PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
PÓS-GRADUAÇÃO EM MEDICINA E CIÊNCIAS DA SAÚDE
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ESTUDOS DAS ALTERAÇÕES DO COMPORTAMENTO E DA RESPOSTA PSICOFARMACOLÓGICA EM RATOS SOBREVIVENTES DE SEPSE

PORTO ALEGRE
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Tese apresentada como requisito para obtenção do grau de Doutor pelo Curso de Pós-Graduação em Medicina e Ciências da Saúde, com área de Concentração em Neurociências, da Pontifícia Universidade Católica do Rio Grande do Sul.

Orientador: Prof. Dr. Iván Izquierdo

Co-orientador: Prof. Dr. João Quevedo

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Dedico esta tese ao meu marido Evandro,
e às minhas filhas Laís e Leticia.

AGRADECIMENTOS

Ao meu Orientador, Prof. Dr. Ivan Izqueirdo, por ter me aceito como sua orientanda, a maior honra acadêmica de minha vida.

Ao meu co-orientador, Prof. Dr. João Quevedo, por quem tenho muita admiração.

Aos meus amigos e colegas de trabalho, Profa. Dra.Tatiana Barichello, Profa. Luciane Ceretta e Prof. Dr. Felipe Dal-Pizzol.

Aos meus colaboradores Larissa Constantino, Roberta Machado e Fabrícia Petronilho e, em especial a Clarissa Comim, pelo companheirismo e dedicação na realização dessa pesquisa.

Aos órgãos de fomento (CNPq, FAPESC, UNESC e Instituto Cérebro e Mente) pelo auxílio financeiro à execução dos experimentos que compõem esta Tese.

RESUMO

A sepse é um estado fisiopatológico multifatorial agressivo, caracterizado por uma resposta inflamatória decorrente da reação do sistema imunológico a infecções, causando, assim, uma doença com implicações clínicas relevantes, cujas taxas de incidência vêm aumentando nos últimos anos, com alto índice de mortalidade, se tratando de uma das maiores causas de óbito nas unidades de terapia intensiva. Estudos recentes demonstram que mesmo os sobreviventes à sepse de unidades de terapia intensiva apresentam alterações de memória, de atenção e de concentração. Nesse contexto, além dos estudos que envolvem humanos, os modelos animais de ligação e perfuração cecal (CLP) são clinicamente relevantes, pois produzem uma inflamação generalizada similar àquela observada durante a síndrome da resposta inflamatória sistêmica e a sepse, mimetizando diversos aspectos da sepse polimicrobiana em humanos e permitindo um estudo mais aprofundado da relação entre alterações metabólicas e inflamação, e alterações do sistema nervoso central. Através de três experimentos se avaliou: as alterações comportamentais em ratos sobreviventes de sepse após 10, 30 e 60 dias; a resposta dos facilitadores de memória em ratos sobreviventes de sepse após 10 e 30 dias; e o efeito do antidepressivo imipramina no nado forçado em ratos sobreviventes de sepse após 10 dias. No primeiro experimento foram utilizados seis testes comportamentais: habituação ao campo aberto; memória de reconhecimento de objetos; esquiva inibitória; esquiva inibitória de treinos contínuos; labirinto em cruz elevado e de nado forçado. No segundo experimento os animais receberam injeções de salina, de epinefrina (25µ/kg), de nolaxone (0,4,mg/kg), de dexametazona (0,3,mg/kg) e de glicose (320mg/kg), e realizaram o teste de esquiva inibitória. No terceiro experimento receberam injeções de imipramina (10mg/kg) e realizaram o teste do nado forçado. No primeiro experimento, após 10 dias da sepse os ratos apresentaram déficits nos testes habituação ao campo aberto, esquiva inibitória, memória de reconhecimento de objetos, esquiva inibitória de treinos contínuos e nado forçado; após 30 dias de sepse apresentaram alterações nos testes esquiva inibitória de treinos contínuos, esquiva inibitória e nado forçado; e após 60 dias todos os déficits de comportamento foram revertidos. No segundo experimento houve reversão do dano de memória com a utilização dos facilitadores de memória após 10 e 30 dias de sepse. No terceiro experimento observou-se o aumento no tempo de

imobilidade do rato e a reversão após o uso de imipramina. Assim, os resultados indicam uma piora no aprendizado e na memória dos ratos após 10 dias da sepse, que se reverteu parcialmente em 30 dias, e completamente após 60 dias; que as vias de formação de memória nos ratos sobreviventes de sepse responderam aos facilitadores de memória; e que a imipramina reverteu os sintomas de depressão avaliados no teste de nado forçado nos ratos sobreviventes de sepse.

Palavras-chave: Sepse. Memória. Facilitadores de Memória. Imipramina.

