effective in reducing short-term memory damage after sepsis. There was no effect of MCC950 on the LTM recognition index (9d).

Discussion

The present study aimed to evaluate the involvement of the NLRP3 inflammasome pathway on early and late sepsis brain changes using the inflammasome formation inhibitor, MCC950. Regarding the early changes caused by sepsis, the interruption of excessive elevation of inflammatory mediators was initially detected at 24 h after sepsis induction and the use

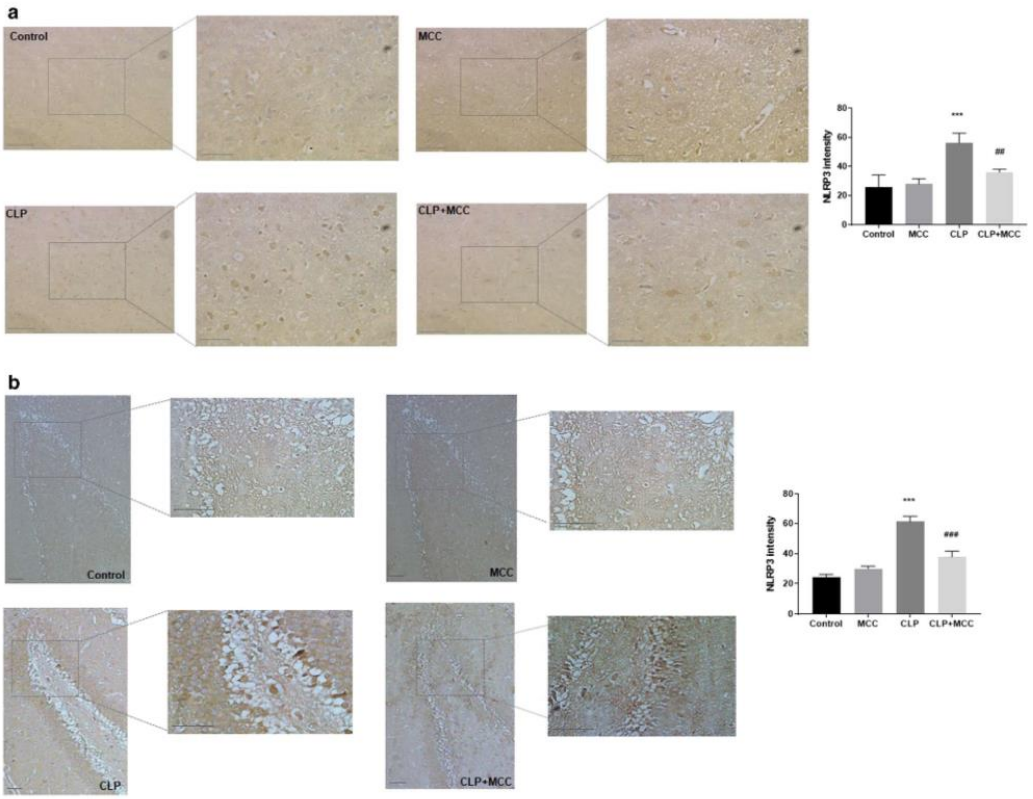


Fig. 3 Immunohistochemical staining of NLRP3 in rat prefrontal cortex (**a**) and hippocampus (**b**) representative microscopic field images (magnification, ×400, scale = 100 μm) immunostained with NLRP3. Data presented as the mean ± SEM (n = 3–4). ***$p < 0.001$ as compared with sham + saline group. ##$p < 0.01$ as compared with CLP + saline group

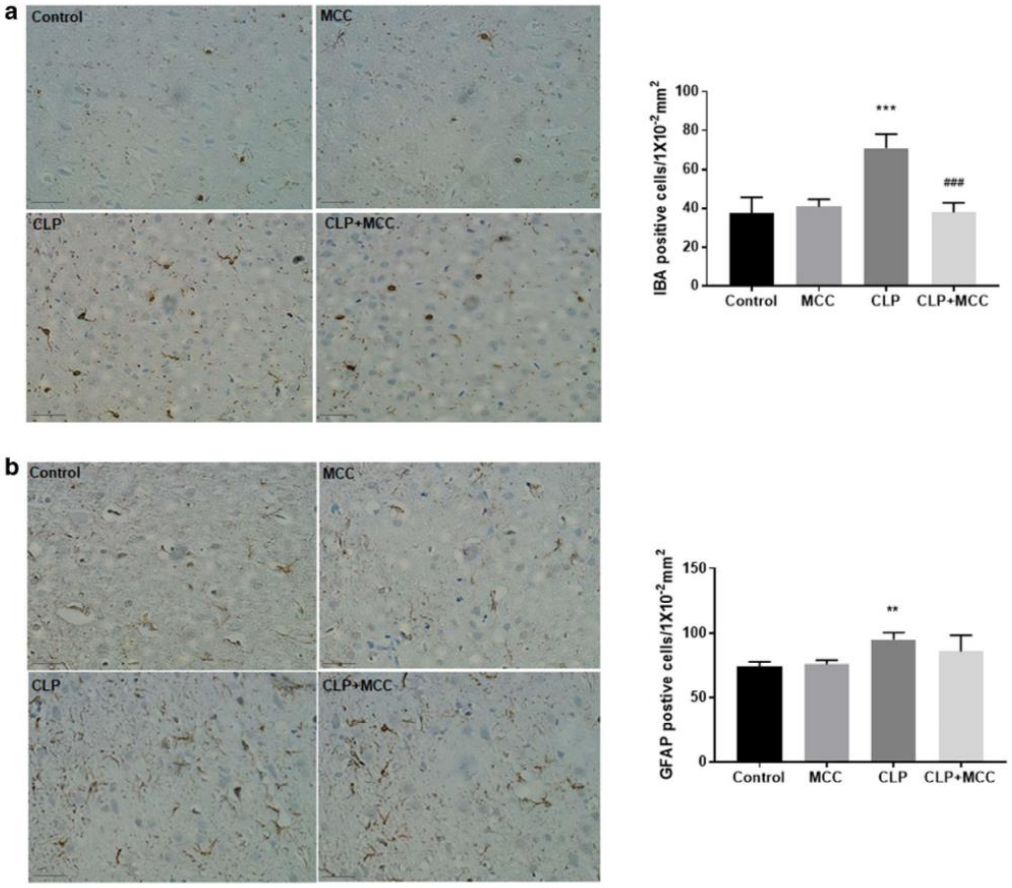


Fig. 4 Immunohistochemical staining of IBA-1 (**a**, **c**) and GFAP (**b**, **d**) in rat prefrontal cortex and hippocampus, respectively. Representative microscopic field images (magnification, ×400 and ×200, respectively, scale = 100 μm) immunostained with IBA-1 or GFAP. Data presented as the mean ± SEM (n = 3–4). $^{***}p < 0.001$ as compared with sham + saline group. $^{###}p < 0.001$ as compared with CLP + saline group

of the MCC950 inhibitor. Moreover, there was an increase in NLRP3 receptor levels in the brain associated with an increase in microglial activation. However, the administration of MCC950 decreased microglial activation, thus reducing oxidative and nitrosative stress, besides altering the cellular energetic activity. The inhibition of NLRP3 inflammasome formation increased the survival of rats undergoing sepsis, as well as restored early memory impairment.

In the last 10 years, studies involving the modulation of the formation of inflammasome NLRP3 increased significantly [38], initially just by relating its action on the immune system [39], then generating links of the influence of its activation in neurodegenerative diseases [17], and more recently looking for evidence of its participation in the neurological damage of inflammatory/infectious diseases, such as sepsis [40]. Since the first studies about sepsis pointed to the peak permeability of the blood–brain barrier within 24 h after sepsis onset, several authors have been using this reference point for other studies [41], including the present study. Even though sepsis is a syndrome derived from an infection, its inflammatory response is the main cause of organ dysfunction typically observed in septic individuals. The release of proinflammatory cytokines due to their high capacity for cell signaling can be described as the start for other changes, including CNS changes.

Most cases of sepsis derive from peripheral infections such as pneumonia; however, sepsis-associated encephalopathy is already accepted, although it remains a poorly understood

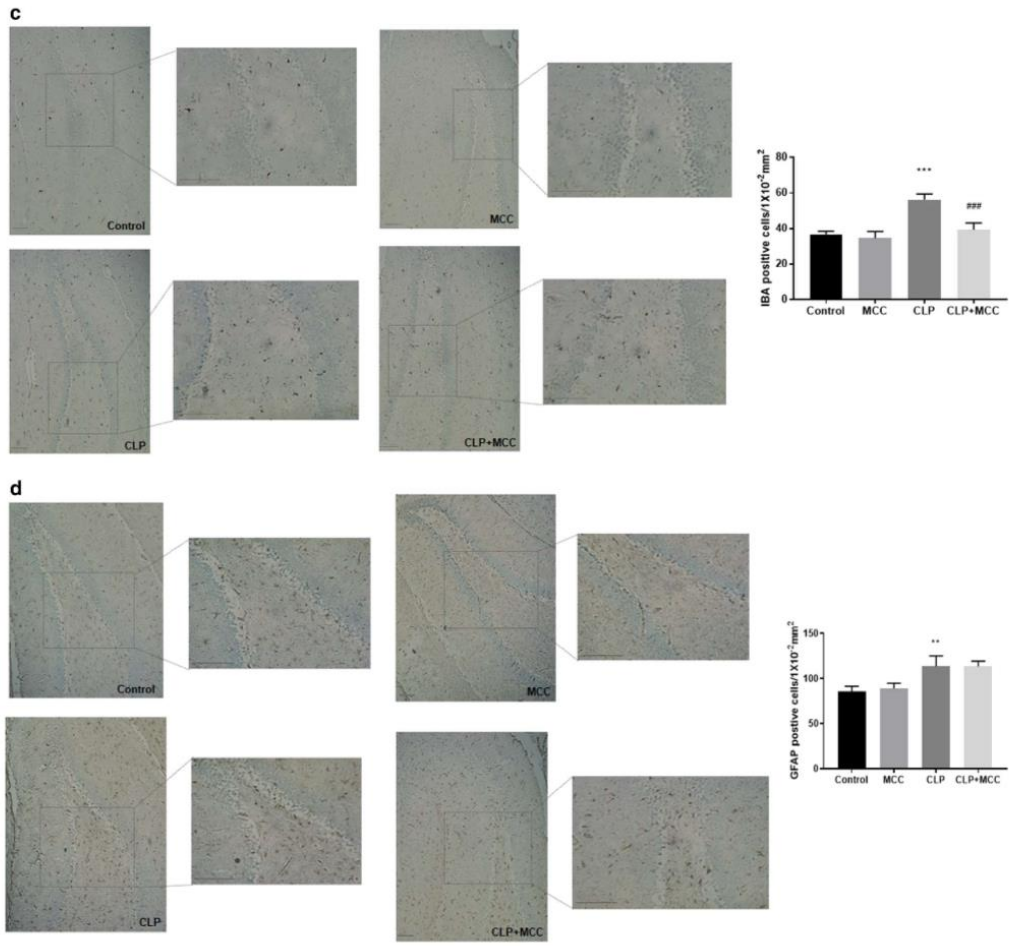


Fig. 4 continued.

consequence in physiopathological terms. What is known about neurological damage in sepsis involves the signaling of peripheral cytokines, which is potentiated by and/or potentiates the release of inflammatory mediators by microglia. In this work, sepsis induction by ligation and perforation of the cecum increased the levels of the main cytokines involved in CNS signaling during sepsis. Both IL-1β and IL-6 increased after sepsis when compared with the control group, and the same profile was previously found in another recent study conducted by our laboratory [42]. With the initial objective of determining the best dose that could inhibit the NLRP3 formation, we used as a basis the doses of 1.4, 14, and 140 ng/kg of MCC950 inhibitor [22], which allowed us to determine the highest dose as more effective in restoring the elevation of these cytokines in the prefrontal cortex and hippocampus.

Previous studies using the MCC950 inhibitor have also shown a reduction in the levels of IL-1β in an animal model of intracerebral hemorrhage [43], in cranioencephalic trauma [44, 45], and stroke [46]. Blocking the formation of the NLRP3 inflammasome impacts the levels of this cytokine because IL-1β turns to its active form after NLRP3 formation [17]. Thus, the reduction in IL-1β levels observed after the administration of the NLRP3 inflammasome formation inhibitor was expected. Consequently, since the levels of pro-

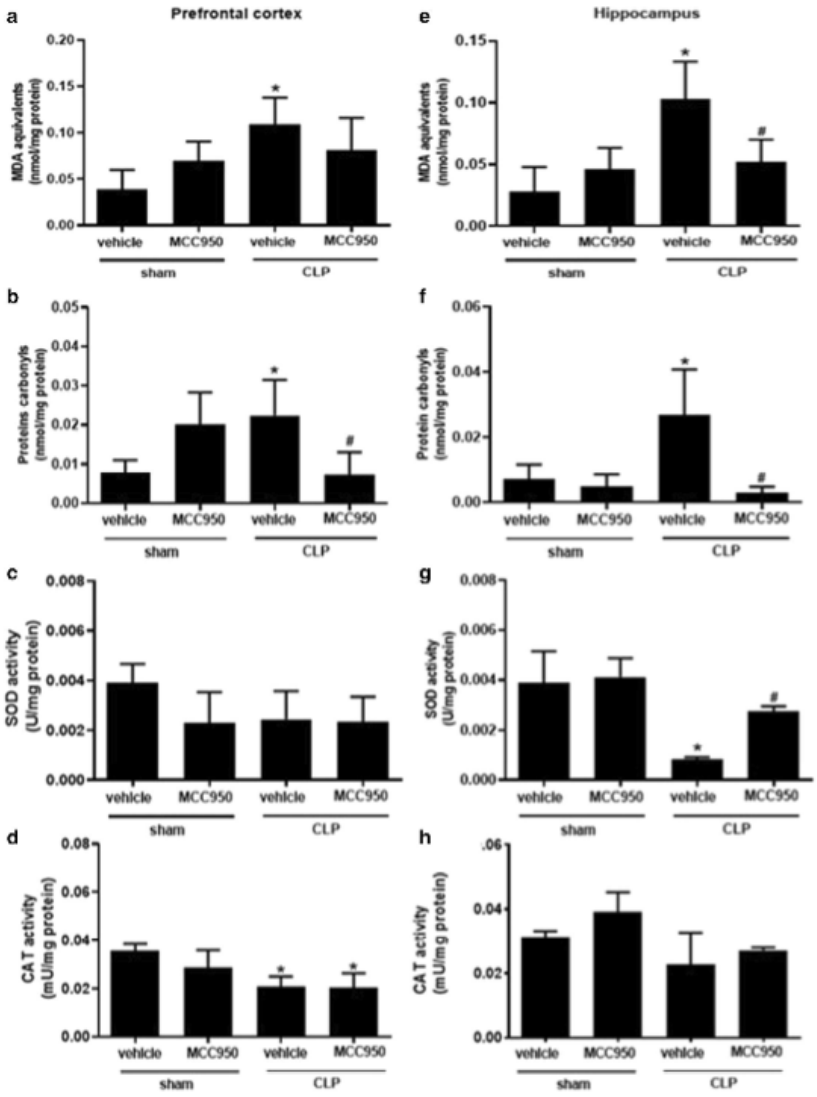


Fig. 5 Lipid peroxidation (**a**, **e**), protein carbonylation (**b**, **f**), SOD (**c**, **g**), and CAT (**d**, **h**) activities in the prefrontal cortex and hippocampus of rats subjected to polymicrobial sepsis and treated with MCC950 (140 ng/kg). Data expressed as mean ± SD, analyzed by one-way ANOVA and Tukey post hoc tests. $*p < 0.05$ in relation to sham + saline group and $\#p < 0.05$ in relation to CLP + saline group

inflammatory cytokines were reduced, one expected to observe no increases in IL-10 levels, which possesses anti-inflammatory characteristics.

TNF-α levels did not follow a logical behavior when compared with IL-1β, for example. Possible explanations for these results may be an insufficient sample for statistical

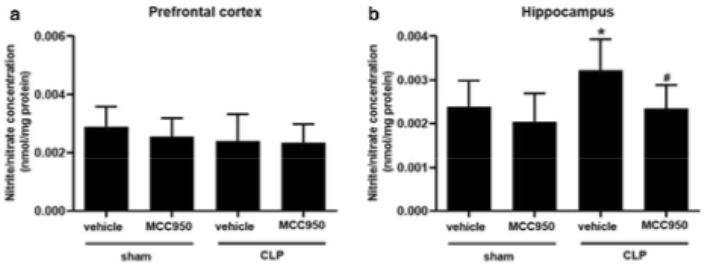


Fig. 6 Nitrite/nitrate concentration in the prefrontal cortex (**a**) and hippocampus (**b**) of rats subjected to polymicrobial sepsis and treated with MCC950 (140 ng/kg). Data expressed as mean ± SD, analyzed by one-way ANOVA with Tukey post hoc test. *$p < 0.05$ in relation to sham + saline group and #$p < 0.05$ in relation to CLP + saline group

significance, or the variation that may occur, since we are measuring cytokine levels released in the brain, and these can be derived both from the periphery as well as released in situ. Another study by our group showed elevation of TNF-α in the prefrontal cortex but not in the hippocampus [47], reinforcing the complexity of sepsis.

Our results show for the first time that, at 24 h after induction, sepsis caused an early increase in the levels of the NLRP3 receptor in the hippocampus and in the prefrontal cortex; two previous studies demonstrated that NLRP3 levels are elevated in the hippocampus of mice at 7 days after sepsis induction [48, 49]. However, we believe that the early elevation of NLRP3 levels can logically explain the neurological damage caused by sepsis. Thus, one can conclude that the levels of NLRP3 receptor rise early and can remain elevated for up to 7 days after sepsis onset, at least in an experimental model.

The NLRP3 receptor is visualized in cells derived from the myeloid lineage, such as microglia, in healthy conditions, but not in astrocytes [50]. In our study, we showed that sepsis activates microglia and astrocytes, but the use of MCC950 in sepsis was effective to decrease only microglial activation. In this sense, we dare to say that the presence of NLRP3 receptor in the studied structures may have derived from an enhanced microglial expression. Still, we could not identify whether the presence of the NLRP3 receptor was a cause or a consequence of the observed microglial activation.

Microglia has played an almost central role in the pathophysiology of neurological dysfunction after sepsis [51]. Its ability to change the profile according to the stimuli can generate a neurotoxic form, capable of releasing substances that cause damage directly or indirectly to neurons [52]. Among the released substances are IL-1β [53], adhesion molecules, and oxidizing substances that can harm cellular macromolecules in the brain.

In our study, we observed increased levels of oxidative damage markers for lipids and proteins in the hippocampus and prefrontal cortex of septic animals, and this finding is consistent with previous reports [42, 54]. This event was avoided with the administration of the MCC950 inhibitor, thus supporting the implication of this inflammasome in brain oxidative damage.

Concerning the activity of antioxidant enzymes, we expected an increase in their levels; however, we noted that SOD activity was decreased in the hippocampus while CAT activity diminished in the prefrontal cortex. Using the MCC950 had a minor act in hippocampal SOD activity, but no results in the prefrontal cortex. The brain has characteristics that make it more susceptible to oxidative damage, such as high oxygen consumption, high concentrations of lipids, and low levels of antioxidant enzymes [55]. Previous studies reveal that sepsis causes fluctuating variation in the activity of the antioxidant enzymes studied here, sometimes with a decreased [42, 47, 56] and sometimes with an increased activity [57]. There are different theories that explain this fluctuation, one of which would be a possible inhibition of its gene expression, a data that we cannot affirm in this work, but it would cause a decrease in its activity. Another plausible explanation would be the structural damage that reactive oxygen species can directly cause in these enzymes, impairing their activity. However, by blocking the formation of the NLRP3 inflammasome, the brain structures of septic rats were preserved from oxidative and nitrosative damage, where a single administration of MCC950 effectively avoided the increase in nitrite and nitrate concentrations, thus indicating the reduction of nitric oxide levels in the hippocampus after sepsis.