ABSTRACT

Recently, some studies demonstrated that survivors from Intensive Care Units (ICU) presented cognitive impairment in long stated period, including alterations in the memory, attention, in the concentration and/or the global loss of the cognitive function. In these studies the majority of the patients showed an improvement in some of the measured cognitive functions. The mechanisms associated to these findings are not well understood but can include brain. In this context, murine models of cecal ligation and perforation (CLP) are clinically relevant since they induce a polymicrobial sepsis that mimics human sepsis contributing to the elucidation of the pathogenesis and to the determination of new therapies in sepsis. Objectives: the first evaluates cognitive performance in rats that survived from sepsis after ten, thirty and sixty days induced by cecal ligation and puncture (CLP); the second evaluates the enhanced-memory effect in sepsis survivors rats after ten and thirty days surgery; the third evaluates the antidepressant effect of imipramine on depressive symptoms observed in sepsis survivors rats. Measurements: the first: the animals separately underwent six behavioral tasks: habituation to an open field, step-down inhibitory avoidance, continuous multiple-trials step-down inhibitory avoidance task, object recognition, elevated plus-maze and forced swimming test; demonstrates that rat survivors 10 days after the surgery presents memory incapacity, learning and depression-like symptoms when submitted to the tests: habituation to the open field, step-down inhibitory avoidance, continuous multiple-trials step down inhibitory avoidance, object recognition and forced swimming. However, after 30 days of induction of sepsis, survivors rats had presented incapacities of memory, learning and depression-like symptoms when submitted to the tests of the step-down inhibitory avoidance, continuous multiple-trials step down inhibitory avoidance and forced swimming. To the 60 days after the surgery was observed sepsis rats survivors had not presented cognitive incapacity in the cited tests previously; the second: after ten and thirty days of recovery rats were submitted a inhibitory avoidance task and after training received injections of saline, epinephrine(25 µg/kg), naloxone (0,4,mg/kg), dexamethasone (0,3,mg/kg), or glucose (320mg/kg) and after 24 hours were submitted to the inhibitory avoidance test; enhanced-memory reversed impairment present per group CLP that received saline after ten and thirty days of surgery;the third: After 10 days of recovery rats received intraperitoneal injection of

imipramine (10 mg/kg) and were subjected to the forced swimming test; It has observed an increase in the immobility time in the forced swimming test in animals subjected to CLP, as a parameter of depressive behavior, was reversed by imipramine. Conclusions: These results indicate that the incapacity of memory, learning and depression-like symptoms demonstrated 10 days after the induction of sepsis, persist 30 days after the CLP; the cognitive incapacities had not persisted 60 days of the surgery; using different pharmacologic approaches we conclude that the memory formation pathways are responsible in sepsis survivors animals; the depressive symptoms evaluated by forced swimming test had been reversed after imipramine administration.

Key Words: Rat sepsis survivors. Memory. Enhanced-memory. Imipramine.

LISTA DE ILUSTRAÇÕES

LISTA DE SIGLAS

ACTH – Hormônio adrenocorticitrófico

BDNF – Fator Neurotrófico Derivado do Cérebro

CAT – Catalase

CLP – Ligação e Perfuração Cecal

CRH – Corticotrofina

GABA – Ácido gama-aminobutírico

HPA – Hipotalâmico-pituitário-adrenal

NAL – Naloxone

NMDA – N-methyl-d-aspartate

SIRS – Síndrome da resposta inflamatória sistêmica

SNC – Sistema nervoso central

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1 INTRODUÇÃO

1.2 SEPSE

A sepse é um estado fisiopatológico multifatorial agressivo, caracterizado por uma resposta inflamatória decorrente da reação do sistema imunológico a infecções, causando assim uma doença com implicações clínicas relevantes (Vandijck et al., 2006).

O termo sepse significa putrefação, decomposição da matéria orgânica por um agente agressor (bactérias, fungos, parasitas, vírus). Os termos infecção e sepse são geralmente utilizados de forma independente, entretanto a terminologia acaba simplificando uma relação complexa. O termo infecção está relacionado à presença de agente agressor em uma localização (tecido, cavidade ou fluido corporal) normalmente estéril, e o termo sepse está relacionado à conseqüente manifestação do hospedeiro; i.e. a reação inflamatória desencadeada frente uma infecção grave. A distinção entre os dois não é fácil, pois todo processo infeccioso desencadeia uma resposta do hospedeiro, e cada indivíduo apresenta um tipo de reação com magnitudes diferentes frente um determinado insulto (Granja et al., 2002).

O progresso no diagnóstico e no tratamento da sepse foi crescente nas últimas décadas, porém com pouca influência no prognóstico da doença. Um papel importante na patogenia da sepse é realizado pela resposta inflamatória, que pode causar danos aos tecidos, conduzindo à falência orgânica. Esta reação é controlada pela resposta antiinflamatória, que pode ser conduzida exageradamente, aumentando as infecções secundárias, e levando à síndrome da resposta antiinflamatória compensatória (Abraham, 2007).