Nitric oxide has a clear relationship with microglial activation; consequently, these cells may have iNOS enzymes activated causing a massive production of nitric oxide and its metabolites [58]. On the other hand, nitric oxide combined with hydrogen peroxide form peroxynitrite, a reactive species that possesses a high oxidizing capacity and may negatively contribute to brain homeostasis in elevated levels [58]. The main source of reactive cellular oxygen species is the mitochondria [59], which actively participates in inflammatory processes since the energy demand in these conditions is very elevated and, consequently, requires larger activity of the mitochondrial respiratory chair complexes. A previous finding points to the role of this organelle in the activation

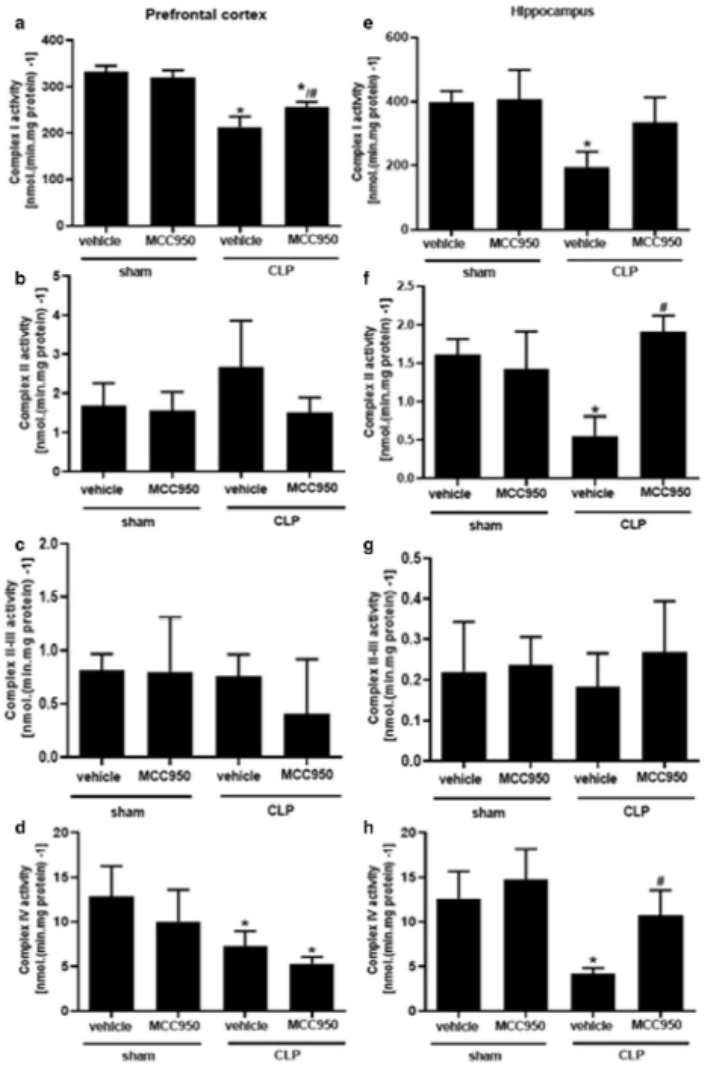


Fig. 7 Activity of complex I (**a**, **e**), complex II (**b**, **f**), complex II–III (**c**, **g**), and complex IV (**d**, **h**) in the prefrontal cortex and hippocampus of rats 24 h after subjected to polymicrobial sepsis and treated with MCC950 (140 ng/kg). Data expressed as mean ± SD, analyzed by one-way ANOVA and Tukey post hoc tests. *$p < 0.05$ in relation to sham + saline group and #$p < 0.05$ in relation to CLP + saline group

of NLRP3 since one of the secondary signs for the NLRP3 inflammasome to be formed would be ROS generation in the process of cellular respiration [38]. In our study, we verified an impairment in the activity of the respiratory chain complexes in animals subjected to sepsis, and in some situations, the inhibition of NLRP3 inflammasome formation was enough to increase the activity in different complexes. Complex I–III activity was the only one that did not show statistically significant variation. The decrease in the activity of the complexes can be explained mainly by the oxidative damage that affected their structural proteins, in this sense their biochemical conformation would be modified, resulting in decreased activity and ATP synthesis [60]. In summary,

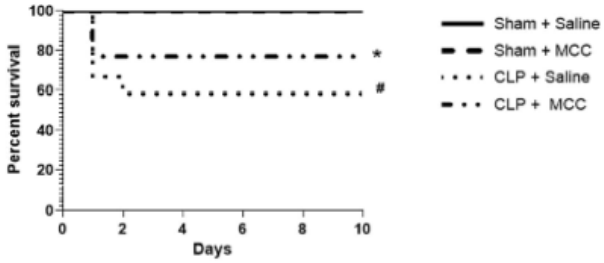


Fig. 8 Kaplan–Meier curve for survival analysis of rats subjected to polymicrobial sepsis and treated with MCC950 (140 ng/kg). Data expressed as mean ± SD, analyzed by one-way ANOVA and Tukey post hoc tests. *$p < 0.05$ in relation to sham + saline group and #$p < 0.05$ in relation to CLP + saline group

the inhibition of NLRP3 inflammasome formation was effective in reducing microglial activation, resulting in less production of oxidizing and damaging products to the CNS.

The survival analysis demonstrated that control animals had 100% survival despite their submission to a surgical procedure, while non-treated septic animals showed the highest mortality rates. On the contrary, corroborating with Fu and colleagues, septic animals that received MCC950 presented a significant reduction in the mortality rates with 10 days of follow-up. Our findings, along with prior evidence, reinforce the fact that sepsis-associated encephalopathy increases mortality both in animals and humans [61], and the formation of NLRP3 may be negatively implicated in neurological damage in sepsis.

During sepsis, the recognition memory is impaired, and substances with antioxidant capacity directly or indirectly contribute by reducing short- and long-term damages [47, 60, 62]. Neurological impairment in septic or sepsis survival patients is an increasing concern in the medical literature due to an important health impact and late manifestations of cognitive injury [2, 61, 63, 64]. In order to assess whether the inhibition of NLRP3 formation could contribute to the reduction of cognitive damage, we used two tests to assess novel object recognition and passive memory.

In the inhibitory avoidance task, aversive memory was evaluated; the animals in the CLP group showed no difference between the test and training sessions, showing impairment of short- and long-term memory. When evaluating the effect of MCC950 administration, it was effective in restoring memory damage for LTM only, evidenced by the increased latency between training and testing sessions. Novel object recognition is a hippocampus-dependent memory test based on the natural tendency of rodents to investigate a novel object rather

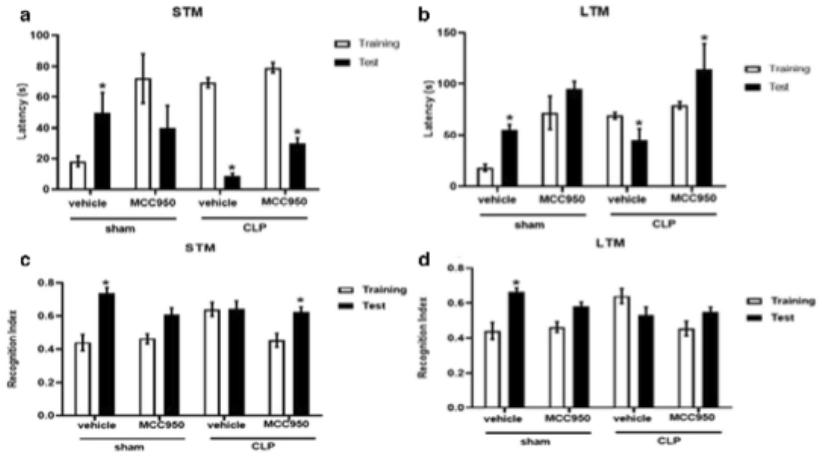


Fig. 9 Latency in short- (**a**) and long-term memory evaluation (**b**), and object recognition index in short- (**c**) and long-term memory evaluation (**d**) of rats 24 h after being subjected to polymicrobial sepsis and treated with MCC950 (140 ng/kg) 10 days after sepsis induction. Data presented as mean and interquartile range, analyzed by Mann–Whitney *U* test. Comparisons between groups in the recognition index objects were performed using Wilcoxon test. *$p < 0.05$ compared with the training session

than a familiar object, when both objects are simultaneously present in an open field arena [65]. This is a test that also allows the investigation of early or late memory damage, and we found the hippocampal damage was responsible for the behavioral changes that CLP animals displayed. However, the administration of MCC950 was effective in restoring only STM damage.

As already described in the literature, the NLPR3 receptor can remain at high levels for up to 7 days after sepsis [48], becoming available to form the inflammasome; in our study, we used a single dose of the inhibitor immediately after sepsis induction, which generated different responses in two behavioral tests performed at 10 days after sepsis. Wu and colleagues revealed a reduction for long-term memory in the freezing time of animals submitted to the fear conditioning test at 7 days after sepsis and treatment with peptide SS-31 [49], and the same pattern was observed in another study that tested animals at 2 weeks after inducing sepsis and treating with MCC950 or a caspase-1 inhibitor [66]. In 2018, Zarbato and colleagues exhibited the protective effect of dimethylfumarate on the short-term object recognition memory of rats submitted to the CLP model [42]. In contrast, Della Giustina and colleagues demonstrated that fish oil–treated rats had positive effects only on long-term memory [67].

These results reveal that the neurological damage in sepsis does not derive from a single factor, but from a set of mechanisms that may need several pharmacological interventions in order to have a beneficial effect for patients. In summary, the formation of the NLRP3 inflammasome is involved with cognitive damage after sepsis, but further studies are needed to show which regions of the hippocampus and prefrontal cortex may be mostly injured.

Conclusion

Animals with sepsis present high brain levels of the NLRP3 receptor activation when compared with the control group, showing the probable influence of the NLRP3 inflammasome on the progression of neurological damage after sepsis. The inhibition of NLRP3 inflammasome formation, with the use of MCC950, was effective to reduce the acute neuroinflammatory response, mitochondrial complex activity, and oxidative damage; reduce long-term cognitive damage; and increase survival after experimental sepsis.

Funding

This research was supported by the National Council for Scientific and Technological Development (CNPq) (F.P.), and the Coordination for the Improvement of Higher Education Personnel (CAPES) (L.G.D.). T.B. and F.D.-P. are CNPq Research Fellows. The funding sources were not involved in the conduction of the research, preparation of the article, nor in the decision to submit the article for publication.

Compliance with ethical standards

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

References

1. Rudd KE, Johnson SC, Agesa KM, Shackelford KA, Tsoi D, Kievlan DR, Colombara DV, Ikuta KS et al (2020) Global, regional, and national sepsis incidence and mortality, 1990–2017: analysis for the Global Burden of Disease Study. Lancet 395(10219): 200–211. https://doi.org/10.1016/S0140-6736(19)32989-7
2. Iwashyna TJ, Ely W, Smith DM (2010) Long-term cognitive impairment and functional disability among survivors of severe sepsis. 304:1787–1794. https://doi.org/10.1001/jama.2010.1553.Long-term
3. Michels M, Ávila P, Pescador B, Vieira A, Abatti M, Cucker L, Borges H, Goulart AI et al (2019) Microglial cells depletion increases inflammation and modifies microglial phenotypes in an animal model of severe sepsis. Mol Neurobiol 56:7296–7304. https://doi.org/10.1007/s12035-019-1606-2
4. Zrzavy T, Höftberger R, Berger T, Rauschka H, Butovsky O, Weiner H, Lassmann H (2019) Pro-inflammatory activation of microglia in the brain of patients with sepsis. Neuropathol Appl Neurobiol 45:278–290. https://doi.org/10.1111/nan.12502
5. Westhoff D, Engelen-Lee JY, Hoogland ICM, Aronica EMA, van Westerloo DJ, van de Beek D, van Gool WA (2019) Systemic infection and microglia activation: a prospective postmortem study in sepsis patients. Immun Ageing 16:1–10. https://doi.org/10.1186/s12979-019-0158-7
6. Griton M, Dhaya I, Nicolas R, et al (2019) Brain, behavior, and immunity experimental sepsis-associated encephalopathy is accompanied by altered cerebral blood perfusion and water diffusion and related to changes in cyclooxygenase-2 expression and glial cell morphology but not to blood–brain. https://doi.org/10.1016/j.bbi.2019.10.012
7. Hart BA 't, Harris RA, Lühder F, et al (2018) Microglia and CNS interleukin-1: beyond immunological concepts. 9:1. https://doi.org/10.3389/fneur.2018.00008
8. Cunningham C (2013) Microglia and neurodegeneration: the role of systemic inflammation. Glia 61:71–90. https://doi.org/10.1002/glia.22350
9. Kitagawa Y, Nakaso K, Horikoshi Y, Morimoto M, Omotani T, Otsuki A, Inagaki Y, Sato H et al (2019) System xc− in microglia is a novel therapeutic target for post-septic neurological and psychiatric illness. Sci Rep 9:1–13. https://doi.org/10.1038/s41598-019-44006-8
10. Brown GC, Vilalta A (2015) How microglia kill neurons. Brain Res 1628:288–297. https://doi.org/10.1016/j.brainres.2015.08.031
11. Garc J, Alonso-bellido IM, Burguillos MA (2019) Reformulating pro-oxidant microglia in neurodegeneration. J Clin Med Rev 8
12. Michels M, Vieira LGDA, Florentino D et al (2015) CD40–CD40 ligand pathway is a major component of acute neuroinflammation and contributes to long-term cognitive dysfunction after sepsis. Mol Med 21:226–226. https://doi.org/10.2119/molmed.2015.00070
13. Danielski LG, Della Giustina A, Goldim MP et al (2017) Vitamin B6 reduces neurochemical and long-term cognitive alterations after polymicrobial sepsis: involvement of the kynurenine pathway modulation. Mol Neurobiol 55:1–14. https://doi.org/10.1007/s12035-017-0706-0
14. Gustin A, Kirchmeyer M, Koncina E, Felten P, Losciuto S, Heurtaux T, Tardivel A, Heuschling P et al (2015) NLRP3 inflammasome is expressed and functional in mouse brain

microglia but not in astrocytes. PLoS One 10:1–19. https://doi.org/10.1371/journal.pone.0130624
15. He Y, Hara H, Núñez G (2016) Mechanism and regulation of NLRP3 inflammasome activation. Trends Biochem Sci 41:1012–1021. https://doi.org/10.1016/j.tibs.2016.09.002
16. Wang S, Yuan YH, Chen NH, Wang HB (2019) The mechanisms of NLRP3 inflammasome/pyroptosis activation and their role in Parkinson's disease. Int Immunopharmacol 67:458–464
17. Song L, Pei L, Yao S, Wu Y, Shang Y (2017) NLRP3 inflammasome in neurological diseases, from functions to therapies. Front Cell Neurosci 11:1–17. https://doi.org/10.3389/fncel.2017.00063
18. Pan Y, Chen X, Zhang Q, Kong L (2014) Brain, behavior, and immunity microglial NLRP3 inflammasome activation mediates IL-1b-related inflammation in prefrontal cortex of depressive rats. Brain Behav Immun 1–11. https://doi.org/10.1016/j.bbi.2014.04.007
19. Dansokho C, Heneka MT (2018) Neuroinflammatory responses in Alzheimer's disease. J Neural Transm 125:771–779. https://doi.org/10.1007/s00702-017-1831-7
20. Haque ME, Akther M, Jakaria M, Kim IS, Azam S, Choi DK (2019) Targeting the microglial NLRP3 inflammasome and its role in Parkinson's disease. Mov Disord 35:20–33. https://doi.org/10.1002/mds.27874
21. Rittirsch D, Huber-Lang MS, Flierl MA, Ward PA (2009) Immunodesign of experimental sepsis by cecal ligation and puncture. Nat Protoc 4:31–36. https://doi.org/10.1038/nprot.2008.214
22. Dempsey C, Rubio Araiz A, Bryson KJ, Finucane O, Larkin C, Mills EL, Robertson AAB, Cooper MA et al (2017) Inhibiting the NLRP3 inflammasome with MCC950 promotes non-phlogistic clearance of amyloid-β and cognitive function in APP/PS1 mice. Brain Behav Immun 61:306–316. https://doi.org/10.1016/j.bbi.2016.12.014
23. CD40-CD40L 2015
24. Draper HH, Hadley M (1990) Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 186:421–431
25. Levine RL, Garland D, Oliver CN et al (1990) Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 186:464–478
26. Bannister JV, Calabrese L (1987) Assays for superoxide dismutase. Methods Biochem Anal 32:279–312
27. Aebi H (1984) Catalase in vitro. Methods Enzymol:121–126
28. Green LC, Wagner DA, Glogowski J, et al (1982) Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. 131–138
29. Cassina A, Radi R (1996) Differential inhibitory action of nitric oxide and peroxynitrite on mitochondrial electron transport. Arch Biochem Biophys 328:309–316. https://doi.org/10.1006/abbi.1996.0178
30. Fischer J, Ruitenbeek W, Ja B et al (1985) Differential investigation of the capacity of succinate oxidation in human skeletal muscle. Clin Chim Acta 153:23–36
31. Rustin P, Chretien D, Bourgeron T, Gérard B, Rötig A, Saudubray JM, Munnich A (1994) Biochemical and molecular investigations in respiratory chain. Clin Chim Acta 228:35–51
32. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275
33. Carvalho D, Petronilho F, Vuolo F, Machado RA, Constantino L, Guerrini R, Calo G, Gavioli EC et al (2008) The nociceptin/orphanin FQ-NOP receptor antagonist effects on an animal model of sepsis. Intensive Care Med 34:2284–2290. https://doi.org/10.1007/s00134-008-1313-3
34. Imanishi T, Sawa A, Ichimaru Y, Miyashiro M, Kato S, Yamamoto T, Ueki S (1997) Ameliorating effects of rolipram on experimentally induced impairments of learning and memory in rodents. Eur J Pharmacol 321:273–278. https://doi.org/10.1016/S0014-2999(96)00969-7
35. Tuon L, Comim CM, Petronilho F, Barichello T, Izquierdo I, Quevedo J, Dal-Pizzol F (2008) Time-dependent behavioral recovery after sepsis in rats. Intensive Care Med 34:1724–1731. https://doi.org/10.1007/s00134-008-1129-1
36. Barichello T, Martins MR, Reinke A, Feier G, Ritter C, Quevedo J, Dal-Pizzol F (2005) Cognitive impairment in sepsis survivors from cecal ligation and perforation. Crit Care Med 33:221–223 discussion 262-3
37. Vianna MR, Izquierdo LA, Barros DM et al (2000) Short- and long-term memory: differential involvement of neurotransmitter systems and signal transduction cascades. An Acad Bras Cienc 72:353–364
38. Hughes MM, O'Neill LAJ (2018) Metabolic regulation of NLRP3. 281:88–98. https://doi.org/10.1111/imr.12608
39. Abderrazak A, Syrovets T, Couchie D, el Hadri K, Friguet B, Simmet T, Rouis M (2015) NLRP3 inflammasome: from a danger signal sensor to a regulatory node of oxidative stress and inflammatory diseases. Redox Biol 4:296–307. https://doi.org/10.1016/j.redox.2015.01.008
40. Zhai Y, Meng X, Ye T, Xie W, Sun G, Sun X (2018) Inhibiting the NLRP3 inflammasome activation with MCC950 ameliorates diabetic encephalopathy in db/db mice. Molecules 23:1–14. https://doi.org/10.3390/molecules23030522
41. Danielski LG, Della Giustina A, Badawy M et al (2017) Brain barrier breakdown as a cause and consequence of neuroinflammation in sepsis. Mol Neurobiol 55:1–9. https://doi.org/10.1007/s12035-016-0356-7
42. Zarbato GF, de Souza Goldim MP, Della Giustina A et al (2018) Dimethyl fumarate limits neuroinflammation and oxidative stress and improves cognitive impairment after polymicrobial sepsis. Neurotox Res 34:418–430. https://doi.org/10.1007/s12640-018-9900-8
43. Ren H, Kong Y, Liu Z, Zang D, Yang X, Wood K, Li M, Liu Q (2018) Selective NLRP3 (pyrin domain-containing protein 3) inflammasome inhibitor reduces brain injury after intracerebral hemorrhage. Stroke 49:184–192. https://doi.org/10.1161/STROKEAHA.117.018904
44. Xu X, Yin D, Ren H, Gao W, Li F, Sun D, Wu Y, Zhou S et al (2018) Selective NLRP3 inflammasome inhibitor reduces neuroinflammation and improves long-term neurological outcomes in a murine model of traumatic brain injury. Neurobiol Dis 117:15–27. https://doi.org/10.1016/j.nbd.2018.05.016
45. Ismael S, Nasoohi S, Ishrat T (2018) MCC950, the selective NLRP3 inflammasome inhibitor protects mice against traumatic brain injury. J Neurotrauma. https://doi.org/10.1089/neu.2017.5344
46. Ismael S, Zhao L, Nasoohi S, Ishrat T (2018) Inhibition of the NLRP3-inflammasome as a potential approach for neuroprotection after stroke. Sci Rep 8:1–9. https://doi.org/10.1038/s41598-018-24350-x
47. Della Giustina A, Goldim MP, Danielski LG, Florentino D, Mathias K, Garbossa L, Oliveira Junior AN, Fileti ME et al (2017) Alpha-lipoic acid attenuates acute neuroinflammation and long-term cognitive impairment after polymicrobial sepsis. Neurochem Int 108:436–447. https://doi.org/10.1016/j.neuint.2017.06.003
48. Fu Q, Wu J, Zhou XY, Ji MH, Mao QH, Li Q, Zong MM, Zhou ZQ et al (2019) NLRP3/Caspase-1 pathway-induced pyroptosis mediated cognitive deficits in a mouse model of sepsis-associated encephalopathy. Inflammation 42:306–318. https://doi.org/10.1007/s10753-018-0894-4
49. Wu J, Zhang M, Hao S, Jia M, Ji M, Qiu L, Sun X, Yang J et al (2014) Mitochondria-targeted peptide reverses mitochondrial dysfunction and cognitive deficits in sepsis-associated encephalopathy.

Mol Neurobiol 52:783–791. https://doi.org/10.1007/s12035-014-8918-z
50. Gustin A, Kirchmeyer M, Koncina E, et al (2015) NLRP3 inflammasome is expressed and functional in mouse brain microglia but not in astrocytes. https://doi.org/10.1371/journal.pone.0130624
51. Michels M, Sonai B, Dal-Pizzol F (2017) Polarization of microglia and its role in bacterial sepsis. J Neuroimmunol 303:90–98. https://doi.org/10.1016/j.jneuroim.2016.12.015
52. Danielski LG, Della Giustina A, Bonfante S et al (2019) The NLRP3 inflammasome and its role in sepsis development. Inflammation. 43:24–31. https://doi.org/10.1007/s10753-019-01124-9
53. Moraes CA, Santos G, De Sampaio E, Spohr TCL, et al (2015) Activated microglia-induced deficits in excitatory synapses through IL-1β: implications for cognitive impairment in sepsis. Mol Neurobiol 653–663. https://doi.org/10.1007/s12035-014-8868-5
54. Petronilho F, Périco SR, Vuolo F, Mina F, Constantino L, Comim CM, Quevedo J, Souza DO et al (2012) Protective effects of guanosine against sepsis-induced damage in rat brain and cognitive impairment. Brain Behav Immun 26:904–910. https://doi.org/10.1016/j.bbi.2012.03.007
55. Sbodio JI, Snyder SH, Paul BD, Sciences M (2017) Redox mechanisms in neurodegeneration: from disease. 1–136. https://doi.org/10.1089/ars.2017.7321
56. Barichello T, Lemos JC, Generoso JS, Cipriano AL, Milioli GL, Marcelino DM, Vuolo F, Petronilho F et al (2011) Oxidative stress, cytokine/chemokine and disruption of blood–brain barrier in neonate rats after meningitis by Streptococcus agalactiae. Neurochem Res 36:1922–1930. https://doi.org/10.1007/s11064-011-0514-2
57. Savio LEB, Andrade MGJ, de Andrade Mello P et al (2017) P2X7 receptor signaling contributes to sepsis-associated brain dysfunction. Mol Neurobiol 54:6459–6470. https://doi.org/10.1007/s12035-016-0168-9
58. Tse JKY (2017) Gut microbiota, nitric oxide, and microglia as prerequisites for neurodegenerative disorders. ACS Chem Neurosci 8:1438–1447
59. Pool R, Gomez H, Kellum JA (2018) Mechanisms of organ dysfunction in sepsis. Crit Care Clin 34:63–80
60. Danielski LG, Della Giustina A, Goldim MP et al (2018) Vitamin B6 reduces neurochemical and long-term cognitive alterations after polymicrobial sepsis: involvement of the kynurenine pathway modulation. Mol Neurobiol 55:5255–5268. https://doi.org/10.1007/s12035-017-0706-0
61. Rengel KF, Hayhurst CJ, Pandharipande PP, Hughes CG (2019) Long-term cognitive and functional impairments after critical illness. Anesth Analg 128:772–780. https://doi.org/10.1213/ANE.0000000000004066
62. Danielski LG, Michels M, Florentino D, Viera A, Lauriano A, Petronilho F (2017) Alpha lipoic acid attenuates oxidative stress-induced damage macromolecules in the brain of rats with sepsis-associated encephalopathy. Crit Care 18. https://doi.org/10.1186/cc14074
63. Götz T, Günther A, Witte OW, Brunkhorst FM, Seidel G, Hamzei F (2014) Long-term sequelae of severe sepsis: cognitive impairment and structural brain alterations - an MRI study (LossCog MRI). BMC Neurol 14:145. https://doi.org/10.1186/1471-2377-14-145
64. Semmler A, Widmann CN, Okulla T et al (2013) Persistent cognitive impairment, hippocampal atrophy and EEG changes in sepsis survivors. J Neurol Neurosurg Psychiatry 84:62–69. https://doi.org/10.1136/jnnp-2012-302883
65. Antunes M, Biala G (2012) The novel object recognition memory: neurobiology, test procedure, and its modifications. Cogn Process 13:93–110
66. Fu Q, Wu J, Zhou X-Y, et al (2019) NLRP3/caspase-1 pathway-induced pyroptosis mediated cognitive deficits in a mouse model of sepsis-associated encephalopathy. Inflammation 42:. https://doi.org/10.1007/s10753-018-0894-4
67. Della Giustina A, Goldim MP, Danielski LG, Florentino D, Garbossa L, Joaquim L, Oliveira Junior AN, Mathias K et al (2019) Fish oil–rich lipid emulsion modulates neuroinflammation and prevents long-term cognitive dysfunction after sepsis. Nutrition. 70:110417. https://doi.org/10.1016/j.nut.2018.12.003

5. CONSIDERAÇÕES FINAIS

Através deste trabalho buscou-se avaliar o envolvimento da via regulada pelo inflamassoma NLRP3 sobre as alterações encefálicas precoces e tardias na sepse, utilizando como estratégia química a inibição da formação do inflamassoma com o uso de MCC950. A respeito das alterações precoces causadas pela sepse, inicialmente detectou-se a interrupção da elevação excessiva de mediadores inflamatórios 24h após a indução de sepse e uso do inibidor MCC950. Em um segundo momento, verificou-se a elevação dos níveis do receptor NLRP3 no encéfalo, associado a elevação de ativação microglial. Contudo, a administração de MCC950, evitou a ativação microglial, gerando redução no estresse oxidativo e nitrosativo, além de, reverter a atividade energética celular alterada. Por fim, a inibição da formação do inflamassoma NLRP3, contribuiu para a melhora da sobrevida de ratos submetidos a sepse, bem como evitou o dano em memória nos sobreviventes.

REFERÊNCIAS

1. Hotchkiss R KI. The pathophysiology and treatment of sepsis. N Engl J Med 2003:1–19.

2. Ely EW, Speroff T, Gordon SM, Harrell FE, Inouye SK, Bernard GR, Page P. Delirium as a Predictor of Mortality in Mechanically Ventilated Patients in the Intensive Care Unit. 2015;291(14):1753–1762.

3. Lazosky A, Young GB, Ba SZ, Phillips R. Quality of life after septic illness ☆. J Crit Care 2010;25(3):406–412.

4. Biff D, Petronilho F, Constantino L, Vuolo F, Zamora-Berridi GJ, Dall'Igna DM, Comim CM, Quevedo J, Kapczinski F, Dal-Pizzol F. Correlation of acute phase inflammatory and oxidative markers with long-term cognitive impairment in sepsis survivors rats. Shock 2013;40(1):45–8.

5. Dal-Pizzol F, Ritter C, Cassol-Jr OJ, Rezin GT, Petronilho F, Zugno AI, Quevedo J, Streck EL. Oxidative mechanisms of brain dysfunction during sepsis. Neurochem Res 2010; 35:1–12.

6. Conway-Morris A, Wilson J, Shankar-Hari M. Immune Activation in Sepsis. Crit Care Clin 2018;34(1):29–42.

7. Danielski LG, Giustina A Della, Bonfante S, Barichello T, Petronilho F. The NLRP3 Inflammasome and Its Role in Sepsis Development. Inflammation 2019 Nov 18.

8. Swanson K V., Deng M, Ting JPY. The NLRP3 inflammasome: molecular activation and regulation to therapeutics. Nat Rev Immunol; 19(8):477–489. https://pubmed.ncbi.nlm.nih.gov/31036962/

9. Goldmann T, Tay TL, Prinz M. Love and death: Microglia, NLRP3 and the Alzheimer's brain. Cell Res 2013;23(5):595–596.

10. Mao Z, Liu C, Ji S, Yang Q, Ye H. The NLRP3 Inflammasome is Involved in the Pathogenesis of Parkinson's Disease in Rats. Neurochem Res 2017;0(0):0.

11. Mira JC, Gentile LF, Mathias BJ, Efron PA, Brakenridge SC, Mohr AM, Moore FA, Moldawer LL. Sepsis Pathophysiology, Chronic Critical Illness and PICS. Crit Care Med 2017;45(2):253–262.

12. Funk DJ, Parrillo JE, Kumar A. Sepsis and Septic Shock: A History. Crit Care Clin 2009;25(1):83–101.

13. Bone R, Balk R, Cerra F, Dellinger R, Fein A, Knaus W, Schein R, Sibbald W. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepise. Chest 1992;101:1644–1655.

14. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche J-D, Coopersmith CM, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 2016;315(8):801–10.

15. Verboom DM, Frencken JF, Ong DSY, Horn J, Poll T Van Der, Bonten MJM, Cremer OL, Klouwenberg PMCK. Robustness of sepsis-3 criteria in critically ill patients. J Intensive Care 2019;7(46):1–9.

16. Shankar-Hari M, Phillips GS, Levy ML, Seymour CW, Liu VX, Deutschman CS, Angus DC, Rubenfeld GD, Singer M; Sepsis Definitions Task Force. Developing a New Definition and Assessing New Clinical Criteria for Septic Shock: For the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016 Feb 23;315(8):775-87. doi: 10.1001/jama.2016.0289.

17. Sartelli M, Kluger Y, Ansaloni L, Hardcastle TC, Rello J, Watkins RR, Bassetti M, Giamarellou E, Coccolini F, Abu-Zidan FM, et al. Raising concerns about the Sepsis-3 definitions. World J Emerg Surg 2018;13(1):1–9.

18. Reinhart K, Daniels R, Kissoon N, Machado FR, Schachter RD, Finfer S. Recognizing Sepsis as a Global Health Priority - A WHO Resolution. N Engl J Med. 2017;377(5):414-417. doi: 10.1056/NEJMp1707170.

19. Fleischmann C, Scherag A, Adhikari NKJ, Hartog CS, Tsaganos T, Schlattmann P, Angus DCA, Reinhart K. Assessment of Global Incidence and Mortality of treated sepsis-currente estimates and limitations. 2016. 259–272 p.

20. Machado FR, Cavalcanti AB, Bozza FA, Ferreira EM, Angotti Carrara FS, Sousa JL, Caixeta N, Salomao R, Angus DC, Pontes Azevedo LC, et al. The epidemiology of sepsis in Brazilian intensive care units (the Sepsis PREvalence Assessment Database, SPREAD): an observational study. Lancet Infect Dis 2017;17(11):1180–1189.

21. Healthcare Cost and Utilization Project (HCUP) Search Page. https://hcup-us.ahrq.gov/search/keyword.jsp

22. Iwashyna TJ, Cooke CR, Wunsch H. The Population Burden of Long-Term Survivorship after Severe Sepsis Among Older Americans. J Am Geriatr Soc 2012;60(6):1070–1077.

23. Prescott HC, Angus DC. Enhancing Recovery From Sepsis: A Review. JAMA. 2018;319(1):62–75. doi:10.1001/jama.2017.17687

24. Prescott HC, Osterholzer JJ, Langa KM, Angus DC, Iwashyna TJ. Late mortality after sepsis: propensity matched cohort study. BMJ 2016:i2375.

25. Yende S, Kellum JA, Talisa VB, Palmer OMP, Chang CH, Filbin MR. Long-term Host Immune Response Trajectories Among Hospitalized Patients With Sepsis. 2019;2(8):1–15.

26. Medzhitov R. Recognition of microorganisms and activation of the immune response. Nature 2007;449(7164):819–826. https://www.nature.com/articles/nature06246

27. Rosales C, Uribe-Querol E. Phagocytosis: A Fundamental Process in Immunity. Biomed Res Int 2017;2017. https://pubmed.ncbi.nlm.nih.gov/28691037/

28. Mills KHG. TLR-dependent T cell activation in autoimmunity. Nat Rev Immunol 2011;11(12):807–822.

29. Chousterman BG, Swirski FK, Weber GF. Cytokine storm and sepsis disease pathogenesis. Semin Immunopathol. 2017;39(5):517-528. doi: 10.1007/s00281-017-0639-8.

30. Buchanan MM, Hutchinson M, Watkins LR, Yin H. Toll-like receptor 4 in CNS pathologies. J Neurochem 2010;114(1):13–27. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2909662&tool=pmcentrez&rendertype=abstract

31. Fukata M, Vamadevan AS, Abreu MT. Toll-like receptors (TLRs) and Nod-like receptors (NLRs) in inflammatory disorders. Semin Immunol. 2009 ;21(4):242-53. doi: 10.1016/j.smim.2009.06.005. PMID: 19748439.

32. Weighardt H, Holzmann B. Role of Toll-like receptor responses for sepsis pathogenesis. Immunobiology. 2007;212(9-10):715-22. doi: 10.1016/j.imbio.2007.09.010.

33. Kong Y, Le Y. Toll-like receptors in inflammation of the central nervous system. Int Immunopharmacol 2011 [accessed 2013 Sep 18];11(10):1407–14. http://www.ncbi.nlm.nih.gov/pubmed/21600311

34. Janeway C A. How the immune system protects the host from infection. Microbes Infect 2001; 3:1167–1171.

35. Venet F, Monneret G. Advances in the understanding and treatment of sepsis-induced immunosuppression. Nat Rev Nephrol 2018;14(2):121–137.

36. Lewis AJ, Billiar TR, Rosengart MR. Biology and Metabolism of Sepsis: Innate Immunity, Bioenergetics, and Autophagy. Surg Infect (Larchmt) 2016;17(3):286–293.

37. Taeb AM, Hooper MH, Marik PE. Sepsis: Current definition, pathophysiology, diagnosis, and management. Nutr Clin Pract 2017;32(3):296–308.

38. Boomer JS, To K, Chang KC, Ii SDJ, Kreisel D. Immunosuppression in Patients Who Die of Sepsis and Multiple Organ Failure. 2012;306(23):2594–2605.

39. Tuon L, Comim CM, Petronilho F, Barichello T, Izquierdo I, Quevedo J, Dal-pizzol F. Memory-enhancing treatments reverse the impairment of inhibitory avoidance retention in sepsis-surviving rats. 2008;12(5):1–6.

40. Annane D, Sharshar T. Cognitive decline after sepsis. Lancet Respir Med 2015;3(1):61–69.

41. Nikayin S, Rabiee A, Hashem MD, Huang M, Bienvenu OJ, Turnbull AE, Needham DM. Anxiety symptoms in survivors of critical illness: a systematic review and meta-analysis. Gen Hosp Psychiatry 2016;43:23–29. https://pubmed.ncbi.nlm.nih.gov/27796253/

42. Rabiee A, Nikayin S, Hashem MD, Huang M, DInglas VD, Bienvenu OJ, Turnbull AE, Needham DM. Depressive Symptoms after Critical Illness: A Systematic Review

and Meta-Analysis. Crit Care Med 2016;44(9):1744–1753. https://pubmed.ncbi.nlm.nih.gov/27153046/

43. Parker AM, Sricharoenchai T, Raparla S, Schneck KW, Bienvenu OJ, Needham DM. Posttraumatic stress disorder in critical illness survivors: A metaanalysis. Crit Care Med 2015;43(5):1121–1129. https://pubmed.ncbi.nlm.nih.gov/25654178/

44. Sharshar T, Hopkinson NS, Orlikowski D, Annane D. Science review: The brain in sepsis--culprit and victim. Crit Care 2005;9(1):37–44. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1065096&tool=pmcentrez&rendertype=abstract

45. Feng Q, Ai YH, Gong H, Wu L, Ai ML, Deng SY, Huang L, Peng QY, Zhang LN. Characterization of Sepsis and Sepsis-Associated Encephalopathy. J Intensive Care Med 2017.

46. Lamar CD, Hurley RA, Hayman LA, Taber KH. Sepsis-Associated Encephalopathy: Review of the Neuropsychiatric Manifestations and Cognitive Outcome. J Neuropsychiatry Clin Neurosci 2011;(23):23–3.

47. Lisman J, Buzsáki G, Eichenbaum H, Nadel L, Rangananth C, Redish AD. Viewpoints: How the hippocampus contributes to memory, navigation and cognition. Nat Neurosci 2017;20(11):1434–1447. /pmc/articles/PMC5943637/?report=abstract

48. Di Paola M, Caltagirone C, Fadda L, Sabatini U, Serra L, Carlesimo GA. Hippocampal atrophy is the critical brain change in patients with hypoxic amnesia. Hippocampus 2008;18(7):719–728. http://doi.wiley.com/10.1002/hipo.20432

49. Esen F, Orhun G, Özcan PE, Brenes Bastos AR, Tüzün E. Diagnosing acute brain dysfunction due to sepsis. Neurol Sci 2020;41(1):25–33. http://link.springer.com/10.1007/s10072-019-04069-x

50. Mazeraud A, Pascal Q, Verdonk F, Heming N, Chrétien F, Sharshar T. Neuroanatomy and Physiology of Brain Dysfunction in Sepsis. Clin Chest Med 2016;37(2):333–345. https://linkinghub.elsevier.com/retrieve/pii/S0272523116300120

51. Barter J, Kumar A, Stortz JA, Hollen M, Nacionales D, Efron PA, Moldawer LL, Foster TC. Age and Sex Influence the Hippocampal Response and Recovery Following Sepsis. Mol Neurobiol. 2019 Dec;56(12):8557-8572. doi: 10.1007/s12035-019-01681-y.

52. Robba C, Crippa IA, Taccone FS. Septic Encephalopathy. Curr Neurol Neurosci Rep 2018 ;18(12):82. http://link.springer.com/10.1007/s11910-018-0895-6

53. Mazeraud A, Righy C, Bouchereau E, Benghanem S, Bozza FA, Sharshar T. Septic-Associated Encephalopathy: a Comprehensive Review. Neurotherapeutics 2020 ;17(2):392–403. http://link.springer.com/10.1007/s13311-020-00862-1

54. Danielski LG, Giustina A Della, Badawy M, Barichello T, Quevedo J, Dal-Pizzol F, Petronilho F. Brain Barrier Breakdown as a Cause and Consequence of Neuroinflammation in Sepsis. Mol Neurobiol 2018;55(2):1045–1053.

55. Sonneville R, Verdonk F, Rauturier C, Klein IF, Wolff M, Annane D, Chretien F, Sharshar T. Understanding brain dysfunction in sepsis. Ann Intensive Care 2013 ;3(1):15. http://www.ncbi.nlm.nih.gov/pubmed/23718252

56. Dal-Pizzol F, Rojas HA, Dos Santos EM, Vuolo F, Constantino L, Feier G, Pasquali M, Comim CM, Petronilho F, Gelain DP, et al. Matrix metalloproteinase-2 and metalloproteinase-9 activities are associated with blood-brain barrier dysfunction in an animal model of severe sepsis. Mol Neurobiol 2013;48(1):62–70.

57. Michels M, Steckert A V, Quevedo J, Barichello T, Dal-Pizzol F. Mechanisms of long-term cognitive dysfunction of sepsis: from blood-borne leukocytes to glial cells. Intensive care Med Exp 2015;3(1):30.

58. Brown GC, Vilalta A. How microglia kill neurons. Brain Res;1628(Pt B):288–297. http://www.ncbi.nlm.nih.gov/pubmed/26341532

59. Rana M, Fei-Bloom Y, Son M, La Bella A, Ochani M, Levine YA, Chiu PY, Wang P, Chavan SS, Volpe BT, et al. Constitutive Vagus Nerve Activation Modulates Immune Suppression in Sepsis Survivors. Front Immunol;9:2032. http://www.ncbi.nlm.nih.gov/pubmed/30237803

60. Sisó S, Jeffrey M, González L. Sensory circumventricular organs in health and disease. Acta Neuropathol 2010;120(6):689–705.

61. Comim C, Vilela M, Constantino L, Petronilho F, Vuolo F, Lacerda-Queiroz N, Rodrigues D, Rocha J DA, Teixeria A, Quevedo J, et al. Traffic of leukocytes and cytokine up-regulation in the central nervous system in sepsis. 2011;28(4):368–370.

62. Akrout N, Sharshar T, Annane D. Mechanisms of brain signaling during sepsis. Curr Neuropharmacol 2009;7(4):296–301.

63. Villabona-Rueda A, Erice C, Pardo CA, Stins MF. The Evolving Concept of the Blood Brain Barrier (BBB): From a Single Static Barrier to a Heterogeneous and Dynamic Relay Center. Front Cell Neurosci 2019;13.

64. Goldim MP, Danielski LG, Rodrigues JF, Joaquim L, Garbossa L, de Oliveira Junior AN, Metzker KLL, Giustina A Della, Cardoso T, Barichello T, et al. Oxidative stress in the choroid plexus contributes to blood-cerebrospinal fluid barrier disruption during sepsis development. Microvasc Res 2019;123:19–24. http://www.ncbi.nlm.nih.gov/pubmed/30552905

65. Sonar SA, Lal G. Blood-brain barrier and its function during inflammation and autoimmunity. J Leukoc Biol 2018;(January):1–15.

66. Manicone AM, McGuire JK. Matrix Metalloproteinases as Modulators of Inflammation. Semin Cell Dev Biol 2008;72(2):181–204.

67. Bojic S, Kotur-Stevuljevic J, Aleksic A, Gacic J, Memon L, Simic-Ogrizovic S. Matrix Metalloproteinase-9 and Tissue Inhibitor of Matrix Metalloproteinase-1 in Sepsis after Major Abdominal Surgery. Dis Markers. 2018 May 16;2018:5064684. doi: 10.1155/2018/5064684. PMID: 29861795; PMCID: PMC5976929.