A síndrome da resposta inflamatória sistêmica é um estado fisiopatológico

multifatorial agressivo, com uma taxa elevada de mortalidade, em índices que chegam a até 51%, se constituindo na 10ª principal causa de morte nas unidades de terapia intensiva (Hough, 2005). O diagnóstico inclui uma exigência para dois ou mais dos seguintes sintomas: febre ou hipotermia, taquicardia, e leucocitose ou leucopenia (Bone, 1992). A diferença principal entre a síndrome da resposta inflamatória sistêmica e a sepse é que esta última é produzida por microorganismos infecciosos, visto que a síndrome da resposta inflamatória sistêmica pode ocorrer na ausência de uma fonte documentada de infecção (Granja et al., 2002). Embora a causa seja difícil de verificar em doenças médicas complexas, há ampla evidência clínica e experimental para suportar o conceito da severidade da resposta inflamatória clínica e o resultado na sobrevivência (Sasse et al., 1995). Ainda, a resposta inflamatória produzida durante a sepse pode estar dentro do espectro da resposta inflamatória sistêmica, e envolve a amplificação rápida dos sinais e respostas além do tecido invadido (Granja et al., 2002).

A sepse leve caracteriza-se por uma síndrome da resposta inflamatória sistêmica (SIRS) e decorre de um processo infeccioso comprovado, enquanto a sepse grave caracteriza-se pela associação da sepse a manifestações de hipoperfusão tecidual e disfunção orgânica, caracterizada por acidose lática, oligúria ou alteração do nível de consciência, ou hipotensão arterial (Heard et al., 1991).

Um dos componentes principais que envolvem a fisiopatologia da sepse é a exacerbada ativação da resposta imune inata. O papel central do sistema imune inato durante a síndrome da resposta inflamatória sistêmica e a sepse é documentado pelo aumento dos fatores pró-inflamatórios após a infecção (Mastronardi et al., 2007), com conseqüente aumento de citocinas pró-inflamatórias, tais como o IL-1 e o TNF para o sistema nervoso central (Abraham, 2007). Como a maioria dos estudos envolvendo sepse focalizou órgãos periféricos, a participação do cérebro durante este processo ainda não está muito clara (Young et al., 1990).

Os pacientes de unidade de terapia intensiva que sobreviveram a um quadro de sepse podem ter a função de alguns órgãos comprometida, podendo resultar em sintomas tais como dispnéia, fadiga, depressão e alterações funcionais (Brun-Buisson, 2000).

A grande incidência, associado à gravidade da sepse, seria, por si só, o suficiente para justificar o aprofundamento no conhecimento de sua fisiopatologia e a tentativa de novas possibilidades terapêuticas. Associando-se esses elementos a grande mortalidade envolvida, decorre que os gastos diretos e indiretos para o tratamento da sepse é bastante elevado em nosso país. A possibilidade de diminuir a mortalidade por sepse e reduzir os gastos com internação em UTIs (alto custo com antibióticos de largo espectro, recursos técnicos, humanos e tecnológicos associados com o manejo do paciente) justificam a necessidade de maior investimento no estudo desta patologia.

1.2.1 Sepse e Sistema Nervoso Central

Os efeitos biológicos causados pela liberação de citocinas no organismo refletem no sistema nervoso central causando febre, anorexia, e ativação do eixo hipotâlamo-pituitária-adrenal, tendo por resultado o aumento da produção dos corticóides adrenais. As interações recíprocas entre o sistema nervoso central e o sistema imunológico são consideradas os componentes principais da resposta inflamatória à sepse, o que acaba causando alterações nos sistemas neuroendócrino, autonômico (Chrousos, 1995), comportamental (Gordon et al., 2004) e distúrbios em quaisquer funções adaptáveis, como as respostas imuno-inflamatórias e hemodinâmica (Saper e Breder, 1994).

No caso da sepse e da síndrome da resposta inflamatória sistêmica há alteração na permeabilidade da barreira cerebral sanguínea e o processo inflamatório acaba afetando os sistemas de controle do sistema nervoso central, causando alteração nas funções fisiológicas cruciais à homeostase, levando a encefalopatia (Chrousos e Gold,1992). A encefalopatia séptica pode vir a ocorrer em 8-70% dos pacientes sépticos, dependendo dos critérios de inclusão empregados (Sprung et al., 1990; Young et al., 1990), sendo a encefalopatia mais comum nas

unidades de terapia intensiva (Bleck et al., 1993). O conceito de encefalopatia séptica como uma patologia que não possa ser explicada pela disfunção, hipotensão, ou pela hipóxia hepática ou renal, é relativamente novo, porém já está claro que a sepse e suas reações podem ser associadas a um largo espectro de danos e disfunções cerebrais (Papadopoulos et al., 2000). A disfunção da barreira hematoencefálica parece ter efeito central na fisiopatologia da encefalopatia séptica, uma vez que permite a passagem para o SNC (sistema nervoso central) de endotoxinas que parecem influenciar diversos aspectos do metabolismo cerebral (Orlikowsk et al., 2003), além de alterar a função de células endoteliais, astrócitos e neurônios (Papadopoulos et al., 2000).

De qualquer maneira, a fisiopatologia da encefalopatia séptica está longe ser perfeitamente conhecida e vários aspectos merecem ser esclarecidos, em especial o início de seu desenvolvimento. Posteriormente, as disfunções ou as insuficiências dos órgãos contribuem para o seu agravamento. Além disso, numerosos fatores de origem iatrogênicos também podem agravar o quadro.