68. Nishioku T, Dohgu S, Takata F, Eto T, Ishikawa N, Kodama KB, Nakagawa S, Yamauchi A, Kataoka Y. Detachment of brain pericytes from the basal lamina is involved in disruption of the blood-brain barrier caused by lipopolysaccharide-induced sepsis in mice. Cell Mol Neurobiol 2009;29(3):309–316.

69. Sewal RK, Modi M, Saikia UN, Chakrabarti A, Medhi B. Increase in seizure susceptibility in sepsis like condition explained by spiking cytokines and altered adhesion molecules level with impaired blood brain barrier integrity in experimental model of rats treated with lipopolysaccharides. Epilepsy Res. 2017 ;135:176-186. doi: 10.1016/j.eplepsyres.2017.05.012

70. Hofer S, Bopp C, Hoerner C, Plaschke K, Faden RM, Martin E, Bardenheuer HJ, Weigand M a. Injury of the Blood Brain Barrier and Up-Regulation of ICAM-1 in Polymicrobial Sepsis. J Surg Res 2008;146(2):276–281.

71. Wang Y, Chen Z, Zhang Y, Fang S, Zeng Q. Mitochondrial biogenesis of astrocytes is increased under experimental septic conditions. Chin Med J (Engl) 2014;127(10):1837–42.

72. Aktas O, Ullrich O, Infante-Duarte C, Nitsch R, Zipp F. Neuronal damage in brain inflammation. Arch Neurol 2007;64(2):185–9.

73. Berg RMG, Møller K, Bailey DM. Neuro-oxidative-nitrosative stress in sepsis. J Cereb Blood Flow Metab 2011; 31(7):1532–44. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3137474&tool=pmcentrez&rendertype=abstract

74. Denes A, Thornton P, Rothwell NJ, Allan SM. Inflammation and brain injury: acute cerebral ischaemia, peripheral and central inflammation. Brain Behav Immun. 2010;24(5):708-23. doi: 10.1016/j.bbi.2009.09.010.

75. Jiang W, Desjardins P, Butterworth RF. Cerebral inflammation contributes to encephalopathy and brain edema in acute liver failure: protective effect of minocycline. J Neurochem 2009 ;109(2):485–93. http://www.ncbi.nlm.nih.gov/pubmed/19220703

76. Nimmo AJ, Cernak I, Heath DL, Hu X, Bennett CJ, Vink R. Neurogenic inflammation is associated with development of edema and functional deficits following traumatic brain injury in rats. 2014;4179(04):3–4.

77. Lawson LJ, Perry VH, Dri P, Gordon S. Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. Neuroscience 1990;39(1):151–170.

78. Hickman S, Izzy S, Sen P, Morsett L, El Khoury J. Microglia in neurodegeneration. Nat Neurosci 2018;21(10):1359–1369.

79. Harrison JF, Rinne ML, Kelley MR, Druzhyna NM, Wilson GL, Ledoux SP. Notch Signaling Modulates the Activation of Microglial Cells. Glia 2007;55(14):1416–1425.

80. Westhoff D, Engelen-Lee JY, Hoogland ICM, Aronica EMA, van Westerloo DJ, van de Beek D, van Gool WA. Systemic infection and microglia activation: a prospective postmortem study in sepsis patients. Immun Ageing 2019;16(1):1–10.

81. Moraes CA., Santos G, De Sampaio E Spohr TCL, Davila JC, Souza Lima FR, Benjamim CF, Bozza F a., Alcantara Gomes FC. Activated Microglia-Induced Deficits in Excitatory Synapses Through IL-1B: Implications for Cognitive Impairment in Sepsis. Mol Neurobiol 2015:653–663.

82. Trettel F, Di Castro MA, Limatola C. Chemokines: Key Molecules that Orchestrate Communication among Neurons, Microglia and Astrocytes to Preserve Brain Function. Neuroscience 2019 Jul 31. http://www.ncbi.nlm.nih.gov/pubmed/31376422

83. Wolf SA, Boddeke HWGM, Kettenmann H. Microglia in Physiology and Disease. Annu Rev Physiol 2017;79(1):619–643.

84. García-Revilla, J.; Alonso-Bellido, I.M.; Burguillos, M.A.; Herrera, A.J.; Espinosa-Oliva, A.M.; Ruiz, R.; Cruz-Hernández, L.; García-Domínguez, I.; Roca-Ceballos, M.A.; Santiago, M.; Rodríguez-Gómez, J.A.; Soto, M.S.; de Pablos, R.M.; Venero, J.L. Reformulating Pro-Oxidant Microglia in Neurodegeneration. J. Clin. Med. 2019, 8, 1719.

85. Michels M, Danieslki LG, Vieira A, Florentino D, Igna DD. CD40 – CD40 Ligand Pathway Is a Major Component of Acute Neuroinflammation and Contributes to Long-term Cognitive Dysfunction after Sepsis. 2015;(15):219–226.

86. Hong S, Beja-Glasser VF, Nfonoyim BM, Frouin A, Li S, Ramakrishnan S, Merry KM, Shi Q, Rosenthal A, Barres BA, et al. Complement and microglia mediate early synapse loss in Alzheimer mouse models. Science 2016 ;352(6286):712–716. http://www.ncbi.nlm.nih.gov/pubmed/27033548

87. Hickman SE, El Khoury J. The neuroimmune system in Alzheimer's disease: the glass is half full. J Alzheimers Dis 2013;33 Suppl 1:S295-302. http://www.ncbi.nlm.nih.gov/pubmed/22751176

88. Wang LM, Wu Q, Kirk RA, Horn KP, Ebada Salem AH, Hoffman JM, Yap JT, Sonnen JA, Towner RA, Bozza FA, et al. Lipopolysaccharide endotoxemia induces amyloid-β and p-tau formation in the rat brain. Am J Nucl Med Mol Imaging 2018;8(2):86–99. http://www.ncbi.nlm.nih.gov/pubmed/29755842

89. Kirk RA, Kesner RP, Wang LM, et al. Lipopolysaccharide exposure in a rat sepsis model results in hippocampal amyloid-β plaque and phosphorylated tau deposition and corresponding behavioral deficits. Geroscience. 2019;41(4):467-481. doi:10.1007/s11357-019-00089-9

90. Cunningham C. Microglia and neurodegeneration: The role of systemic inflammation. Glia 2013;61(1):71–90.

91. Kitagawa Y, Nakaso K, Horikoshi Y, Morimoto M, Omotani T, Otsuki A, Inagaki Y, Sato H, Matsura T. System x c− in microglia is a novel therapeutic target for post-septic neurological and psychiatric illness. Sci Rep 2019;9(1):1–13.

92. Cenini G, Lloret A, Cascella R. Oxidative Stress in Neurodegenerative Diseases: From a Mitochondrial Point of View. Oxid Med Cell Longev 2019;2019:2105607.

93. Hamed SA, Hamed EA, Abdella MM. Septic encephalopathy: Relationship to serum and cerebrospinal fluid levels of adhesion molecules, lipid peroxides and S-100B protein. Neuropediatrics 2009;40(2):66–72.

94. Davies DC. Blood-brain barrier breakdown in septic encephalopathy and brain tumours. J Anat 2002;200(6):639–646.

95. Zhu T, Zhao Y, Hu H, Zheng Q, Luo X, Ling Y, Ying Y, Shen Z, Jiang P, Shu Q. TRPM2 channel regulates cytokines production in astrocytes and aggravates brain disorder during lipopolysaccharide-induced endotoxin sepsis. Int Immunopharmacol 2019 ;75:105836. https://linkinghub.elsevier.com/retrieve/pii/S1567576919311762

96. Gebicki JM. Oxidative stress, free radicals and protein peroxides. Arch Biochem Biophys 2016;595:33–39. https://pubmed.ncbi.nlm.nih.gov/27095212/

97. Kehrer JP, Klotz LO. Free radicals and related reactive species as mediators of tissue injury and disease: Implications for Health. Crit Rev Toxicol 2015 ;45(9):765–798. https://pubmed.ncbi.nlm.nih.gov/26610815/

98. Lushchak VI. Free radicals, reactive oxygen species, oxidative stress and its classification. Chem Biol Interact 2014;224:164–175. https://pubmed.ncbi.nlm.nih.gov/25452175/

99. Ortiz GG, Pacheco Moisés FP, Mireles-Ramírez M, Flores-Alvarado LJ, González-Usigli H, Sánchez-González VJ, Sánchez-López AL, Sánchez-Romero L, Díaz-Barba EI, Santoscoy-Gutiérrez JF, et al. Oxidative Stress: Love and Hate History in Central Nervous System. In: Advances in Protein Chemistry and Structural Biology. Vol. 108. Academic Press Inc.; 2017. p. 1–31.

100. Hopkins RO. Sepsis, oxidative stress, and brain injury. Crit Care Med 2007 ;35(9):2233–2234. https://pubmed.ncbi.nlm.nih.gov/17713382/

101. Turrens JF. Mitochondrial formation of reactive oxygen species. J Physiol 2003 [accessed 2020 Oct 5];552(2):335–344. https://pubmed.ncbi.nlm.nih.gov/14561818/

102. Brieger K, Schiavone S, Miller FJ, Krause KH. Reactive oxygen species: From health to disease. Swiss Med Wkly 2012;142. https://pubmed.ncbi.nlm.nih.gov/22903797/

103. Sies H. Oxidative stress: A concept in redox biology and medicine. Redox Biol 2015;4:180–183. https://pubmed.ncbi.nlm.nih.gov/25588755/

104. Van Acker H, Coenye T. The Role of Reactive Oxygen Species in Antibiotic-Mediated Killing of Bacteria. Trends Microbiol 2017 ;25(6):456–466. https://pubmed.ncbi.nlm.nih.gov/28089288/

105. Poprac P, Jomova K, Simunkova M, Kollar V, Rhodes CJ, Valko M. Targeting Free Radicals in Oxidative Stress-Related Human Diseases. Trends Pharmacol Sci 2017;38(7):592–607. https://linkinghub.elsevier.com/retrieve/pii/S0165614717300974

106. Adams L, Franco MC, Estevez AG. Reactive nitrogen species in cellular signaling. Exp Biol Med 2015;240(6):711–717. https://pubmed.ncbi.nlm.nih.gov/25888647/

107. Hancock JT, Brouquisse R. Considerations of the importance of redox state for reactive nitrogen species action. J Exp Bot 2019;70(17):4323–4331. https://pubmed.ncbi.nlm.nih.gov/30793204/

108. Spiller F, Oliveira Formiga R, Fernandes da Silva Coimbra J, Alves-Filho JC, Cunha TM, Cunha FQ. Targeting nitric oxide as a key modulator of sepsis, arthritis and pain. Nitric Oxide - Biol Chem 2019;89:32–40

109. Brown GC. The endotoxin hypothesis of neurodegeneration. J Neruroinflammation 2019;16(1):180.

110. Semmler A, Okulla T, Sastre M, Dumitrescu-Ozimek L, Heneka MT. Systemic inflammation induces apoptosis with variable vulnerability of different brain regions. J Chem Neuroanat 2005;30(2–3):144–57. http://www.ncbi.nlm.nih.gov/pubmed/16122904

111. Chien HF, Yeh KY, Jiang-Shieh YF, Wei IH, Chang CY, Chang ML, Wu CH. Signal transduction pathways of nitric oxide release in primary microglial culture challenged with gram-positive bacterial constituent, lipoteichoic acid. Neuroscience 2005;133(2):423–36. http://www.ncbi.nlm.nih.gov/pubmed/15878805

112. Culmsee C, Michels S, Scheu S, Arolt V, Dannlowski U, Alferink J. Mitochondria, Microglia, and the Immune System-How Are They Linked in Affective Disorders?. Front Psychiatry. 2019;9:739. Published 2019 Jan 9. doi:10.3389/fpsyt.2018.00739

113. Zmijewski JW, Park DW. Mitochondrial Dysfunction and Immune Cell Metabolism in Sepsis. Infect Chemother Infect Chemother 2017;49149(1):10–21.

114. Barichello T, Fortunato JJ, Vitali AM, Feier G, Reinke A, Moreira JCF, Quevedo J, Dal-Pizzol F. Oxidative variables in the rat brain after sepsis induced by cecal ligation and perforation. Crit Care Med 2006;34(3):886–9.

115. Comim CM, Cassol-Jr OJ, Constantino LS, Felisberto F, Petronilho F, Rezin GT, Scaini G, Daufenbach JF, Streck EL, Quevedo J, et al. Alterations in inflammatory mediators, oxidative stress parameters and energetic metabolism in the brain of sepsis survivor rats. Neurochem Res 2011;36(2):304–311.

116. Brealey D, Brand M, Hargreaves I, Heales S, Land J, Smolenski R, Davies N a., Cooper CE, Singer M. Association between mitochondrial dysfunction and severity and outcome of septic shock. Lancet 2002;360(9328):219–223.

117. Kumar V, Sharma A. Is neuroimmunomodulation a future therapeutic approach for sepsis? Int Immunopharmacol 2010;10(1):9–17.

118. Kira J-I, Masaki K, Ohyagi Y, Takeuchi H, Katsumoto A, Takahashi K, Tanaka F. Microglia in Alzheimer's Disease: Risk Factors and Inflammation. Front Neurol | www.frontiersin.org 2018;9:978. www.frontiersin.org

119. Song L, Pei L, Yao S, Wu Y, Shang Y. NLRP3 Inflammasome in Neurological Diseases, from Functions to Therapies. Front Cell Neurosci. 2017;11:63. doi: 10.3389/fncel.2017.00063. PMID: 28337127; PMCID: PMC5343070.

120. Freeman L, Guo H, David CN, Brickey WJ, Jha S, Ting JP-Y. NLR members NLRC4 and NLRP3 mediate sterile inflammasome activation in microglia and astrocytes. J Exp Med 2017;214(5):1351–1370.

121. Olsen I, Singhrao SK. Inflammasome involvement in Alzheimer's disease. Handb Infect Alzheimer's Dis 2017; 54:17–25.
122. Mamik MK, Power C. Inflammasomes in neurological diseases: emerging pathogenic and therapeutic concepts. Brain. 2017 Sep 1;140(9):2273-2285. doi: 10.1093/brain/awx133.

123. Hughes MM, Neill LAJO. Metabolic regulation of NLRP3. 2018; 281:88–98.

124. He Y, Hara H, Núñez G. Mechanism and Regulation of NLRP3 Inflammasome Activation. Trends Biochem Sci 2016;41(12):1012–1021.

125. Place DE, Kanneganti TD. Recent advances in inflammasome biology. Curr Opin Immunol 2018;50:32–38.

126. Kim JJ, Jo EK. NLRP3 inflammasome and host protection against bacterial infection. J Korean Med Sci 2013;28(10):1415–1423.
127. Jo E-K, Kim JK, Shin D-M, Sasakawa C. Molecular mechanisms regulating NLRP3 inflammasome activation. Cell Mol Immunol 2016;13(2):148–159.

128. Shao BZ, Xu ZQ, Han BZ, Su DF, Liu C. NLRP3 inflammasome and its inhibitors: A review. Front Pharmacol 2015;6(NOV):1–9.

129. Zhou K, Shi L, Wang Y, Chen S, Zhang J. Recent Advances of the NLRP3 Inflammasome in Central Nervous System Disorders. J Immunol Res. 2016;2016:9238290. doi: 10.1155/2016/9238290.

130. Babelova A, Moreth K, Tsalastra-greul W, Zeng-brouwers J, Eickelberg O, Young MF, Bruckner P, Pfeilschifter J, Schaefer RM, Gro H. Biglycan , a Danger Signal That Activates the NLRP3 Inflammasome via Toll-like and P2X Receptors * □. 2009;284(36):24035–24048.

131. Chaly Y, Fu Y, Marinov A, Hostager B, Yan W, Campfield B. Follistatin-like protein 1 enhances NLRP3 inflammasome- mediated IL-1β secretion from monocytes and macrophages Yury. 2015;44(5):1467–1479.

132. Moon J, Lee S, Park M, Siempos II, Haslip M, Lee PJ, Yun M, Kim CK, Howrylak J, Ryter SW, et al. UCP2-induced fatty acid synthase promotes NLRP3 inflammasome activation during sepsis. 2015;125(2):665–680.

133. Zhang W, Xu X, Kao R, Mele T, Kvietys P, Martin CM. Cardiac Fibroblasts Contribute to Myocardial Dysfunction in Mice with Sepsis: The Role of NLRP3 Inflammasome Activation. 2014;9(9).

134. Rahim I, Djerdjouri B, Sayed RK, Fernández-Ortiz M, Fernández-Gil B, Hidalgo-Gutiérrez A, López LC, Escames G, Reiter RJ, Acuña-Castroviejo D. Melatonin administration to wild-type mice and nontreated NLRP3 mutant mice share similar inhibition of the inflammatory response during sepsis. J Pineal Res. 2017;63(1). doi: 10.1111/jpi.12410.

135. Zhang BO, Liu YUE, Sui Y, Cai H, Liu W, Zhu M, Yin X. Cortistatin Inhibits NLRP3 Inflammasome Activation of Cardiac Fibroblasts During Sepsis. J Card Fail 2015;21(5):426–433.

136. Wu Y, Ren J, Zhou B, Ding C, Chen J, Wang G, Gu G, Wu X, Liu S, Hu D, et al. Gene silencing of non-obese diabetic receptor family (NLRP3) protects against the sepsis-induced hyper-bile acidaemia in a rat model. 2014;2:277–293.

137. Chen YL, Xu G, Liang X, et al. Inhibition of hepatic cells pyroptosis attenuates CLP-induced acute liver injury. Am J Transl Res. 2016;8(12):5685-5695. Published 2016 Dec 15.

138. Zhao W, Zhang L, Sui M, Zhu Y, Zeng L. Protective effects of sirtuin 3 in a murine model of sepsis-induced acute kidney injury. Nat Publ Gr 2016;(August):1–11.

139. Luo Y, Jiang L, Kang K, Fei D, Meng X, Nan C, Pan S, Zhao M, Zhao M. International Immunopharmacology Hemin inhibits NLRP3 in flammasome activation in sepsis-induced acute lung injury, involving heme oxygenase-1. Int Immunopharmacol 2014;20(1):24–32.

140. Mao K, Chen S, Chen M, Ma Y, Wang Y, Huang B, He Z, Zeng Y, Hu Y, Sun S, et al. Nitric oxide suppresses NLRP3 inflammasome activation and protects against LPS-induced septic shock. 2013:201–212.

141. Ahn H, Kim J, Jeung E, Lee G. Immunobiology Dimethyl sulfoxide inhibits NLRP3 inflammasome activation. Immunobiology 2014;219(4):315–322.

142. Gustin A, Kirchmeyer M, Koncina E, Felten P, Losciuto S, Heurtaux T, Tardivel A, Heuschling P, Dostert C. NLRP3 inflammasome is expressed and functional in mouse brain microglia but not in astrocytes. PLoS One 2015;10(6):1–19.

143. Sbodio JI, Snyder SH, Paul BD. Redox Mechanisms in Neurodegeneration: From Disease Outcomes to Therapeutic Opportunities. Antioxid Redox Signal. 2019;30(11):1450-1499. doi:10.1089/ars.2017.7321

144. Dinarello CA. Historical insights into cytokines. Eur J Immunol. 2007;37 Suppl 1(Suppl 1):S34-S45. doi:10.1002/eji.200737772

145. McAfoose J, Baune BT. Evidence for a cytokine model of cognitive function. Neurosci Biobehav Rev. 2009;33(3):355-66. doi: 10.1016/j.neubiorev.2008.10.005.

146. Aricioğlu F, Yalcinkaya C, Ozkartal CS, et al. NLRP1-Mediated Antidepressant Effect of Ketamine in Chronic Unpredictable Mild Stress Model in Rats. Psychiatry Investig. 2020;17(4):283-291. doi:10.30773/pi.2019.0189

147. Lénárt N, Brough D, Dénes Á. Inflammasomes link vascular disease with neuroinflammation and brain disorders. J Cereb Blood Flow Metab 2016;36(10):1668–1685.

148. Fu Q, Wu J, Zhou XY, Ji MH, Mao QH, Li Q, Zong MM, Zhou ZQ, Yang JJ. NLRP3/Caspase-1 Pathway-Induced Pyroptosis Mediated Cognitive Deficits in a Mouse Model of Sepsis-Associated Encephalopathy. Inflammation 2019;42(1):306–318.

149. Petronilho F, Périco SR, Vuolo F, Mina F, Constantino L, Comim CM, Quevedo J, Souza DO, Dal-Pizzol F. Protective effects of guanosine against sepsis-induced damage in rat brain and cognitive impairment. Brain Behav Immun 2012;26(6):904–910.

150. Dempsey C, Rubio Araiz A, Bryson KJ, Finucane O, Larkin C, Mills EL, Robertson AAB, Cooper MA, O'Neill LAJ, Lynch MA. Inhibiting the NLRP3 inflammasome with MCC950 promotes non-phlogistic clearance of amyloid-β and cognitive function in APP/PS1 mice. Brain Behav Immun 2017;61:306–316.
151. Fink MP, Heard SO. Laboratory models of sepsis and septic shock. J Surg Res 1990;49(2):186–196.

152. Rittirsch D, Huber-Lang MS, Flierl MA, Ward PA. Immunodesign of experimental sepsis by cecal ligation and puncture. Nat Protoc 2009;4(1):31–36.