1.2.2 Sepse e Comportamento

Recentemente, diversos estudos vêm mostrando que pacientes de unidade de terapia Intensiva que sobreviveram a um quadro de sepse apresentam incapacidade cognitiva por um longo prazo, incluindo alterações de memória, de atenção, de concentração e a perda global da função cognitiva (Granja et al., 2004; Hopkins et al., 2005; Hough, 2005; Jackson et al., 2004).

O termo incapacidade cognitiva representa anormalidades clínicas significativas em uma ou mais funções do cérebro, incluindo memória, atenção, função executiva, anormalidades espaciais e visuais, e a função intelectual. A incapacidade cognitiva pode ser suave, moderada ou severa, e pode limitar a

habilidade de um indivíduo em pensar, raciocinar e executar tarefas diárias.

O termo declínio cognitivo relaciona-se a deterioração das habilidades cognitivas e não é necessariamente sinônimo de incapacidade cognitiva, pois não implica em um nível absoluto do funcionamento. Entretanto, este tipo de declínio pode causar limitações significativas nas atividades diária de uma pessoa que deseja executar níveis elevados da cognição em áreas ocupacionais (Gordon et al., 2004).

Sabe-se, ainda, que estas alterações comportamentais não estão claramente caracterizadas do ponto de vista psicopatológico e de duração. Estudos precedentes, que descreveram os sintomas de pacientes de unidade de terapia Intensiva que sobreviveram a um quadro de sepse, demonstram que a maioria destes pacientes apresentou alguma incapacidade cognitiva na alta hospitalar (Hopkins et al., 1999; Angus et al., 2001; Granja et al., 2004). Após um ano a maioria dos pacientes mostrou uma melhora na função cognitiva total; entretanto, algumas habilidades cognitivas, como a memória, não melhoraram completamente. É bem caracterizada a participação de vias inflamatórias apoptóticas e danos neuronais secundários à encefalopatia séptica (Messaris et al., 2004).

Muitos pacientes criticamente doentes apresentaram incapacidades neurocognitivas crônicas significativas em 2 meses, 6 meses (Jackson et al., 2003), 9 meses (Hopkins et al., 2006), 1 ano (Hopkins et al., 1999), 2 anos (Hopkins et al., 2005), e em até 6 anos após a alta hospitalar. As incapacidades neurocognitivas melhoram durante os primeiros 6 a 12 meses da saída dos pacientes da unidade de terapia intensiva, podendo ser permanente ou não, bem como associadas, ou não, com as incapacidades na função diária, a baixa qualidade de vida, e a inabilidade de retornar ao trabalho (Sukantarat et al., 2005; Jackson et al., 2004).

Estudos concluíram que, dos pacientes de unidade de terapia intensiva que sobreviveram a um quadro de sepse e que tiveram incapacidades neurocognitivas na alta hospitalar, somente 45% mantiveram as incapacidades neurocognitivas após 1 ano (Hopkins et al., 1999). Não havia nenhuma melhoria adicional nas seqüelas neurocognitivas após 2 anos da alta hospitalar (Hopkins et al., 2005).

Outros significativos sintomas que pacientes de unidade de terapia intensiva que sobreviveram a um quadro de sepse apresentam são a depressão e a ansiedade (Scragg et al., 2001). A prevalência e a severidade dos transtornos afetivos, incluindo sintomas de depressão e ansiedade, variam de 10% a 58% (Jackson et al., 2003; Hopkins et al., 1999; Al-Saidi et al., 2003; Schelling et al., 1998). A depressão foi relatada em até 30% dos sobreviventes (Jackson et al., 2003), e estima-se que 47% têm ansiedade clinicamente significativa. Em alguns casos a depressão severa pode imitar sintomas de incapacidade cognitiva, embora existam diferenças entre estas circunstâncias. No geral, os indivíduos com depressão retêm a habilidade de aprender, não se esquecem rapidamente, e não indicam decréscimos significativos na linguagem (Scragg et al., 2001).

A neurobiologia da depressão pode ser explicada, em sua maior parte, pela desregulação do eixo hipotalâmico-pituitário-adrenal (HPA), que possui um papel central no sistema neuroendócrino, que libera o hormônio corticotrofina (CRH), considerado o mediador central na resposta ao estresse (Nestler et al., 2002). A liberação da CRH está sob o controle serotoninérgico, noradrenérgico e colinérgico e é inibida pelo GABA. A CRH ainda libera o hormônio adrenocorticitrófico (ACTH), que age diretamente na glândula adrenal, liberando cortisol. Um estudo apontou níveis de cortisol elevados no sangue em pacientes deprimidos, sugerindo uma anormalidade no eixo HPA (Máxime et al., 2007).

Em laboratório, sabe-se que ratos sobreviventes de sepse, após 10 e 30 dias, apresentavam aumento no tempo de imobilidade no teste de natação forçada (Barichello et al., 2005), sugerindo um “comportamento do tipo depressivo”, mas, como se trata de um modelo animal, não se pode afirmar que apresentam depressão. Verificando parâmetros como anedonia, perda de peso, aumento da glândula adrenal, aumento dos níveis circulantes de ACTH e cortisol, e ainda, a diminuição dos níveis de BDNF, poderemos afirmar se ratos sobreviventes de sepse possuem, ou não, parâmetros relacionados a depressão.

Os antidepressivos são a primeira linha de tratamento para depressão. São substâncias eficazes na remissão de sintomas característicos da depressão, e entre os antidepressivos se encontra a imipramina.

A imipramina, droga clássica antidepressiva, é uma amina terciária do grupo dos tricíclicos. É responsável por inibir a receptação da noradrenalina e da serotonina. Possui também afinidade por receptores colinérgicos (Ach), adrenérgicos (alfa-1), histamínicos (h1) e 5HT2 (receptor da serotonina). Provoca, ainda, uma redução na sensibilidade dos receptores beta-adrenérgicos. A droga está indicada em estados de depressão maior, episódios depressivos do transtorno bipolar, transtorno do pânico, fobia-social e distimia (Stahl, 2002).

2 MODELO ANIMAL DE SEPSE

Estudos de sepse em humanos são difíceis devido a severidade da doença, a necessidade de intervenções terapêuticas imediatas e a heterogeneidade dos pacientes.

Assim, modelos animais têm sido usados extensivamente para explorar a patogênese e gerar dados pré-clínicos de intervenções terapêuticas. Para tanto deve-se utilizar um modelo animal que reproduza a vasodilatação, hipotensão, aumento do débito cardíaco, resposta ao tratamento e mortalidade vistos em pacientes sépticos. Tem-se utilizado para isto modelo de sepse abdominal, sepse cutânea, sepse induzida pela administração de lipopolissacarídeo (LPS) ou fator de necrose tumoral.

Porém, os modelos que induzem peritonite são mais amplamente usados. A peritonite pode ser induzida por inoculação direta de bactérias ou de conteúdo fecal na cavidade peritoneal. Entretanto o modelo mais aceito na literatura, e que parece simular mais adequadamente o quadro clínico de sepse, é o chamado CLP - Cecal Ligation and Perforation (Ligação e Perfuração Cecal).

A CLP se baseia na ligação do ceco logo abaixo da válvula ileo-cecal

(mantendo desta maneira o trânsito intestinal), perfuração do ceco com tamanho padronizado e liberação de conteúdo fecal para a cavidade peritoneal, conforme classicamente descrito por Wichterman e Cols (1980). Desta maneira, além da peritonite, se induz isquemia mesentérica, simulando as grandes síndromes clínicas de sepse abdominal (p.ex. apendicite, isquemia mesentérica). Recentemente este modelo foi modificado para melhor simular as características clínicas dos pacientes com sepse abdominal, introduzindo desta maneira a ressuscitação volêmica e emprego de antibióticos de amplo espectro (Hubbard et al., 2005).

3 MEMÓRIA

Memória é conceituada como a capacidade de guardar a informação aprendida para posteriormente ser utilizada, possuindo diversas fases de processamento e consolidação (Cahil and Mcgaugh, 1996).

Os tipos de memórias podem ser classificados de acordo com a sua função, e dentre esses tipos encontramos a memória de trabalho, que serve para manter durante segundos ou minutos as informações que são processadas no momento. A memória de trabalho e a memória imediata podem ser consideradas sinônimas. A memória de trabalho é processada fundamentalmente no córtex pré-frontal, que atua agrupado com o córtex entorrinal, parietal superior e cingulado anterior, e com o hipocampo (Vianna et al., 2000).

A memória também pode ser classificada, de acordo do seu conteúdo, como memória declarativa ou explícita, que está relacionada a fatos, eventos ou conhecimentos. Neste tipo de memória se observa que a consolidação da memória necessita de um amplo circuito neural, onde as principais estruturas envolvidas são a amígdala, o hipocampo e o córtex entorrinal. Já as memórias procedurais, ou implícitas, são associadas aos comportamentos, hábitos e habilidades do indivíduo,

sendo que este tipo de memória é evocado principalmente para eventos não-verbalizados. As redes neurais que estão mais ligadas à memória não-declarativa são: as aferências corticais de áreas sensoriais de associação, estriado, caudado, putâmen, cerebelo e as estruturas que envolvam os núcleos da base ou estruturas extrapiramidais (Izquierdo e Medina, 1997).

Segundo Izquierdo (2002), as memórias podem ser classificadas pelo tempo que duram. Com exceção da memória de trabalho, as memórias declarativas podem durar minutos, horas, dias ou algumas décadas, enquanto as memórias procedurais, em sua grande maioria, duram por toda vida.