153. Buras JA, Holzmann B, Sitkovsky M. Model organisms: Animal models of sepsis: Setting the stage. Nat Rev Drug Discov 2005;4(10):854–865.

154. Olivieri R, Michels M, Pescador B, Ávila P, Abatti M, Cucker L, Burger H, Dominguini D, Quevedo J, Dal-Pizzol F. The additive effect of aging on sepsis-induced cognitive impairment and neuroinflammation. J Neuroimmunol 2018;314(November 2017):1–7.

155. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 1990;186:421–31.

156. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn BW, Shaltiel S, Stadtman ER. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 1990;186:464–78.

157. Bannister J V, Calabrese L. Assays for superoxide dismutase. Methods Biochem Anal 1987;32:279–312.

158. Aebi H. Catalase in vitro. Methods Enzym 1984;(105):121–6.

159. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. 1982:131–138.

160. Cassina A, Radi R. Differential inhibitory action of nitric oxide and peroxynitrite on mitochondrial electron transport. Arch Biochem Biophys 1996;328(2):309–316.

161. Fischer J, Ruitenbeek W, Ja B, Jm T, Jh V, Am S, Rc S. Differential investigation of the capacity of succinate oxidation in human skeletal muscle. Clin Chim Acta 1985;153(1):23–36.

162. Rustin P, Chretien D, Bourgeron T, Gerard B, Rotig A, Saudubray J, Minnich A. Biochemical and molecular investigations in respiratory chain. Clin Chim Acta 1994;228(1):35–51.

163. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein Measurement With The Folin Phenol Reagent. J Biol Chem 1951;193:265–275.

164. Zhu F, Liu Y, Zhao J, Zheng Y. Minocycline alleviates behavioral deficits and inhibits microglial activation induced by intrahippocampal administration of Granulocyte-Macrophage Colony-Stimulating Factor in adult rats. Neuroscience. 2014;266:275–81.

165. Imanishi T, Sawa A, Ichimaru Y, Miyashiro M, Kato S, Yamamoto T, et al. Ameliorating effects of rolipram on experimentally induced impairments of learning and memory in rodents. Eur J Pharmacol. 1997;321(3):273–8.

166. BRASIL. Ministério da Ciência, Tecnologia e Inovação. Conselho Nacional De Controle de Experimentação Animal – CONCEA. Diretriz Brasileira para o Cuidado e a utilização de animais para fins científicos e didáticos (DBCA). Brasília; 2013.

ANEXO

ANEXO A- Parecer Aprovação da Comissão de Ética no Uso de Animais

UNIVERSIDADE DO SUL DE SANTA CATARINA
COMISSÃO DE ÉTICA NO USO DE ANIMAIS – CEUA/UNISUL

Palhoça, 30 de novembro de 2016
Registro na CEUA (código): 16.032.5.01.IV

Ao Pesquisador/Professor(a): Fabrícia Cardoso Petronilho

Prezado(a),

Viemos por meio deste, certificar que a proposta de estudo e/ou projeto de pesquisa intitulada "Investigação do envolvimento do inflamassoma NLRP3 sobre alterações cerebrais precoces e tardias na sepse", registrada com o nº16.032.5.01.IV, sob a responsabilidade de Fabrícia Cardoso Petronilho - que envolve a manutenção ou utilização de modelos animais pertencentes ao filo *Chordata*, subfilo *Vertebrata* (exceto humanos), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da Lei Federal nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi **aprovado** pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) desta Instituição, em reunião de 13/12.

A CEUA/UNISUL tem por finalidade cumprir e fazer cumprir, no âmbito da UNISUL e nos limites de suas atribuições, os dispostos na legislação Federal aplicável à criação, manutenção e a utilização de animais em atividades de ensino e de pesquisa, realizadas pelos corpos docente, discente e técnico-administrativo da UNISUL e pesquisadores de outras instituições, caracterizando-se a sua atuação como educativa, consultiva, de assessoria e fiscalização nas questões relativas à matéria, sob os aspectos: I - Ético; II - Legal: enquadramento na legislação vigente.

Gostaríamos de salientar que, embora aprovado, qualquer alteração dos procedimentos e metodologias que houver durante a realização do projeto em questão, deverá ser informada imediatamente à Comissão.

Atenciosamente,

Prof. Sandro Melim Sgrott
Coordenador da Comissão

Campus Florianópolis - Unidade Pedra Branca - Avenida Pedra Branca, 25 - Cidade Universitária Pedra Branca - CEP 88137-270 - Palhoça - SC - Fone 55-48-3279-1036 - ceua@unisul.br

ANEXO B – Produção científica publicada durante o período do Doutorado

Inflammation (© 2017)
DOI: 10.1007/s10753-017-0689-z

ORIGINAL ARTICLE

Dimethyl Fumarate Modulates Oxidative Stress and Inflammation in Organs After Sepsis in Rats

Amanda Della Giustina,[1] Sandra Bonfante,[1] Graciela Freitas Zarbato,[1] Lucinéia Gainski Danielski,[1] Khiany Mathias,[1] Aloir Neri de OliveiraJr,[1] Leandro Garbossa,[1] Taise Cardoso,[1] Maria Eduarda Fileti,[1] Raquel Jaconi De Carli,[1] Mariana Pereira Goldim,[1] Tatiana Barichello,[2,3] and Fabricia Petronilho[1,4,5]

Abstract— Sepsis is defined as life-threatening organ dysfunction induced by a disrupted host response to infecting pathogens. Evidences suggest that oxidative stress is intrinsically related to sepsis progression. Dimethyl fumarate (DMF) is a novel oral therapeutic agent with anti-oxidant properties which exerts protective effects through activation of nuclear factor erythroid 2 (NFE2)-related factor 2 (Nrf2). Thus, the aim of this study is to evaluate the effect of DMF in different organs of rats submitted to an animal model of sepsis. Adult male Wistar rats were subjected to sepsis by cecal ligation and puncture (CLP) procedure and sham-operated rats was considered control group. The experimental groups were divided into sham + vehicle, sham + DMF, sham + NAC, CLP + vehicle, CLP + DMF, and CLP + NAC. Rats were treated by oral gavage with DMF immediately after and 12 h after surgery, or NAC (s.c.) at 3, 6, and 12 h after surgery. Twenty-four hours after sepsis induction, neutrophil infiltration, nitrite/nitrate concentrations, oxidative damage to lipids and proteins, superoxide dismutase (SOD), and catalase (CAT) activities were evaluated in the heart, liver, lung, and kidney. Septic animals presented increased neutrophil infiltration, NO metabolism, oxidative damage to lipids and proteins, and decreases of SOD and CAT activities, mainly in the heart, liver, and lung, while DMF-treated animals showed significant reduction in neutrophil infiltration, NO metabolism, and oxidative damage followed by increased SOD and CAT activities. DMF is effective in preventing oxidative stress and inflammation in rats 24 h after sepsis induction.

KEY WORDS: sepsis; oxidative stress; inflammation; organ injury.

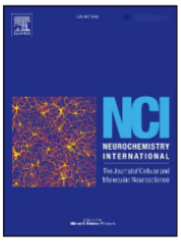
[1] Laboratory of Neurobiology of Inflammatory and Metabolic Processes, Graduate Program in Health Sciences, University of South Santa Catarina, Tubarao, SC, Brazil
[2] Laboratory of Neurosciences, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina (UNESC), Criciúma, SC, Brazil
[3] Department of Psychiatry and Behavioral Sciences, McGovern Medical School, The University of Texas Health Science Center at Houston (UTHealth), Houston, TX, USA
[4] Laboratório de Neurobiologia de Processos Inflamatórios e Metabólicos, Programa de Pós-graduação em Ciências da Saúde, Universidade do Sul de Santa Catarina, Tubarão, SC, Brazil
[5] To whom correspondence should be addressed at Laboratório de Neurobiologia de Processos Inflamatórios e Metabólicos, Programa de Pós-graduação em Ciências da Saúde, Universidade do Sul de Santa Catarina, Tubarão, SC, Brazil. E-mail: fabricia.petronilho@unisul.br

INTRODUCTION

Sepsis is currently defined as life-threatening organ dysfunction induced by a disrupted host response to infecting pathogens [1]. Despite improvements in therapeutic approaches and intensive care, the incidence and mortality rates of sepsis continue to increase [2] and this disease is considered a major health issue in the USA [3]. Also, sepsis is the most common cause of death in non-coronary intensive care units (ICU) patients [4]. Septic patients usually develop multiple organ failures that may evolve to death if the sepsis origin

Published online: 09 November 2017

Accepted Manuscript



Alpha-lipoic acid attenuates acute neuroinflammation and long-term cognitive impairment after polymicrobial sepsis

Amanda Della Giustina, Mariana Pereira Goldim, Lucinéia Gainski Danielski, Drielly Florentino, Khiany Mathias, Leandro Garbossa, Aloir Neri Oliveira Junior, Maria Eduarda Fileti, Graciela Freitas Zarbato, Naiana da Rosa, Ana Olívia Martins Laurentino, Jucélia Jeremias Fortunato, Francielle Mina, Tatiani Bellettini-Santos, Josiane Budni, Tatiana Barichello, Felipe Dal-Pizzol, Fabricia Petronilho

PII: S0197-0186(17)30121-3

DOI: 10.1016/j.neuint.2017.06.003

Reference: NCI 4090

To appear in: *Neurochemistry International*

Received Date: 23 February 2017

Revised Date: 31 May 2017

Accepted Date: 7 June 2017

Please cite this article as: Della Giustina, A., Goldim, M.P., Danielski, Luciné.Gainski., Florentino, D., Mathias, K., Garbossa, L., Oliveira Junior, A.N., Fileti, M.E., Zarbato, G.F., da Rosa, N., Martins Laurentino, Ana.Olí., Fortunato, Jucé.Jeremias., Mina, F., Bellettini-Santos, T., Budni, J., Barichello, T., Dal-Pizzol, F., Petronilho, F., Alpha-lipoic acid attenuates acute neuroinflammation and long-term cognitive impairment after polymicrobial sepsis, *Neurochemistry International* (2017), doi: 10.1016/j.neuint.2017.06.003.

Accepted Manuscript

Title: Mechanism of synergistic action on behavior, oxidative stress and inflammation following co-treatment with ketamine and different antidepressant classes

Authors: Gislaine Z. Réus, Beatriz I. Matias, Amanda L. Maciel, Helena M. Abelaira, Zuleide M. Ignácio, Airam B. de Moura, Danyela Matos, Lucineia G. Danielski, Fabricia Petronilho, André F. Carvalho, João Quevedo

PII: S1734-1140(16)30459-5
DOI: http://dx.doi.org/doi:10.1016/j.pharep.2017.04.021
Reference: PHAREP 717

To appear in:

Received date: 17-12-2016
Accepted date: 28-4-2017

Please cite this article as: {http://dx.doi.org/

LC/Q-TOF profile and preliminary stability studies of an enriched flavonoid fraction of *Cecropia pachystachya* Trécul leaves with potential antidepressant-like activity

Caroline F. Ortmann[#a], Helena M. Abelaira[#b], Gislaine Z. Réus[b#], Zuleide M. Ignácio[b], Vitor Clasen Chaves[a], Talitha Caldas dos Santos[a], Pâmela de Carvalho[a], Anelise S. Carlessi[b], Livia Bruchchen[b], Lucineia G. Danielski[c], Simone Gonçalves Cardoso[a], Angela M. de Campos[a], Fabricia Petronilho[c], Joyce Rebelo[e], Meline O. S. Morais[e], Francieli Vuolo[f], Felipe Dal-Pizzol[f], Emilio L. Streck[e], João Quevedo[b,d], and Flávio H. Reginatto*[a]

[a] Programa de Pós-graduação em Farmácia, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil;
[b] Laboratório de Neurociências, Programa de Pós-Graduação em Ciências da Saúde, Unidade Acadêmica de Ciências da Saúde, Universidade do Extremo Sul Catarinense, Criciúma, SC, Brazil;
[c] Laboratório de Fisiopatologia Clínica e Experimental, Programa de Pós-graduação em Ciências da Saúde, Universidade do Sul de Santa Catarina, Tubarão, SC, Brazil;
[d] Center for Translational Psychiatry, Department of Psychiatry and Behavioral Sciences, The University of Texas Medical School at Houston, Houston, TX, USA;
[e] Laboratório de Bioenergética, Programa de Pós-Graduação em Ciências da Saúde, Unidade Acadêmica de Ciências da Saúde, Universidade do Extremo Sul Catarinense, Criciúma, SC, Brazil;
[f] Laboratório de Fisiopatologia Experimental, Programa de Pós-Graduação em Ciências da Saúde, Unidade Acadêmica de Ciências da Saúde, Universidade do Extremo Sul Catarinense, Criciúma, SC, Brazil.

[#r]**These authors equally contributed to this work.**

***Corresponding author:**
Flávio Henrique Reginatto
Laboratório de Farmacognosia,
Universidade Federal de Santa Catarina
Florianópolis, SC 88040-900, Brazil.
Phone: +55 48 3721-3499
E-mail: flavio.reginatto@ufsc.br

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/bmc.3982

Mol Neurobiol
DOI 10.1007/s12035-016-0071-4

Ketamine Exhibits Different Neuroanatomical Profile After Mammalian Target of Rapamycin Inhibition in the Prefrontal Cortex: the Role of Inflammation and Oxidative Stress

Helena M. Abelaira[1] · Gislaine Z. Réus[1] · Zuleide M. Ignácio[1] · Maria Augusta B. dos Santos[1] · Airam B. de Moura[1] · Danyela Matos[1] · Júlia P. Demo[1] · Júlia B. I. da Silva[1] · Lucineia G. Danielski[2] · Fabricia Petronilho[2] · André F. Carvalho[3] · João Quevedo[1,4,5,6]

Received: 25 March 2016 / Accepted: 18 August 2016

Abstract Studies indicated that mammalian target of rapamycin (mTOR), oxidative stress, and inflammation are involved in the pathophysiology of major depressive disorder (MDD). Ketamine, an *N*-methyl-D-aspartate (NMDA) receptor antagonist, has been identified as a novel MDD therapy; however, the antidepressant mechanism is not fully understood. In addition, the effects of ketamine after mTOR inhibition have not been fully investigated. In the present study, we examined the behavioral and biochemical effects of ketamine in the prefrontal cortex (PFC), hippocampus, amygdala, and nucleus accumbens after inhibition of mTOR signaling in the PFC. Male adult Wistar rats received pharmacological mTOR inhibitor, rapamycin (0.2 nmol) or vehicle into the PFC and then a single dose of ketamine (15 mg/kg, i.p.). Immobility was assessed in forced swimming tests, and then oxidative stress parameters and inflammatory markers were evaluated in the brain and periphery. mTOR activation in the PFC was essential to ketamine's antidepressant-like effects. Ketamine increased lipid damage in the PFC, hippocampus, and amygdala. Protein carbonyl was elevated in the PFC, amygdala, and NAc after ketamine administration. Ketamine also increased nitrite/nitrate in the PFC, hippocampus, amygdala, and NAc. Myeloperoxidase activity increased in the hippocampus and NAc after ketamine administration. The activities of superoxide dismutase and catalase were reduced after ketamine administration in all brain areas studied. Inhibition of mTOR signaling pathways by rapamycin in the PFC was required to protect against oxidative stress by reducing damage and increasing antioxidant enzymes. Finally, the TNF-α level was increased in serum by ketamine; however, the rapamycin plus treatment group was not able to block this increase. Activation of mTOR in the PFC is involved in the antidepressant-like effects of ketamine; however, the inhibition of this pathway was able to protect certain brain areas against oxidative stress, without affecting inflammation parameters.

Keywords mTOR · Oxidative stress · Inflammation · Ketamine · Animal model · Major depressive disorder

✉ Gislaine Z. Réus
gislainezilli@hotmail.com

[1] Laboratory of Neurosciences, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina, Criciuma, SC 88806-000, Brazil

[2] Laboratory of Clinical and Experimental Pathophysiology, Postgraduate Program in Health Sciences, University of Southern Santa Catarina (UNISUL), Tubarão, SC, Brazil

[3] Department of Clinical Medicine and Translational Psychiatry Research Group, Faculty of Medicine, Federal University of Ceará, Fortaleza, CE, Brazil

[4] Center for Translational Psychiatry, Department of Psychiatry and Behavioral Sciences, Medical School, The University of Texas Health Science Center at Houston, Houston, TX, USA

[5] Center of Excellence on Mood Disorders, Department of Psychiatry and Behavioral Sciences, Medical School, The University of Texas Health Science Center at Houston, Houston, TX, USA

[6] Neuroscience Graduate Program, Graduate School of Biomedical Sciences, The University of Texas Health Science Center at Houston, Houston, TX, USA

Introduction

Major depressive disorder (MDD) is a significant public health threat, accounting for 65.5 million disability-adjusted

Published online: 02 September 2016

Neurotoxicity Research
https://doi.org/10.1007/s12640-018-9900-8

ORIGINAL ARTICLE

Dimethyl Fumarate Limits Neuroinflammation and Oxidative Stress and Improves Cognitive Impairment After Polymicrobial Sepsis

Graciela Freitas Zarbato[1] · Mariana Pereira de Souza Goldim[1] · Amanda Della Giustina[1] · Lucinéia Gainski Danielski[1] · Khiany Mathias[1] · Drielly Florentino[1] · Aloir Neri de Oliveira Junior[1] · Naiana da Rosa[1] · Ana Olivia Laurentino[1] · Taina Trombetta[1] · Maria Luiza Gomes[2] · Amanda Valnier Steckert[3] · Ana Paula Moreira[3] · Patricia Fernanda Schuck[2] · Jucelia Jeremias Fortunato[1] · Tatiana Barichello[3,4] · Fabricia Petronilho[1]

Received: 23 October 2017 / Revised: 2 April 2018 / Accepted: 6 April 2018

Abstract
Sepsis is caused by a dysregulated host response to infection, often associated with acute central nervous system (CNS) dysfunction, which results in long-term cognitive impairment. Dimethyl fumarate (DMF) is an important agent against inflammatory response and reactive species in CNS disorders. Evaluate the effect of DMF on acute and long-term brain dysfunction after experimental sepsis in rats. Male Wistar rats were submitted to the cecal ligation and puncture (CLP) model. The groups were divided into sham (control) + vehicle, sham + NAC, sham + DMF, CLP + vehicle, CLP + NAC, and CLP + DMF. The animals were treated with DMF (15 mg/kg at 0 and 12 h after CLP, per gavage) and the administration of *n*-acetylcysteine (NAC) (20 mg/kg; 3, 6, and 12 h after CLP, subcutaneously) was used as positive control. Twenty-four hours after CLP, cytokines, myeloperoxidase (MPO), nitrite/nitrate (N/N), oxidative damage to lipids and proteins, and antioxidant enzymes were evaluated in the hippocampus, total cortex, and prefrontal cortex. At 10 days after sepsis induction, behavioral tests were performed to assess cognitive damage. We observed an increase in cytokine levels, MPO activity, N/N concentration, and oxidative damage, a reduction in SOD and GPx activity in the brain structures, and cognitive damage in CLP rats. DMF treatment was effective in reversing these parameters. DMF reduces sepsis-induced neuroinflammation, oxidative stress, and cognitive impairment in rats subjected to the CLP model.

Keywords Sepsis · Neuroinflammation · Cognitive impairment · Oxidative stress · Dimethyl fumarate

Introduction

Sepsis is the most frequent cause of death in intensive care units (ICU) (Mayr et al. 2014), and it is determined by a systemic inflammatory response associated with an infection (Singer et al. 2016). The central nervous system (CNS) is rapidly damaged during sepsis, causing sepsis-associated encephalopathy (SAE), which is clinically characterized by disorientation, *delirium*, or coma (Wassmer et al. 2006). Also, long-term memory disturbance and impaired learning ability frequently affect sepsis survivors (Iwashyna et al. 2010).

The pathophysiological mechanisms associated with SAE development comprise the production of proinflammatory mediators, oxidative stress, and mitochondrial dysfunction (Dal-Pizzol et al. 2014). Proinflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6, activate target cells and stimulate the production of other cytokines, chemokines, reactive oxygen species (ROS), reactive nitrogen species (RNS), eicosanoids, and proteolytic enzymes (Doyle and O'Neill 2006). In fact, the production of inflammatory mediators is exacerbated in sepsis, leading to microcirculation dysfunction, tissue damage, and multiple organ failure (Comim et al. 2011b).