A memória de curta e de longa duração são fenômenos independentes, paralelos, com os mesmos conteúdos, que ocorrem em áreas com similaridades, como a região de CA1 do hipocampo, o córtex entorrinal, e o córtex parietal, mas os mecanismos são diferentes. Além das áreas, os axônios liberam neurotransmissores-facilitadores de memórias. Os facilitadores de memória agem nos receptores glutamatérgicos NMDA (N-methyl-d-aspartate) responsáveis pelos mecanismos de formação da memória (Izquierdo et al., 1997).

Algumas situações causam um aumento na circulação de epinefrina e corticoesteróides. Ambos são conhecidos como moduladores de memória. A dexametasona (glicocorticóide sintético) pertence à classe dos corticosteróides, como outros hormônios esteróides, que atuam controlando a velocidade de síntese de proteínas. Seu principal efeito se refere a uma profunda alteração na resposta imune linfocitária, representada pela ação antiinflamatória e imunossupressora, podendo prevenir ou suprimir processos inflamatórios de várias naturezas, inclusive resultantes de radiações, mecânicos, químicos e infecciosos (Kaplan, 1999). Embora o efeito não atinja a doença de base, seu uso é muitas vezes de vital importância na supressão do processo histopatológico natural ou para combater efeitos secundários de outras drogas. Todavia, a inibição quase instantânea da liberação do hormônio adenocorticotrófico (ACTH) ocasionada pelos corticosteróides representa provavelmente uma excreção. Os mesmos reagem com proteínas receptoras no citoplasma das células sensíveis de muitos tecidos, formando um complexo esteróide-receptor. O complexo sofre uma modificação, que se manifesta por aumento da constante de sedimentação; a seguir, move-se para o núcleo, onde se

liga à cromatina e regula a transcrição de genes específicos (Stahl, 2002).

Estudos prévios revelam que injeções de dexametasona facilitaram a retenção da memória testada em ratos com o uso da esquiva inibitória. Isto se deu pelo mecanismo mediado pela ativação dos núcleos basolateral e medial da amígdala (Roozendaal & McGaugh, 1996).

A glicose é uma das substâncias básicas dos seres vivos. Constituinte normal do sangue humano, provem de absorção alimentar no tubo digestivo, ou por decomposição do glicogênio, sua forma de armazenamento do organismo. Uma vez dentro da célula, a glicose é prontamente fosforilada, formando a *Glicose-6-fosfato*, que logo se polimeriza em glicogênio, ou é catabolizada (Stahl, 2002).

Brandt et al. (2006) realizou pesquisa que investigou os efeitos da administração da glicose sobre o desempenho cognitivo e encontrou efeitos benéficos em adultos jovens saudáveis, adultos mais velhos, e mesmo em adultos com patologias cognitivas severas como a doença de Alzheimer. Embora os benefícios no desempenho cognitivo que foram encontrados ocorram em uma escala de tarefas cognitivas, no geral parece que a administração de glicose tem um efeito pronunciado em testes da memória a longo prazo.

Outros sistemas também participam da regulação da memória, como os receptores opióides. Injeções pós-treino de naloxone (antagonista opióide) melhorou a resposta dos ratos submetidos ao teste de esquiva inibitória (Izquierdo & Dias, 1985). Sun et al. (2005) relata que danos no sistema endógeno opióide poderiam danificar significativamente a aprendizagem e a memória, mas que os mesmos podem ser invertidos pelo naloxone (NAL), um receptor antagonista. Ficou demonstrado que o NAL pode exercer efeitos benéficos em déficits da memória e que o NAL pode facilitar a aprendizagem e a memória nos ratos com demência.

É importante ressaltar que os sintomas de dano cognitivo, como a afetação da memória, a depressão e a ansiedade, devem ser melhor relacionados com a sepse para posteriormente justificar-se os mecanismos que envolvem o dano cognitivo.

Estudo anterior (Barichello et al., 2005) comparou os resultados de ratos sobreviventes de sepse (10 dias após) com os resultados de um grupo controle sadio quando submetidos a testes de memória, atenção, concentração e

aprendizagem, constatando que os animais sobreviventes de sepse apresentaram déficits cognitivos significativos.

Sendo assim, mostra-se importante verificar as possíveis alterações comportamentais em ratos sobreviventes de sepse em um modelo de ligação e perfuração cecal (CLP) após 10, 30 e 60 dias da cirurgia, e os efeitos da aplicação de facilitadores de memória e da imipramina, para melhor compreender o mecanismo neurobiológico envolvidos nesta síndrome, possibilitando que se possam determinar novas terapias da sepse.

4 OBJETIVOS

4.1 Objetivo Geral

Avaliar as alterações de comportamento e a resposta aos psicofármacos em ratos sobreviventes de sepse.

4.2 Objetivos Específicos

- Avaliar as alterações comportamentais em ratos sobreviventes de sepse

após 10, 30 e 60 dias da CLP.

- Avaliar as respostas dos facilitadores de memória em ratos sobreviventes de sepse após 10 e 30 dias da CLP.

- Avaliar o efeito do antidepressivo imipramina no teste do nado forçado em ratos sobreviventes de sepse após 10 dias da CLP.

5 MATERIAIS E MÉTODOS

Este trabalho apresenta a compilação de três experimentos, desenvolvidos ao longo dos anos de 2006 a 2008, que utilizaram ratos sobreviventes de sepse para verificar as possíveis alterações comportamentais que se apresentaram e os efeitos da aplicação de facilitadores de memória e da imipramina.

No primeiro experimento os animais foram submetidos a seis testes comportamentais depois de decorridos 10, 30 e 60 dias da CLP: Habituação ao Campo Aberto (Open-Field), Memória de Reconhecimento de Objetos (Object Recognition), Esquiva Inibitória (Step-down Inhibitory Avoidance Task), Esquiva Inibitória de Treinos Contínuos (CMIA), Labirinto em Cruz Elevado (Elevated Plus-Maze) e o Teste de Nado Forçado (Forced Swimming Test) - (Anexo1).

No segundo experimento, após 10 e 30 dias da CLP os animais foram submetidos ao teste de Esquiva Inibitória, e após o treino receberam injeções de epinefrina (25µg/kg), nolaxone (0,4 mg/kg), dexametazona (0,3 mg/kg), glicose (320 mg/kg) e salina (controle) - (Anexo 2).

No terceiro experimento, após 10 dias da CLP os animais receberam imipramina (10 mg/kg) ou salina, e foram submetidos ao teste do Nado Forçado - (Anexo 3).

5.1 Modelo Animal de Sepse

Esta técnica intra-abdominal foi produzida usando a técnica de ligação cecal e perfuração (CLP), conforme previamente descrito (Fink et al., 1990). Os ratos foram anestesiados com fenobarbital, sendo submetidos a laparotomia com incisão mediana abdominal. O ceco foi ligado logo abaixo da junção íleo-cecal com fio seda 3-0, mantendo assim a continuidade intestinal. O ceco foi perfurado com uma agulha número 14 na face antimesentérica, e foi gentilmente comprimido até a extrusão de conteúdo fecal. Os planos cirúrgicos foram fechados e os ratos observados em caixa de recuperação por 2 horas. O grupo controle foi submetido a laparotomia, o ceco manipulado, mas não houve ligação ou perfuração. A principal vantagem deste modelo é sua simplicidade e a semelhança com diversos problemas clínicos, como, por exemplo, apendicite e diverticulite (Finketal.,1990).