✉ Fabricia Petronilho
fabricia.petronilho@unisul.br

[1] Laboratory of Neurobiology of Inflammatory and Metabolic Processes, Graduate Program in Health Sciences, Health Sciences Unit, University of South Santa Catarina, Tubarao, SC, Brazil

[2] Laboratory Inborn Errors of Metabolism, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina (UNESC), Criciuma, SC, Brazil

[3] Laboratory of Neurosciences, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina (UNESC), Criciuma, SC, Brazil

[4] Department of Psychiatry and Behavioral Sciences, McGovern Medical School, The University of Texas Health Science Center at Houston (UTHealth), Houston, TX, USA

Published online: 30 April 2018

Mol Neurobiol
DOI 10.1007/s12035-017-0706-0

Vitamin B_6 Reduces Neurochemical and Long-Term Cognitive Alterations After Polymicrobial Sepsis: Involvement of the Kynurenine Pathway Modulation

Lucinéia Gainski Danielski[1] · Amanda Della Giustina[1] · Mariana Pereira Goldim[1] · Drielly Florentino[1] · Khiany Mathias[1] · Leandro Garbossa[1] · Rosiane de Bona Schraiber[1] · Ana Olívia Martins Laurentino[1] · Marina Goulart[1] · Monique Michels[2] · Karina Barbosa de Queiroz[3] · Markus Kohlhof[3] · Gislaine Tezza Rezin[1] · Jucélia Jeremias Fortunato[1] · Joao Quevedo[4,5,6,7] · Tatiana Barichello[4,5,6,7] · Felipe Dal-Pizzol[2] · Roney S. Coimbra[3] · Fabricia Petronilho[1]

Received: 7 May 2017 / Accepted: 1 August 2017

Abstract Neurological dysfunction as a result of neuroinflammation has been reported in sepsis and cause high mortality. High levels of cytokines stimulate the formation of neurotoxic metabolites by kynurenine (KYN) pathway. Vitamin B_6 (vit B_6) has anti-inflammatory and antioxidant properties and also acts as a cofactor for enzymes of the KYN pathway. Thus, by using a relevant animal model of polymicrobial sepsis, we studied the effect of vit B_6 on the KYN pathway, acute neurochemical and neuroinflammatory parameters, and cognitive dysfunction in rats. Male Wistar rats (250–300 g) were submitted to cecal ligation and perforation (CLP) and divided into sham + saline, sham + vit B_6, CLP + saline, and CLP + vit B_6 (600 mg/kg, s.c.) groups. Twenty-four hours later, the prefrontal cortex and hippocampus were removed for neurochemical and neuroinflammatory analyses. Animals were followed for 10 days to determine survival rate, when cognitive function was assessed by behavioral tests. Vitamin B_6 interfered in the activation of kynurenine pathway, which led to an improvement in neurochemical and neuroinflammatory parameters and, consequently, in the cognitive functions of septic animals. Thus, the results indicate that vit B_6 exerts neuroprotective effects in acute and late consequences after sepsis.

Keywords Sepsis · Vitamin B_6 · Brain damage · Tryptophan · Oxidative stress · Neuroinflammation

Introduction

Sepsis is a common clinical syndrome in intensive care units (ICUs), with mortality rates that reach 60% [1]. The development of organ dysfunction is a complication that

✉ Fabricia Petronilho
fabricia.petronilho@unisul.br

[1] Laboratory of Neurobiology of Inflammatory and Metabolic Processes, Graduate Program in Health Sciences, Health Sciences Unit, University of South Santa Catarina, Tubarão, SC, Brazil

[2] Laboratory of Experimental Pathophysiology, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina, Criciúma, SC, Brazil

[3] Laboratory of Neurogenomics, Research Center René Rachou/CPqRR, Oswaldo Cruz Foundation, Minas Gerais, Belo Horizonte, MG, Brazil

[4] Translational Psychiatry Program, Department of Psychiatry and Behavioral Sciences, The University of Texas Health Science Center at Houston, McGovern Medical School, Houston, TX, USA

[5] Center of Excellence on Mood Disorders, Department of Psychiatry and Behavioral Sciences, The University of Texas Health Science Center at Houston, McGovern Medical School, Houston, TX, USA

[6] Neuroscience Graduate Program, The University of Texas Graduate School of Biomedical Sciences at Houston, Houston, TX, USA

[7] Laboratory of Neurosciences, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina, Criciúma, SC, Brazil

Published online: 06 September 2017

Mol Neurobiol
DOI 10.1007/s12035-016-0356-7

REVIEW

Brain Barrier Breakdown as a Cause and Consequence of Neuroinflammation in Sepsis

Lucineia Gainski Danielski[1] · Amanda Della Giustina[1] · Marwa Badawy[2] · Tatiana Barichello[2,3,4,5] · João Quevedo[2,3,4,6] · Felipe Dal-Pizzol[7] · Fabrícia Petronilho[1]

Received: 30 August 2016 / Accepted: 22 December 2016

Abstract The blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB) are important for the maintenance of brain homeostasis**.** During sepsis, peripheral production of proinflammatory cytokines and reactive oxygen species are responsible for structural alterations in those brain barriers. Thus, an increasing permeability of these barriers can lead to the activation of glial cells such as microglia and the production of cytotoxic mediators which in turn act on the brain barriers, damaging them further. Thereby, in this review, we try to highlight how the brain barrier's permeability is not only a cause but a consequence of brain injury in sepsis.

Keywords Sepsis · Blood brain barrier · Blood-cerebrospinal fluid barrier · Neuroinflammation · Microglial activation

✉ Fabrícia Petronilho
fabricia.petronilho@unisul.br

[1] Laboratório de Neurobiologia de Processos Inflamatórios e Metabólicos, Programa de Pós-Graduação em Ciências da Saúde, Universidade do Sul de Santa Catarina, Tubarão, SC, Brazil

[2] Translational Psychiatry Program, Department of Psychiatry and Behavioral Sciences, McGovern Medical School, The University of Texas Health Science Center at Houston (UTHealth), Houston, TX, USA

[3] Center of Excellence on Mood Disorders, Department of Psychiatry and Behavioral Sciences, McGovern Medical School, The University of Texas Health Science Center at Houston (UTHealth), Houston, TX, USA

[4] Neuroscience Graduate Program, The University of Texas MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences, Houston, TX, USA

[5] Laboratory of Experimental Microbiology, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina (UNESC), Criciúma, SC, Brazil

[6] Laboratory of Neurosciences, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina (UNESC), Criciúma, SC, Brazil

[7] Laboratory of Experimental Pathophysiology, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina (UNESC), Criciúma, SC, Brazil

Introduction

Sepsis and its complications are among the most common causes of mortality in intensive care units (ICUs) reaching 50–60% rates regardless of treatment used [1]. Despite considerable advances in the treatment of sepsis; it not only remains a major cause of mortality but also produces harmful neurological responses which further aggravate this condition [2]. Given the cognitive sequelae in sepsis survivors, further attention needs to be directed toward an understanding of the neurological dysfunction in sepsis, both in the acute phase of encephalopathy and in its chronic stage [3].

The exact mechanisms involved in neurological damage in patients with sepsis are still not well understood but may include neuroinflammation [4–6]. In response to systemic inflammation, inflammatory mediators have direct access to the brain through the circumventricular organs, as well as by disrupted otherwise intact brain barriers and allow penetration of various mediators and potential neurotoxic factors into the brain [7]. In turn, as response to these mediators and neurotoxic factors, microglial cells, which are the resident immune cells of the brain, are activated. When activated, microglial cells may negatively affect the brain allowing the production of nitric oxide, cytokines, and reactive oxygen species that lead to cell death within vulnerable areas of the brain such as the hippocampus and the prefrontal cortex. This production is in itself responsible for a further increase of the brain barrier

Published online: 14 January 2017

Inflammation (© 2019)
DOI: 10.1007/s10753-019-01124-9

REVIEW

The NLRP3 Inflammasome and Its Role in Sepsis Development

Lucinéia Gainski Danielski,[1] Amanda Della Giustina,[1] Sandra Bonfante,[1] Tatiana Barichello,[2,3] and Fabricia Petronilho[1,4]

Abstract— The pathophysiology of sepsis is extremely complex. During this disease, the exacerbation of the inflammatory response causes oxidative stress, alterations in mitochondrial energy dynamics, and multiple organ failure. Some studies have highlighted the important role of the NLRP3 inflammasome in sepsis. This inflammasome is a macromolecular protein complex that finely regulates the activation of caspase-1 and the production and secretion of potent pro-inflammatory cytokines such as IL-1β and IL-18. In this review, we elucidate evidences to understand the connection between sepsis development and the NLRP3 inflammasome, the most widely investigated member of this class of receptor.

KEY WORDS: sepsis; NLRP3; inflammasome; organ failure.

INTRODUCTION

Sepsis is a syndrome characterized by pathophysiologic and biochemical alterations caused by an infectious insult. These abnormalities are related to an exacerbated systemic inflammation, and they manifest as life-threatening clinical conditions [1] that may lead to multiple organ dysfunction [2] and a mortality rate of approximately 60% [3].

The initial stage of sepsis pathophysiology involves the production of signaling molecules responsible for recruiting immune cells, such as the pathogen-associated molecular patterns (PAMPs) and the damage-associated molecular patterns (DAMPs) and their interaction with germline-encoded receptors called pattern recognition receptors (PRRs) [4]. The involvement of Toll-like receptor-4 (TLR-4) in sepsis is well described [5–7]; however, another class of receptor has drawn attention in recent years: the nucleotide-binding and oligomerization domain (NOD)-like receptor (NLR) class, specifically the pyrin domain-containing 3 (NLRP3) member. NLRP3, along with other structures, generate a complex called inflammasome that can convert pro-inflammatory procaspases into their mature form, thus inducing the release of important pro-inflammatory cytokines [8, 9].

The NLRP3/caspase-1/IL-1 axis has emerged as a critical signaling pathway of the innate immune system

[1] Laboratory of Neurobiology of Inflammatory and Metabolic Processes, Graduate Program in Health Sciences, Health Sciences Unit, University of South Santa Catarina, Tubarão, SC, Brazil
[2] Laboratory of Experimental Microbiology, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina, Criciúma, SC, Brazil
[3] Center for Experimental Models in Psychiatry, Department of Psychiatry and Behavioral Sciences, Medical School, The University of Texas Health Science Center at Houston, Houston, TX, USA
[4] To whom correspondence should be addressed at Laboratory of Neurobiology of Inflammatory and Metabolic Processes, Graduate Program in Health Sciences, Health Sciences Unit, University of South Santa Catarina, Tubarão, SC, Brazil. E-mail: fabricia.petronilho@unisul.br

Published online: 18 November 2019

Microvascular Research 123 (2019) 19–24

Contents lists available at ScienceDirect

Microvascular Research

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

Oxidative stress in the choroid plexus contributes to blood–cerebrospinal fluid barrier disruption during sepsis development

Mariana Pereira Goldim[a], Lucineia Gainski Danielski[a], Judite Filgueiras Rodrigues[a], Larissa Joaquim[a], Leandro Garbossa[a], Aloir Neri de Oliveira Junior[a], Kiuanne Lino Lobo Metzker[a], Amanda Della Giustina[a], Taise Cardoso[a], Tatiana Barichello[b,c], Fabricia Petronilho[a,*]

[a] Laboratory of Neurobiology of Inflammatory and Metabolic Processes, Graduate Program in Health Sciences, Health Sciences Unit, University of South Santa Catarina, Tubarão, SC, Brazil
[b] Center for Experimental Models in Psychiatry, Department of Psychiatry and Behavioral Sciences, The University of Texas Medical School at Houston, Houston, TX, USA
[c] Laboratory of Experimental Microbiology, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina, Criciúma, SC, Brazil

ARTICLE INFO

Keywords:
Sepsis
Choroid plexus
Oxidative stress
Neuroinflammation

ABSTRACT

Background: The choroid plexus (CP), main component of blood–cerebrospinal fluid barrier (BCSFB), protects the brain from peripheral inflammation similar to the blood–brain barrier. Thus, CP is considered a critical target site of oxidative damage, which in sepsis oxidative stress is likely to be a major step in the development of brain damage. Functional alterations in CP may be associated with sepsis-induced brain injury. However, there is no description on the mechanisms associated with BCSFB disruption during sepsis development.
Materials and methods: To test this hypothesis, we examined time-dependent oxidative stress markers in CP and permeability of BCSFB in rats submitted to polymicrobial sepsis by cecal ligation and puncture (CLP) or sham surgery (control). We assessed albumin cerebrospinal fluid/plasma concentration quotient (Qalb), an index of BCSFB dysfunction and in CP samples, the oxidative damage in lipids, proteins, antioxidant enzymes and nitrite/nitrate (N/N) concentration in 12, 24 and 48 h after CLP.
Results: The increase of BCSFB permeability is time-related to the increase of N/N concentration, oxidative damage to lipid and proteins, and decrease of antioxidant enzyme superoxide dismutase activity at 12 h in the CP; and decrease of catalase activity in 12 and 24 h.
Conclusions: In experimental sepsis the BCSFB dysfunction occurs and oxidative stress seems to be a major step in this dysfunction.

1. Introduction

Sepsis associated encephalopathy (SAE) is characterized with delirium, coma, and seizure, being a cause of cognitive dysfunction, morbidity, and mortality in critical illness. Evidences showed that pro-inflammatory cytokines play an important role in its development and in the subsequent production of reactive oxygen species (ROS) that further aggravate this dysfunction (Balusu et al., 2016a). These mediators are involved with blood brain barrier (BBB) alterations, with subsequent increased permeability and cerebral edema, which recent studies shows that are an important step in development of SAE (Balusu et al., 2016b; Kaur et al., 2016). While most studies focus on the BBB and the development of SAE (Balusu et al., 2016a; Ericsson et al., 1995; Marques et al., 2007), few studies address the choroid plexus (CP) participation in inflammatory brain processes.

The CP is an epithelial cell monolayer located along the lateral, third, and fourth ventricles of the brain. CP forms an interface between peripheral blood and cerebrospinal fluid (CSF) and is the main component of the blood–CSF barrier (BCSFB) (Balusu et al., 2016b). Generally, CP is considered to be the route of migration of endogenous and exogenous compounds into and out of the brain in addition to the BBB (Kaur et al., 2016).

Several lines of evidence have implicated the role of CP as an important mediator of acute peripheral inflammation into the central nervous system (CNS) (Balusu et al., 2016a; Marques et al., 2007; Kaur et al., 2016). It has been demonstrated that the structure constitutively

* Corresponding author at: Laboratório de Neurobiologia de Processos Inflamatórios e Metabólicos, Programa de Pós-Graduação em Ciências da Saúde, Universidade do Sul de Santa Catarina, Tubarão, SC, Brazil.
E-mail address: fabricia.petronilho@unisul.br (F. Petronilho).

https://doi.org/10.1016/j.mvr.2018.12.001
Received 3 September 2018; Received in revised form 7 November 2018; Accepted 12 December 2018
Available online 13 December 2018
0026-2862/

brain sciences

MDPI

Article

Manual Therapy Reduces Pain Behavior and Oxidative Stress in a Murine Model of Complex Regional Pain Syndrome Type I

Afonso S. I. Salgado [1,2], Juliana Stramosk [2], Daniela D. Ludtke [2,3], Ana C. C. Kuci [2], Daiana C. Salm [2,3], Lisandro A. Ceci [2,3], Fabricia Petronilho [4], Drielly Florentino [4], Lucineia G. Danielski [4], Aline Gassenferth [5], Luana R. Souza [6], Gislaine T. Rezin [7], Adair R. S. Santos [5,6], Leidiane Mazzardo-Martins [6], William R. Reed [7] and Daniel F. Martins [1,2,*]

1 Coordinator of Integrative Physical Therapy Residency–Philadelphia University Center, Londrina 86020-000, Paraná, Brazil
2 Experimental Neuroscience Laboratory (LaNEx), University of Southern Santa Catarina, Palhoça 88137-270, Santa Catarina, Brazil
3 Postgraduate Program in Health Sciences, University of Southern Santa Catarina, Palhoça 88137-270, Santa Catarina, Brazil
4 Laboratory of Neurobiology of Inflammatory and Metabolic Processes, Graduate Program in Health Sciences, Health Sciences Unit, University of South Santa Catarina, Tubarão 88704-900, Santa Catarina, Brazil
5 Laboratory of Neurobiology of Pain and Inflammation, Department of Physiological Sciences, Centre of Biological Sciences, University Federal of Santa Catarina, Florianópolis 88040-900, Santa Catarina, Brazil
6 Postgraduate Program in Neuroscience, Center of Biological Sciences, Federal University of Santa Catarina, Florianópolis 88040-900, Santa Catarina, Brazil
7 Department of Physical Therapy, School of Health Professions, University of Alabama at Birmingham, Birmingham, AL 35294-1212, USA
* Correspondence: daniel.martins4@unisul.br; Tel.: +55-(48)-3279-1144

Received: 2 July 2019; Accepted: 8 August 2019; Published: 10 August 2019

check for updates

Abstract: Complex regional pain syndrome type I (CRPS-I) is a chronic painful condition. We investigated whether manual therapy (MT), in a chronic post-ischemia pain (CPIP) model, is capable of reducing pain behavior and oxidative stress. Male Swiss mice were subjected to ischemia-reperfusion (IR) to mimic CRPS-I. Animals received ankle joint mobilization 48h after the IR procedure, and response to mechanical stimuli was evaluated. For biochemical analyses, mitochondrial function as well as oxidative stress thiobarbituric acid reactive substances (TBARS), protein carbonyls, antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) levels were determined. IR induced mechanical hyperalgesia which was subsequently reduced by acute MT treatment. The concentrations of oxidative stress parameters were increased following IR with MT treatment preventing these increases in malondialdehyde (MDA) and carbonyls protein. IR diminished the levels of SOD and CAT activity and MT treatment prevented this decrease in CAT but not in SOD activity. IR also diminished mitochondrial complex activity, and MT treatment was ineffective in preventing this decrease. In conclusion, repeated sessions of MT resulted in antihyperalgesic effects mediated, at least partially, through the prevention of an increase of MDA and protein carbonyls levels and an improvement in the antioxidant defense system.

Keywords: chronic pain; Complex Regional Pain Syndrome; manual therapy; osteopathy; oxidative stress

Anais da Academia Brasileira de Ciências (2019) 91(4): e20190434
(Annals of the Brazilian Academy of Sciences)
Printed version ISSN 0001-3765 / Online version ISSN 1678-2690
http://dx.doi.org/10.1590/0001-3765201920190434
www.scielo.br/aabc | www.fb.com/aabcjournal

Vitamin B6 reduces oxidative stress in lungs and liver in experimental sepsis

AMANDA D. GIUSTINA, LUCINÉIA G. DANIELSKI, MICHELE M. NOVOCHADLO, MARIANA P.S. GOLDIM, LARISSA JOAQUIM, KIUANNE L.L. METZKER, RAQUEL JACONI DE CARLI, TAIS DENICOL, THAINA CIDREIRA, THAYNAN VIEIRA and FABRICIA PETRONILHO

Laboratory of Neurobiology of Inflammatory and Metabolic Processes, Graduate Program in Health Sciences, Health Sciences Unit, University of South Santa Catarina, 88704-900 Tubarão, SC, Brazil

Manuscript received on April 24, 2019; accepted for publication on August 2, 2019

How to cite: GIUSTINA AD, DANIELSKI LG, NOVOCHADLO MM, GOLDIM MPS, JOAQUIM L, METZKER KLL, CARLI RJ, DENICOL T, CIDREIRA T, VIEIRA T AND PETRONILHO F. 2019. Vitamin B6 reduces oxidative stress in lungs and liver in experimental sepsis. An Acad Bras Cienc 91: e20190434. DOI 10.1590/0001-3765201920190434.

Abstract: Sepsis is a life-threatening organ dysfunction induced by a disrupted host response to infecting pathogens. Inflammation and oxidative stress are intrinsically related to sepsis progression and organ failure. Vitamin B6 is an important cellular cofactor for metabolic processes and has anti-inflammatory and antioxidant properties. We aimed at evaluating the effect of vit B6 on inflammation and oxidative stress markers in the liver and lung of rats subjected to a relevant animal model of polymicrobial sepsis. Adult male *Wistar* rats were submitted to cecal ligation and perforation model and immediately after sepsis induction, vit B6 was administered as a single dose (600 mg/kg, subcutaneous). Twenty-four hours later, the lung and liver were harvest for neutrophil infiltration, oxidative markers to lipids and protein and antioxidant activity of endogenous enzyme. Vitamin B6 diminished neutrophil infiltration in both organs, oxidative markers in the liver and restored catalase activity levels in the lung of septic animals. Vitamin B6 exerts anti-inflammatory and antioxidant effects in peripheral organs after polymicrobial sepsis.

Key words: sepsis, vitamin B6, oxidative stress, antioxidant.

INTRODUCTION

Sepsis is a life-threatening organ dysfunction induced by a dysregulated host response to infectious stimuli (Singer et al. 2016). The pathologic hallmark of sepsis relies on the failure to maintain a satisfactory balance between excessive and inadequate inflammatory response, leading to a massive generation of reactive oxygen species (ROS) and nitrogen species (RNS) (Andrades et al. 2009). Reactive species promote several proinflammatory effects, such as neutrophil recruitment (Hotchkiss and Karl 2003), and favor the occurrence of oxidative damage due to an imbalanced activity of endogenous enzymes, including catalase (CAT) (Andrades et al. 2005). As oxidative stress plays an important role in the development of sepsis-induced organ dysfunction, the application of antioxidant compounds has been considered a potential and promisor treatment (Ritter et al. 2004).

Vitamin B6 (vit B6) is a water-soluble vitamin easily found as pyridoxal, pyridoxine and pyridoxamine forms in animal and vegetal foods (Salam et al. 2015). After digestion and absorption,

Correspondence to: Fabricia Petronilho
E-mail: fabricia.petronilho@unisul.br
ORCid: https://orcid.org/0000-0003-3240-2808

Randomized Controlled Trial Altern Ther Health Med. 2019 Nov;25(6):34-43.

Antioxidant Effect of Far Infrared Radiation Produced by Bioceramics in Individuals with Intermittent Claudication: A Randomized, Controlled Pilot Study

Fábio Goulart da Silva, Gerli Elenise Gehrke Herr, Francisco José Cidral-Filho, Fabricia Petronilho, Lucineia Gainski Danielski, Drielly Florentino, Franciane Bobinski, Daniela Dero Ludtke, Cintia Vieira, Daniel Fernandes Martins, Eliane Roseli Winkelmann

PMID: 32006455

Abstract

Background: Biomedical research has recently incorporated bioceramics applications into new health care approaches.

Objective: This study evaluated the effect of far infrared-emitting bioceramics wraps in the treatment of intermittent claudication.

Methods: This is a randomized double-blind placebo-controlled pilot study. Thirty-five patients met the criteria and were randomized into either control (placebo wraps) or bioceramics group (far infrared emitting-ceramics wraps) and assessed over a 90-day period for the following outcomes: six-minute walk test (6MWT), ankle-brachial index (ABI), Flow-mediated arterial dilation (FMD), quality of life and claudication. Oxidative stress and inflammatory biomarkers were measured in plasma of patients.

Results: Intervention induced a decrease in oxidative stress, with significant lower levels of reactive substances to thiobarbituric acid (TBARS), as well as increase in superoxide dismutase and catalase enzyme activities. There was an increase in the environment subscale of the quality of life questionnaire. No statistically significant differences were found in the inflammatory cytokines, 6MWT, ABI and FMV evaluations.

Conclusions: In Sum, FIR treatment improved oxidative stress profile and quality-of-life of patients with intermittent claudication. The study was registered into the ensaiosclinicos.gov.br (Registro Brasileiro de Ensaios Clínicos [ReBEC]) (RBR-7nr6sy register number).

Related information

MedGen
PubChem Compound (MeSH Keyword)

LinkOut - more resources

Medical
MedlinePlus Health Information

Research Materials
NCI CPTC Antibody Characterization Program

Miscellaneous
NCI CPTAC Assay Portal

Molecular Neurobiology
https://doi.org/10.1007/s12035-020-02089-9

NLRP3 Activation Contributes to Acute Brain Damage Leading to Memory Impairment in Sepsis-Surviving Rats

Lucineia Gainski Danielski[1] · Amanda Della Giustina[1] · Sandra Bonfante[1] · Mariana Pereira de Souza Goldim[1] · Larissa Joaquim[1] · Kiuanne Lobo Metzker[1] · Erica Bernardo Biehl[1] · Thaynan Vieira[1] · Fabiana Durante de Medeiros[1] · Naiana da Rosa[1] · Jaqueline Generoso[2] · Lutiana Simoes[2] · Hémelin Resende Farias[3] · Isabela da Silva Lemos[3] · Vijayasree Giridharan[4] · Gislaine Tezza Rezin[1] · Jucelia Jeremias Fortunato[1] · Rafael Mariano Bitencourt[1] · Emilio Luiz Streck[3] · Felipe Dal-Pizzol[2] · Tatiana Barichello[2,4] · Fabricia Petronilho[1]

Received: 2 April 2020 / Accepted: 24 August 2020

Abstract
Sepsis survivors present acute and long-term cognitive impairment and the pathophysiology of neurological dysfunction in sepsis involves microglial activation. Recently, the involvement of cytosolic receptors capable of forming protein complexes called inflammasomes have been demonstrated to perpetuate neuroinflammation. Thus, we investigated the involvement of the NLRP3 inflammasome activation on early and late brain changes in experimental sepsis. Two-month-old male Wistar rats were submitted to the sepsis model by cecal ligation and perforation (CLP group) or laparotomy only (sham group). Immediately after surgery, the animals received saline or NLRP3 inflammasome formation inhibitor (MCC950, 140 ng/kg) intracerebroventricularly. Prefrontal cortex and hippocampus were isolated for cytokine analysis, microglial and astrocyte activation, oxidative stress measurements, nitric oxide formation, and mitochondrial respiratory chain activity at 24 h after CLP. A subset of animals was followed for 10 days for survival assessment, and then behavioral tests were performed. The administration of MCC950 restored the elevation of IL-1β, TNF-α, IL-6, and IL-10 cytokine levels in the hippocampus. NLRP3 receptor levels increased in the prefrontal cortex and hippocampus at 24 h after sepsis, associated with microglial, but not astrocyte, activation. MCC950 reduced oxidative damage to lipids and proteins as well as preserved the activity of the enzyme SOD in the hippocampus. Mitochondrial respiratory chain activity presented variations in both structures studied. MCC950 reduced microglial activation, decreased acute neurochemical and behavioral alteration, and increased survival after experimental sepsis.

Keywords Sepsis · NLRP3 · Inflammasome · MCC950 · Neuroinflammation · Cognitive impairment

Introduction

In the last decade, there has been a decrease in mortality in patients with sepsis. Age-standardized sepsis incidence fell by 37% and mortality decreased by 52.8% from 1990 to 2017 [1]. In this sense, other challenges arise, especially those related to quality of life and acute and persistent neurological damage presented by survivors. Surviving an episode of sepsis represents a major risk for the development of long-term neurocognitive dysfunction affecting a patient's ability to live independently, as well as reducing his work ability [2]. Several studies have attributed these behavioral changes to neuronal damage during sepsis and the microglial activation is involved in this process [3–6]. When activated, microglial cells are capable of indirectly causing neuronal damage by releasing inflammatory mediators such as IL-1β and TNF-α, or by reducing their production of neurotrophins. Directly, microglial cells cause neuronal damage by activation of

✉ Fabricia Petronilho
fabricia.petronilho@unisul.br

[1] Laboratory of Neurobiology of Inflammatory and Metabolic Processes, Graduate Program in Health Sciences, Health Sciences Unit, University of South Santa Catarina, Tubarão, SC, Brazil

[2] Laboratory of Experimental Pathophysiology, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina, Criciuma, SC, Brazil

[3] Laboratory of Experimental Neurology, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina, Criciuma, SC, Brazil

[4] Translational Psychiatry Program, Faillace Department of Psychiatry and Behavioral Sciences, McGovern Medical School, The University of Texas Health Science Center at Houston (UTHealth), Houston, TX 77054, USA

Published online: 01 September 2020

Molecular Neurobiology
https://doi.org/10.1007/s12035-020-02032-y

Lipoic Acid and Fish Oil Combination Potentiates Neuroinflammation and Oxidative Stress Regulation and Prevents Cognitive Decline of Rats After Sepsis

Amanda Della Giustina[1] · Mariana Pereira de Souza Goldim[1] · Lucinéia Gainski Danielski[1] · Leandro Garbossa[1] · Aloir Neri Oliveira Junior[1] · Thainá Cidreira[1] · Taís Denicol[1] · Sandra Bonfante[1] · Naiana da Rosa[1] · Jucélia Jeremias Fortunato[1] · Juliete Palandi[2] · Bruna Hoffmann de Oliveira[2] · Daniel Fernandes Martins[2] · Franciane Bobinski[2] · Michelle Garcez[3] · Tatiani Bellettini-Santos[3] · Josiane Budni[3] · Gabriela Colpo[4] · Giselli Scaini[4] · Vijayasree V. Giridharan[4] · Tatiana Barichello[4,5] · Fabricia Petronilho[1,6]

Received: 26 March 2020 / Accepted: 22 July 2020

Abstract
Sepsis causes organ dysfunction due to an infection, and it may impact the central nervous system. Neuroinflammation and oxidative stress are related to brain dysfunction after sepsis. Both processes affect microglia activation, neurotrophin production, and long-term cognition. Fish oil (FO) is an anti-inflammatory compound, and lipoic acid (LA) is a universal antioxidant substance. They exert neuroprotective roles when administered alone. We aimed at determining the effect of FO+LA combination on microglia activation and brain dysfunction after sepsis. Microglia cells from neonatal pups were co-treated with lipopolysaccharide (LPS) and FO or LA, alone or combined, for 24 h. Cytokine levels were measured. *Wistar* rats were subjected to sepsis by cecal ligation and perforation (CLP) and treated orally with FO, LA, or FO+LA. At 24 h after surgery, the hippocampus, prefrontal cortex, and total cortex were obtained and assayed for levels of cytokines, myeloperoxidase (MPO) activity, protein carbonyls, superoxide dismutase (SOD), and catalase (CAT) activity. At 10 days after surgery, brain-derived neurotrophic factor (BDNF) levels were determined and behavioral tests were performed. The combination diminished in vitro levels of pro-inflammatory cytokines. The combination reduced TNF-α in the cortex, IL-1β in the prefrontal cortex, as well as MPO activity, and decreased protein carbonyls formation in all structures. The combination enhanced catalase activity in the prefrontal cortex and hippocampus, elevated BDNF levels in all structures, and prevented behavioral impairment. In summary, the combination was effective in preventing cognitive damage by reducing neuroinflammation and oxidative stress and increasing BDNF levels.

Keywords Sepsis · Fish oil · Lipoic acid · Microglia · Oxidative stress

✉ Fabricia Petronilho
fabricia.petronilho@unisul.br

[1] Laboratory of Neurobiology of Inflammatory and Metabolic Processes, Graduate Program in Health Sciences, University of South Santa Catarina, Tubarão, SC, Brazil

[2] Experimental Neuroscience Laboratory (LaNEx), Postgraduate Program in Health Sciences, University of Southern Santa Catarina, Palhoça, Santa Catarina, Brazil

[3] Laboratory of Neurosciences, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina, Criciúma, SC, Brazil

[4] Faillace Department of Psychiatry and Behavioral Sciences, Translational Psychiatry Program, McGovern Medical School, The University of Texas Health Science Center at Houston (UTHealth), Houston, TX 77054, USA

[5] Laboratory of Experimental Pathophysiology, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina, Criciúma, SC, Brazil

[6] Laboratório de Neurobiologia de Processos Inflamatórios e Metabólicos, Programa de Pós-graduação em Ciências da Saúde, Universidade do Sul de Santa Catarina, Tubarão, SC, Brazil

Published online: 02 August 2020

Experimental Gerontology 140 (2020) 111063

Contents lists available at ScienceDirect

Experimental Gerontology

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

Aging influences in the blood-brain barrier permeability and cerebral oxidative stress in sepsis

Willian Margotti[a], Amanda Della Giustina[a], Mariana Pereira de Souza Goldim[a], Marcos Hubner[a], Thainá Cidreira[a], Taís Luise Denicol[a], Larissa Joaquim[a], Raquel Jaconi De Carli[a], Lucinéia Gainski Danielski[a], Kiuanne Lino Lobo Metzker[a], Sandra Bonfante[a], Tatiana Barichello[b], Fabricia Petronilho[a,*]

[a] Laboratory of Neurobiology of Inflammatory and Metabolic Processes, Postgraduate Program in Health Sciences, University of South Santa Catarina - UNISUL, Tubarao, SC, Brazil

[b] Translational Psychiatry Program, Faillace Department of Psychiatry and Behavioral Sciences, McGovern Medical School, The University of Texas Health Science Center at Houston (UTHealth), Houston, TX 77054, USA

ARTICLE INFO

Section Editor: Stephane Baudry

Keywords:
Sepsis
Brain
Aging
Oxidative stress
Blood-brain barrier

ABSTRACT

Sepsis is a set of serious manifestations throughout the body produced by an infection, leading to changes that compromise cellular homeostasis and can result in dysfunction of the central nervous system. The elderly have a higher risk of developing sepsis than younger peoples. Under the influence of inflammatory mediators and oxidizing agents released in the periphery as a result of the infectious stimulus, changes occur in the blood-brain barrier (BBB) permeability, with neutrophil infiltration, the passage of toxic compounds, activation of microglia and production of reactive species that results in potentiation of neuroimmune response, with the progression of neuronal damage and neuroinflammation. The objective of this study is to compare BBB permeability and the development of oxidative stress in the hippocampus and prefrontal cortex of young and old rats submitted to polymicrobial sepsis induction. Male Wistar rats grouped into sham (60d), sham (210d), cecal ligation and perforation (CLP) (60d) and CLP (210d) with $n = 16$ per experimental group were evaluated using the CLP technique to induce sepsis. The brain regions were collected at 24 h after sepsis induction to determine BBB permeability, myeloperoxidase (MPO) activity as marker of neutrophil activation, nitrite/nitrate (N/N) levels as marker of reactive nitrogen species, thiobarbituric acid reactive substances as marker of lipid peroxidation, protein carbonylation as marker of protein oxidation, and activity of antioxidant enzyme catalase (CAT). There was an increase in the BBB permeability in the CLP groups, and this was enhanced with aging in both brain region. MPO activity in the brain regions increased in the CLP groups, along with a hippocampal increase in the CLP 210d group compared to the 60d group. The concentration of N/N in the brain region was increased in the CLP groups. The damage to lipids and proteins in the two structures was enhanced in the CLP groups, while only lipid peroxidation was higher in the prefrontal cortex of the CLP 210d group compared to the 60d. CAT activity in the hippocampus was decreased in both CLP groups, and this was also influenced by age, whereas in the prefrontal cortex there was only a decrease in CAT in the CLP 60d group compared to the sham 60d. These findings indicate that aging potentiated BBB permeability in sepsis, which possibly triggered an increase in neutrophil infiltration and, consequently, an increase in oxidative stress.

1. Introduction

Sepsis is a set of serious manifestations throughout the body produced by an infection. Its clinical manifestations include those associated with the infectious focus in question (Singer et al., 2016). In terms of epidemiological characteristics, the high rates of sepsis incidence and mortality have not changed in recent decades, leading the World Health Organization (WHO) to include sepsis in global health priorities (WHO, 2018). In the United States, the incidence of sepsis is 300 cases per 100,000 inhabitants, where ¼ of the patients who are

* Corresponding author at: Laboratory of Neurobiology of Inflammatory and Metabolic Processes, Graduate Program in Health Sciences, University of South Santa Catarina - UNISUL, Santa Catarina, SC, Brazil.
E-mail address: fabricia.petronilho@unisul.br (F. Petronilho).

https://doi.org/10.1016/j.exger.2020.111063
Received 10 February 2020; Received in revised form 20 July 2020; Accepted 17 August 2020
Available online 19 August 2020

Microvascular Research 128 (2020) 103956

Contents lists available at ScienceDirect

Microvascular Research

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

Stanniocalcin-1 ameliorates cerebral ischemia by decrease oxidative stress and blood brain barrier permeability

Sandra Bonfante[a], Amanda Della Giustina[a], Lucineia Gainski Danielski[a], Tais Denicol[a], Larissa Joaquim[a], Erica Biehl[a], Gabriel Scopel[a], Raquel Jaconi de Carli[a], Marcos Hubner[a], Taise Cardoso[a], Talita Tuon[b], Jaqueline Generoso[c], Tatiana Barichello[c,d,e], Silvia Terra[f], Fabricia Petronilho[a,*]

[a] Laboratory of Neurobiology of Inflammatory and Metabolic Processes, Graduate Program in Health Sciences, Health Sciences Unit, University of South Santa Catarina, Tubarão, SC, Brazil
[b] Graduate Program in Rehabilitation Sciences, Federal University of Santa Catarina, Araranguá, SC, Brazil
[c] Laboratory of Experimental Pathophysiology, Graduate Program in Health Sciences, University of Southern Santa Catarina, Criciúma, SC, Brazil
[d] Center of Excellence on Mood Disorders, Department of Psychiatry and Behavioral Sciences, McGovern Medical School, The University of Texas Health Science Center at Houston (UTHealth), Houston, TX, USA
[e] Translational Psychiatry Program, Department of Psychiatry and Behavioral Sciences, McGovern Medical School, The University of Texas Health Science Center at Houston (UTHealth), Houston, TX, USA
[f] Postgraduate Program: Biochemistry, Universidade Federal do Rio Grande do Sul (UFRGS), Brazil

ARTICLE INFO

Keywords:
Stroke
Stanniocalcin-1
Oxidative stress
Blood brain barrier

ABSTRACT

Blood brain barrier (BBB) permeability and oxidative stress have been reported to be important mechanisms for brain damage following ischemic stroke and stanniocalcin-1 (STC-1), a neuroprotective protein, has anti-inflammatory and anti-oxidative stress properties. Herein, we report the effect of STC-1 on BBB permeability and brain oxidative stress after stroke in an animal model. Male Wistar received an intracerebroventricularly injection of human recombinant STC-1 (100 ng/kg) or saline and were subjected to sham procedure or global cerebral ischemia/reperfusion (I/R) model. Six and 24 h after I/R, neurological evaluation was performed; at 24 h brain water content was evaluated in the total brain, and BBB permeability, nitrite/nitrate (N/N) concentration, lipid peroxidation, protein carbonyls formation, superoxide dismutase (SOD) and catalase (CAT) activity were determined in the hippocampus, cortex, prefrontal cortex, striatum and cerebellum. Rats exhibited neurological deficit at 6 and 24 h after I/R and STC-1 reduction at 24 h. After I/R there were an increase of brain water content, BBB permeability in the hippocampus, cortex and pre-frontal cortex and N/N in the hippocampus, and STC-1 decreased this level only in the hippocampus. STC-1 decreased lipid peroxidation in the hippocampus, cortex and prefrontal cortex and protein oxidative damage in the hippocampus and cortex. SOD activity decreased in the hippocampus, cortex and prefrontal cortex after I/R and STC-1 reestablished these levels in the hippocampus and cortex. CAT activity decreased only in the hippocampus and cortex and STC-1 increased the CAT activity in the hippocampus. Our data provide the first experimental demonstration that STC-1 reduced brain dysfunction associated with cerebral I/R in rats, by decreasing BBB permeability and oxidative stress parameters.

1. Introduction

Ischemic stroke is the primary cause of long-term disability and is the fourth leading cause of death worldwide (Mukundan and Seidenwurm, 2018). According to the World Health Organization, 50% of ischemic stroke survivors suffer from some degree of physical or cognitive impairment, and about 20% of them require institutional care (Bustamante et al., 2017).

The incidence of ischemic stroke and the subsequent reperfusion decrease blood brain barrier (BBB) integrity and increase paracellular permeability and contribute to cerebral edema (Sadeghian et al., 2019). The destruction of BBB integrity is associated with several possible

* Corresponding author at: Laboratório de Neurobiologia de Processos Inflamatórios e Metabólicos, Programa de Pós-graduação em Ciências da Saúde, Universidade do Sul de Santa Catarina, Tubarão, SC, Brazil.
E-mail address: fabricia.petronilho@unisul.br (F. Petronilho).

https://doi.org/10.1016/j.mvr.2019.103956
Received 16 September 2019; Received in revised form 11 November 2019; Accepted 12 November 2019
Available online 13 November 2019

JIM-03-2019-RCT-0085

Randomized Controlled Trial

Effects of the use of bioceramic wraps in patients with lower limb venous ulcers: a randomized double-blind placebo-controlled trial

Gerli E.G. Herr[1], Fábio Goulart da Silva[1], Francisco José Cidral-Filho[2], Fabricia Petronilho[3], Lucinéia Gainski Danielski[3], Mariana Pereira de Souza Goldim[3], Afonso Shiguemi Inoue Salgado[2,4], Franciane Bobinski[2], Daniel Fernandes Martins[2], Eliane R. Winkelmann[1,5]

1. Integral Health Care, Regional University of the Northwest of the State of Rio Grande do Sul (UNIJUÍ) and University of Cruz Alta (UNICRUZ), Ijuí, 98700-000 RS, Brazil
2. Experimental Neuroscience Laboratory (LaNEx), University of Southern Santa Catarina, Palhoça, 88137-270 SC, Brazil
3. Laboratory of Neurobiology of Inflammatory and Metabolic Processes, University of Southern Santa Catarina, Tubarão, 88704-900 SC, Brazil.
4. Integrative Physical Therapy Residency, Philadelphia University Center, Londrina, 86020-000 PR, Brazil
5. Laboratory of Exercise Physiopathology, Department of Life Sciences (DCVida), Regional University of the Northwest of the State of Rio Grande do Sul (UNIJUÍ), Ijuí, 98700-000 RS, Brazil

ABSTRACT

Background: Venous ulcer represents the most advanced stage of chronic venous insufficiency. It is an important public health problem and has a significant impact on patients' quality of life due to chronic pain, inability to work, need for hospitalization and frequent outpatient follow-up.
Objective: We investigated the treatment benefits of far-infrared ceramic (cFIR), in a 90-day study of lower limb venous ulcers and looked at ulcer healing scores, quality of life, serum bio-markers of oxidative stress and antioxidant defense enzymes.
Design, setting, participants and interventions: This is a randomized double-blind placebo-controlled study conducted in the Vascular Surgery Service of a hospital located in the northwest region of the State of Rio Grande do Sul, Brazil. We included patients with lower limb venous ulcers who were randomized to use either a bioceramics wrap or a placebo wrap for 90 days.
Main outcome measures: The following evaluations were conducted at baseline and after 15, 30, 60 and 90 days: ulcer healing score, quality of life, and serum markers of oxidative stress and antioxidant enzyme activity.
Results: Patients (n = 24) with lower limb venous ulcers were randomized into two treatment groups. cFIR decreased the ulcer size on day 30 (P = 0.042) and 90 (P = 0.034) and the total ulcer healing scale scores on day 30 (P = 0.049) and 90 (P = 0.02) of the treatment, when compared to baseline. Additionally, cFIR improved tissue type (epithelial tissue) on day 60 (P = 0.022) when compared to baseline evaluation.
Conclusion: cFIR clinically improved ulcer healing in patients with lower limb venous ulcers.
Trial registration: RBR-8c7xzn on ReBEC.

Please cite this article as: Herr GEG, Silva FG, Cidral-Filho FJ, Petronilho F, Danielski LG, Goldim MP, Salgado ASI, Bobinski F, Martins DF, Winkelmann ER. Effects of the use of

Accepted Manuscript

FISH OIL-RICH LIPID EMULSION MODULATES NEUROINFLAMMATION AND PREVENTS LONG-TERM COGNITIVE DYSFUNCTION AFTER SEPSIS

Amanda Della Giustina , Mariana Pereira Goldim , Lucinéia Gainski Danielski , Drielly Florentino , Leandro Garbossa , Larissa Joaquim , Aloir Neri Oliveira Junior , Khiany Mathias , Maria Eduarda Fileti , Graciela Freitas Zarbato , Naiana da Rosa , Ana Olívia Martins Laurentino , Jucélia Jeremias Fortunato , Juliete Palandi , Bruna Hoffmann de Oliveira , Daniel Fernandes Martins , Franciane Bonbinski , Tatiani Bellettini-Santos , Josiane Budni , Tatiana Barichello , Fabricia Petronilho

PII: S0899-9007(18)30944-4
DOI: https://doi.org/10.1016/j.nut.2018.12.003
Reference: NUT 10417

To appear in: *Nutrition*

Received date: 15 August 2018
Revised date: 7 November 2018
Accepted date: 4 December 2018

Please cite this article as: Amanda Della Giustina , Mariana Pereira Goldim , Lucinéia Gainski Danielski , Drielly Florentino , Leandro Garbossa , Larissa Joaquim , Aloir Neri Oliveira Junior , Khiany Mathias , Maria Eduarda Fileti , Graciela Freitas Zarbato , Naiana da Rosa , Ana Olívia Martins Laurentino , Jucélia Jeremias Fortunato , Juliete Palandi , Bruna Hoffmann de Oliveira , Daniel Fernandes Martins , Franciane Bonbinski , Tatiani Bellettini-Santos , Josiane Budni , Tatiana Barichello , Fabricia Petronilho , FISH OIL-RICH LIPID EMULSION MODULATES NEUROINFLAMMATION AND PREVENTS LONG-TERM COGNITIVE DYSFUNCTION AFTER SEPSIS, *Nutrition* (2018), doi: https://doi.org/10.1016/j.nut.2018.12.003

Effects of Far-Infrared Emitting Ceramic Materials on Recovery During 2-Week Preseason of Elite Futsal Players

Renan F.H. Nunes,[1] Francisco J. Cidral-Filho,[2] Lucinar J.F. Flores,[3] Fabio Y. Nakamura,[4,5] Harrison F.M. Rodriguez,[6] Franciane Bobinski,[2] Amanda De Sousa,[2] Fabricia Petronilho,[7] Lucineia G. Danielski,[7] Maryane M. Martins,[7] Daniel F. Martins,[2] and Luiz G.A. Guglielmo[1]

[1]Departament of Physical Education, Federal University of Santa Catarina (UFSC), Florianópolis, Santa Catarina, Brazil; [2]Laboratory of Experimental Neurosciences and Postgraduate Program in Health Sciences, University of Southern Santa Catarina, Palhoça, Santa Catarina, Brazil; [3]Department of Physical Education, State University of Western Parana, Marechal Cândido Rondon, Paraná, Brazil; [4]The College of Healthcare Sciences, James Cook University, Douglas, Queensland, Australia; [5]Department of Medicine and Aging Sciences, "G. d'annunzio", University of Chieti-Pescara, Pescara, Italy; [6]Department of Futsal, Sport Club Copagril, Marechal Cândido Rondon, Paraná, Brazil; and [7]Laboratory of Neurobiology of Inflammatory and Metabolic Processes, University of Southern Santa Catarina, Tubarão, Santa Catarina, Brazil

Abstract

Nunes, RFH, Cidral-Filho, FJ, Flores, LJF, Nakamura, FY, Rodriguez, HFM, Bobinski, F, De Sousa, A, Petronilho, F, Danielski, LG, Martins, MM, Martins, DF, and Guglielmo, LGA. Effects of far-infrared emitting ceramic materials on recovery during 2-week preseason of elite futsal players. *J Strength Cond Res* XX(X): 000–000, 2018–We investigated the effects of far-infrared emitting ceramic materials (cFIR) during overnight sleep on neuromuscular, biochemical and perceptual markers in futsal players. Twenty athletes performed a 2-week preseason training program and during sleep wore bioceramic (BIO; $n = 10$) or placebo pants (PL; $n = 10$). Performance (countermovement jump [CMJ]; squat jump [SJ]; sprints 5, 10, and 15-m) and biochemical markers (tumor necrosis factor alpha-TNF-α, interleukin 10-IL-10, thiobarbituric acid–reactive species [TBARS], carbonyl, superoxide dismutase [SOD], catalase [CAT]) were obtained at baseline and after the 1st and 2nd week of training. Delayed-onset muscle soreness (DOMS) and training strain were monitored throughout. Changes in ΔCMJ and ΔSJ were possibly (60/36/4 [week-1]) and likely (76/22/2 [week-2]) higher in BIO. Both groups were faster in 5-m sprint in week 2 compared with baseline ($p = 0.015$), furthermore, BIO was likely faster in 10-m sprint (3/25/72 [week 1]). Significant group × time interaction in %ΔTNF-α were observed ($p = 0.024$ [week-1]; $p = 0.021$ [week-2]) with values possibly (53/44/3 [week 1]) and likely (80/19/1 [week 2]) higher in BIO. The %ΔIL-10 decreased across weeks compared with baseline ($p = 0.019$ [week-1]; $p = 0.026$ [week-2]), showing values likely higher in BIO (81/16/3 [week-1]; 80/17/3 [week-2]). Significant weekly increases in %ΔTBARS ($p = 0.001$ [week-1]; $p = 0.011$ [week-2]) and %ΔCarbonyl ($p = 0.002$ [week-1]; $p < 0.001$ [week-2]) were observed compared with baseline, showing likely (91/5/4 [week-1]) and possibly (68/30/2 [week-2]) higher changes in BIO. Significant weekly decreases in %ΔSOD were observed compared with baseline ($p = 0.046$ [week 1]; $p = 0.011$ [week-2]), and between week 2 and week 1 ($p = 0.021$), in addition to significant decreases in %ΔCAT compared with baseline ($p = 0.070$ [week 1]; $p = 0.012$ [week 2]). Training strain ($p = 0.021$; very -likely [0/2/98]; week 1) and DOMS was lower in BIO (likely; 7 sessions) with differences over time ($p = 0.001$). The results suggest that the daily use of cFIR clothing could facilitate recovery, especially on perceptual markers during the early phases of an intensive training period.

Key Words team sports, muscle damage, inflammation, performance

Introduction

During the preseason period, team sports athletes deal with large physical and physiological demands as a result of training sessions and friendly matches (32,34). The high-intensity efforts typically found in team sports matches and training sessions, encompassing a high load on the eccentric phase of muscle contraction, have been shown to induce inflammatory and oxidative stress responses (23,37), as well as increased passive stiffness, swelling, and edema. All above contribute to some extent to delayed-onset muscle soreness (DOMS), which may result in the reduction in physical performance and disruption in the athlete's regular training routine (37,41).

Address correspondence to Renan F.H. Nunes, nunesrenan85@hotmail.com.

00(00)/1–14

Journal of Strength and Conditioning Research

Neurochemistry International 135 (2020) 104712

Contents lists available at ScienceDirect

Neurochemistry International

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

Early life neuroimmune challenge protects the brain after sepsis in adult rats

Drielly Florentino[a], Amanda Della Giustina[a], Mariana Pereira de Souza Goldim[a], Lucineia Gainski Danielski[a], Aloir Neri de Oliveira Junior[a], Larissa Joaquim[a], Sandra Bonfante[a], Erica Biehl[a], Naiana da Rosa[a], Deisy Fernandes[a], Fernanda Frederico Gava[b], Monique Michels[c], Jucelia Jeremias Fortunato[a], Gislaine Zilli Réus[b], Samira S Valvassori[b], Joao Quevedo[b,d,e,f], Felipe Dal-Pizzol[c], Tatiana Barichello[c,d], Fabricia Petronilho[a,*]

[a] Laboratory of Neurobiology of Inflammatory and Metabolic Processes, Graduate Program in Health Sciences, Health Sciences Unit, University of South Santa Catarina (UNISUL), Tubarão, SC, Brazil
[b] Translational Psychiatry Laboratory, Graduate Program in Health Sciences, University of Southern Santa Catarina (UNESC), Criciúma, SC, Brazil
[c] Laboratory of Experimental Pathophysiology, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina (UNESC), Criciúma, Santa Catarina, Brazil
[d] Center of Excellence on Mood Disorders, Department of Psychiatry and Behavioral Sciences, McGovern Medical School, The University of Texas Health Science Center at Houston (UTHealth), Houston, TX, USA
[e] Neuroscience Graduate Program, The University of Texas MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences, Houston, TX, USA
[f] Translational Psychiatry Program, Department of Psychiatry and Behavioral Sciences, McGovern Medical School, The University of Texas Health Science Center at Houston (UTHealth), Houston, TX, USA

ARTICLE INFO

Keywords:
Sepsis
LPS-preconditioning
Neuroinflammation
Memory

ABSTRACT

Evidences has suggested that in the early life the innate immune system presents plasticity and the time and dose-adequate stimuli in this phase may program long-lasting immunological responses that persist until adulthood. We aimed to evaluate whether LPS challenge in early childhood period may modulate brain alterations after sepsis in adult life. Experiments were performed to evaluate the LPS challenge in early childhood or adult period on acute and long-term brain alterations after model of sepsis by cecal ligation and perforation (CLP) in adult life. Wistar rats were divided in saline + sham, LPS + sham, saline + CLP and LPS + CLP groups to determine cytokine levels and nitrite/nitrate concentration in cerebrospinal fluid (CSF); oxidative damage, activity of antioxidant enzymes (superoxide dismutase-SOD and catalase-CAT); blood brain barrier (BBB) permeability; myeloperoxidase (MPO) and epigenetic enzymes activities in the hippocampus and prefrontal cortex (at 24 h after CLP) and cognitive function, survival and brain-derived neurotrophic factor (BDNF) level (at ten days after CLP). LPS-preconditioning in early life could lead to decreased levels of TNF-α and IL-6 and oxidative damage parameters in the brain after CLP in adult rats. In addition, LPS-preconditioning in early life increase CAT activity, attenuates the BBB permeability and epigenetic enzymes alterations and in long term, improves the memory, BDNF levels and survival. In conclusion, rats submitted to CLP in adulthood displayed acute neuroinflammation, neurochemical and epigenetic alteration improvement accompanied in long term by an increase in survival, neurotrophin level and memory performance when preconditioned with LPS in the early life.

1. Introduction

Early childhood is a period that the individual present high vulnerability to infection but still can have a remarkable resilience that is poorly understood (Kallionpää et al., 2014). In this life phase the innate immune system still presents immaturity and plasticity and the time and dose-adequate stimuli may program long-lasting alterations in immunological responses that persist until adulthood (Georgountzou and Papadopoulos, 2017).

Children growing up in rural areas, around animals and in larger families seem to develop asthma less often than do other children (Ege, 2017). These observations have led to the formulation of the hygiene hypothesis. According to this hypothesis, delays in exposure to normal bacteria in the body as well as disease-causing agents make a weaker

* Corresponding author. Laboratório de Neurobiologia de Processos Inflamatórios e Metabólicos, Programa de Pós-Graduação em Ciências da Saúde, Universidade do Sul de Santa Catarina, Tubarão, SC, Brazil.
E-mail address: fabricia.petronilho@unisul.br (F. Petronilho).

https://doi.org/10.1016/j.neuint.2020.104712
Received 2 September 2019; Received in revised form 24 January 2020; Accepted 27 February 2020
Available online 29 February 2020

Journal of Ethnopharmacology 264 (2021) 113139

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Macroscopic, biochemical and hystological evaluation of topical anti-inflammatory activity of *Casearia sylvestris* (Flacourtiaceae) in mice

Ana Caroline Heymanns [a,b], Micheline N. Albano [c], Márcia R. da Silveira [c], Simony D. Muller [c], Fabrícia C. Petronilho [a], Lucinéia D. Gainski [a], Eduardo Cargnin-Ferreira [d], Anna P. Piovezan [a,b,*]

[a] Post-graduate Programm in Health Science, University of Southern Catarina (UNISUL), Tubarão, SC, Brazil
[b] Laboratory of Experimental Neuroscience (LANEX)-UNISUL, Palhoça, SC, Brazil
[c] Pharmacy Degree Course UNISUL, Tubarão, SC, Brazil
[d] Laboratory of Histological Markers, Federal Institute of Santa Catarina (IFSC), Garopaba, SC, Brazil

ARTICLE INFO

Keywords:
Casearia sylvestris
Inflammation
Skin conditions

ABSTRACT

Ethnopharmacological relevance: Inflammatory skin diseases presents high prevalence and lack of alternatives that can be used for self-care by the population. Casearia *sylvestris* is a plant used topically in different communities in Brazil, to treat wounds or promote cutaneous healing. To evaluate the topical anti-inflammatory activity for the crude hydroalcoholic extract of *Casearia sylvestris* (HCE-CS) in the models of single or multiple administration of chroton oil to induce ear edema in mice.

Materials and methods: Experimental study using male Swiss mice (25–35g) kept under constant conditions in the Laboratory of Experimental Neuroscience (LaNEx)-UNISUL. Edema was induced in both models, respectively, by the single or multiple application of croton oil (CO, 2.5%, in 20 μl) on the external surface of the ear. The different groups of animals (n = 8) received different treatments: vehicle, dexamethasone (DEXA) or different doses of HCE-CS. Edema was evaluated macroscopically for 6 h (early edema) or 8 days (late edema) after the first application of the CO and immediately after the animals were submitted to euthanasia for the collection of the samples (treated ears). For early edema, the tissue was biochemically evaluated for myeloperoxidase activity (MPO) and levels of nitrite/nitrate. In the late edema model, the ears were histologically evaluated for general morphometry, degranulated and non-degranulated mast cells, as well as acanthosis.

Results: Topic treatment with HCE-CS significantly reduced the early and late edema, as well as MPO activity and tissue levels of nitrite/nitrate. Finally, in the late edema model there was a lower density of degranulated mast cells in relation to the vehicle treated group and decreased thickness of the epidermis (acanthosis). Conclusion: These results suggest a possible benefit of topical treatment with HCE-CS in inflammatory conditions of the skin.

1. Introduction

Inflammatory skin diseases or inflammation that accompanies pathophysiologic process triggered by other morbidities are frequent cause of suffering and impairment on patients' quality of life (Hay et al., 2014; Foggin et al., 2017). Mechanisms implicated in inflammatory response for centuries have been the researches focus, involving the most complex techniques; despite of this, we are still so far distant from the complete understanding of such conditions, and even new concepts about this process has been recently proposed (Braconi et al., 2010). Plants are important source of biologically active natural products and they are promisingly considered in antiinflamatory new drugs discovery field, since the phytochemicals derived from them have relatively low toxicity and shown related actions to this property (Shakya, 2016).

Shrubs of *Casearia sylvestris* Sw. (Flacourtiaceae, syn.: *Guidonia sylvestris* (Sw) occurs in forests of Southern Brazil and contains many chemical constituents (Ferreira et al., 2011). These compounds are responsible for a great variety of biological activities, including antioxidant (Albano et al., 2013), antiparasitic and cytotoxic effects (Bou et al., 2014; Ferreira et al., 2014). Beside of these, we have previously registered antinociceptive activity for hydroalcoolic crude extract of

* Corresponding author. Av. Avenida Pedra Branca, 25, Palhoça, SC, CEP 88137-270, Brazil.
E-mail addresses: nutriheymanns@gmail.com (A.C. Heymanns), micheline.albano@gmail.com (M.N. Albano), marcia15_rs@hotmail.com (M.R. da Silveira), simony.davet@unisul.br (S.D. Muller), fabricia.petronilho@unisul.br (F.C. Petronilho), lucineia.gainski@unisul.br (L.D. Gainski), eduardo.cargnin.ferreira@gmail.com (E. Cargnin-Ferreira), appiovezan@yahoo.com.br (A.P. Piovezan).

https://doi.org/10.1016/j.jep.2020.113139
Received 4 February 2020; Received in revised form 24 June 2020; Accepted 29 June 2020
Available online 26 July 2020

Neurotoxicity Research
https://doi.org/10.1007/s12640-020-00293-y

ORIGINAL ARTICLE

Stanniocalcin 1 Inhibits the Inflammatory Response in Microglia and Protects Against Sepsis-Associated Encephalopathy

Sandra Bonfante[1] · Larissa Joaquim[1] · Maria Eduarda Fileti[1] · Amanda Della Giustina[1] · Mariana Pereira de Souza Goldim[1] · Lucinéia Gainski Danielski[1] · Evandro Cittadin[1] · Raquel Jaconi De Carli[1] · Bianca Xavier de Farias[1] · Nicole Alessandra Engel[1] · Naiana da Rosa[1] · Jucélia Jeremias Fortunato[1] · Vijayasree Giridharan[2] · Giselli Scaini[2] · Gislaine Tezza Rezin[1] · Jaqueline Generoso[3] · Rafael Mariano de Bitencourt[1] · Silvia Terra[4] · Tatiana Barichello[2,3] · Fabricia Petronilho[1,5]

Received: 7 July 2020 / Revised: 10 September 2020 / Accepted: 29 September 2020

Abstract
Sepsis-associated encephalopathy is a serious consequence of sepsis, triggered by the host response against an infectious agent, that can lead to brain damage and cognitive impairment. Several mechanisms have been proposed in this bidirectional communication between the immune system and the brain after sepsis as neuroinflammation, oxidative stress, and mitochondrial dysfunction. Stanniocalcin-1 (STC-1), an endogen neuroprotective protein, acts as an anti-inflammatory and suppresses superoxide generation through induction of uncoupling proteins (UCPs) in the mitochondria. Here, we demonstrated a protective role of STC-1 on inflammatory responses in vitro, in activated microglia stimulated with LPS, and on neuroinflammation, oxidative stress, and mitochondrial function in the hippocampus of rats subjected to an animal model of sepsis by cecal ligation and puncture (CLP), as well the consequences on long-term memory. Recombinant human STC-1 (rhSTC1) suppressed the pro-inflammatory cytokine production in LPS-stimulated microglia without changing the UCP-2 expression. Besides, rhSTC1 injected into the cisterna magna decreased acute hippocampal inflammation and oxidative stress and increased the activity of complex I and II activity of mitochondrial respiratory chain and creatine kinase at 24 h after sepsis. rhSTC1 was effective in preventing long-term cognitive impairment after CLP. In conclusion, rhSTC1 confers significant neuroprotection by inhibiting the inflammatory response in microglia and protecting against sepsis-associated encephalopathy in rats.

Keywords Sepsis · Stanniocalcin-1 · Microglia · Brain

Sandra Bonfante and Larissa Joaquim contributed equally to this work.

✉ Fabricia Petronilho
fabricia.petronilho@unisul.br

[1] Laboratory of Neurobiology of Inflammatory and Metabolic Processes (NEUROIMet), Graduate Program in Health Sciences, University of South Santa Catarina, Tubarão, SC, Brazil

[2] Translational Psychiatry Program, Faillace Department of Psychiatry and Behavioral Sciences, McGovern Medical School, The University of Texas Health Science Center at Houston (UTHealth), Houston, TX 77054, USA

[3] Laboratory of Experimental Pathophysiology, Graduate Program in Health Sciences, University of Southern Santa Catarina, Criciúma, SC, Brazil

[4] Graduate Program: Biochemistry, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil

[5] Laboratório de Neurobiologia de Processos Inflamatórios e Metabólicos (NEUROIMet), Programa de Pós-graduação em Ciências da Saúde, Universidade do Sul de Santa Catarina, Tubarão, SC, Brazil

Introduction

Sepsis is a debilitating systemic inflammatory process involving multiple organ systems that is preceded by an infection (Singer et al. 2016). Sepsis-associated encephalopathy (SAE) represents a diffuse cerebral dysfunction during sepsis that frequently causes long-term cognitive impairments, including disrupted attention, memory, executive function, and speed of information processing (Barichello et al. 2019).

SAE is probably the most frequent sepsis-related organ dysfunction, affecting up to 70% of patients with sepsis and regularly precedes any other organ involvement (Sonneville et al. 2017). The development of SAE has been associated with higher mortality, lower quality of life in survivors, and long-term neurological sequelae (Prescott and Angus 2018).

In sepsis, peripheral inflammation stimulates the immune system located in the central nervous system (CNS) via

Published online: 06 October 2020