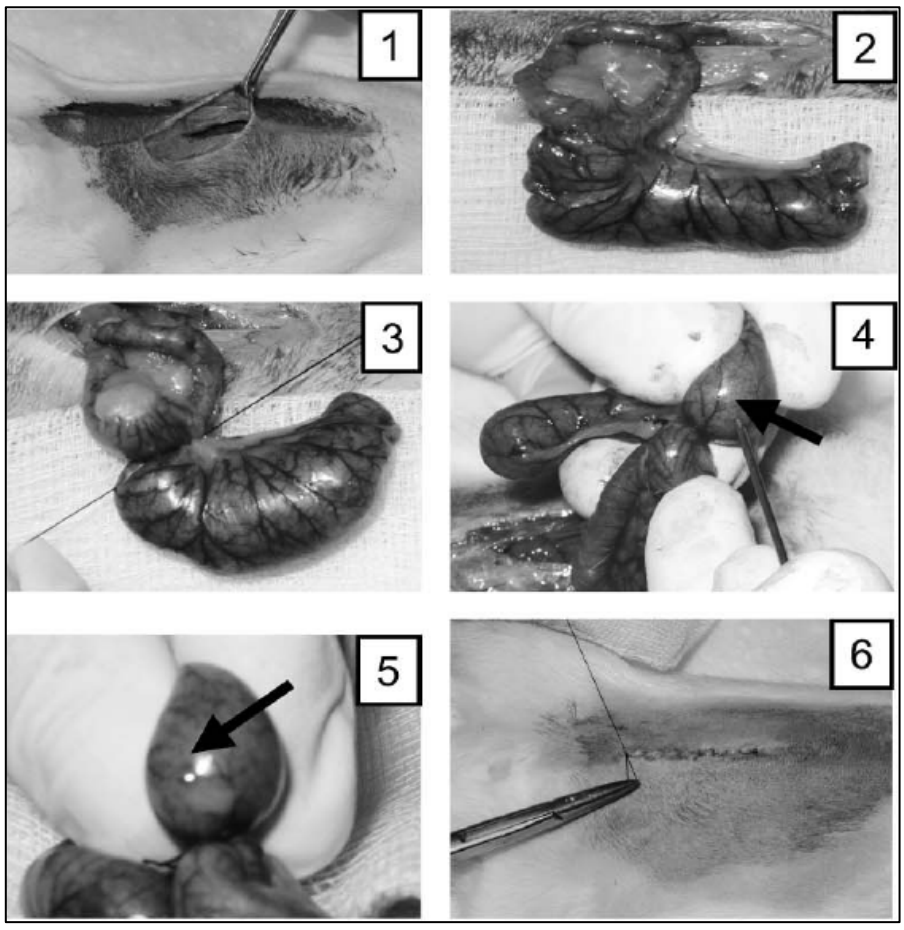


Figura 01: Modelo Animal de Sepse

5.2 Testes Comportamentais

5.2.1 Habituação ao campo aberto (Open-Field Task)

Neste teste comportamental foi avaliada a atividade exploratória. Embora seja ainda de difícil definição, o termo “atividade exploratória” é amplamente utilizado em pesquisas relacionadas ao comportamento animal. Num sentido geral, refere-se a todas as atividades relacionadas à obtenção de informação acerca do ambiente, as quais abrangem não só respostas reflexas atencionais imediatas, como também as respostas voluntárias típicas. A adoção desse tipo de teste apresenta uma clara conveniência pela facilidade de registro comportamental, quando comparado ao estudo no ambiente natural. O pressuposto básico envolvido em estudos de confinamento em um novo ambiente é que no intuito de explorar o ambiente o animal precisa locomover-se nele. Dessa forma, a quantidade de movimento passa a ser um indicador de atividade exploratória. A resposta exploratória de levantar-se nas patas traseiras (rearing) é também muito comum em roedores e tem sido utilizada como medida do nível de excitabilidade, uma vez que esse comportamento freqüentemente se correlacionam com outras atividades como a auto-limpeza corporal (crossing), defesa e reações sexuais.

Este teste avalia o desempenho motor na sessão de treinamento e memória não associativa no teste de retenção de memória.

O teste foi realizado em um campo aberto de 40 x 60 cm, delimitado por 4 paredes com 50 cm de altura, sendo 3 de madeira e uma de vidro transparente. O piso do campo aberto é dividido em 12 quadrados iguais marcados por linhas pretas.

Na sessão de treino os animais foram cuidadosamente colocados no quadrado do canto posterior esquerdo do aparelho, a partir do qual podia explorar livremente o ambiente por 5 minutos. Imediatamente após os animais voltaram para

a caixa-moradia. A sessão de teste foi realizada 24 horas após o treino.

Os números de cruzamentos através das linhas pretas e o número de “rearings” foram avaliados em ambas as sessões.

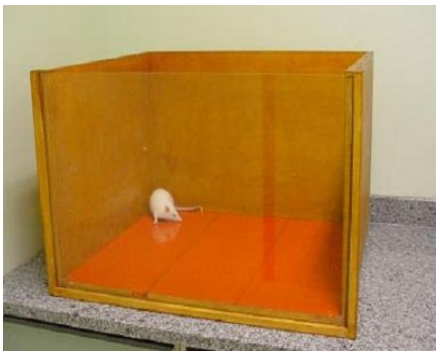
Crossing: atividade motora. Rearings: atividade exploratória (Vianna et al., 2000).

Figura 02: Habituação ao Campo Aberto

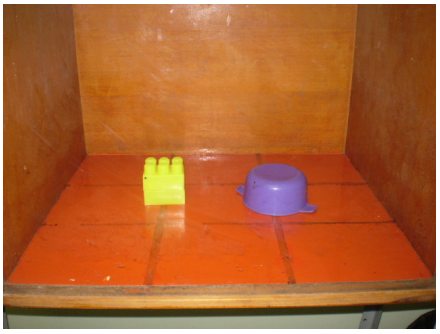
5.2.2 Memória de reconhecimento de objetos (Object Recognition Memory)

Para a realização deste teste utilizou-se o campo aberto. No primeiro dia realiza-se o treino, onde o animal é colocado cuidadosamente no quadrado do canto posterior esquerdo do aparelho, do qual explorará o ambiente por 5 minutos sem nenhum objeto. O primeiro dia serve como habituação do animal. No segundo dia o

animal é recolocado no aparelho, no qual estarão dois objetos iguais, objeto A e objeto B (forma, tamanho e cor) que foram posicionados em dois cantos adjacentes a 10 cm da parede, conta-se o tempo que o animal explora cada objeto (A e B). No mesmo dia, 1 hora 30 minutos depois se testa a memória de curta duração, quando o animal explora novamente o ambiente na presença do primeiro objeto familiar (objeto A) e o novo objeto (objeto C), conta-se novamente o tempo total que o animal explora cada objeto (Lima et al., 2004). No dia seguinte (24 horas após) avalia-se a memória de longa duração onde será feito o mesmo procedimento trocando o objeto C pelo objeto D (diferente do objeto A), conta-se o tempo de exploração de cada objeto. Este teste avalia as memórias de curta e longa duração. Todos os objetos tinham textura (lisa) e tamanho (150-200 gramas) semelhante, mas formas diferentes. Neste teste é utilizado o índice de reconhecimento para calcular o tempo gasto para cada animal para explorar o objeto, expressado como uma razão (TB/(TA+TB) onde TA = tempo gasto para explorar o objeto familiar; e TB = tempo gasto para explorar o novo objeto.


Habituação


Treino


01:30 h


Após 24h

Figura 03: Memória de reconhecimento de objeto

5.2.3 Esquiva inibitória (Step-down Inhibitory Avoidance Task)

Este teste avalia a memória aversiva. O equipamento consiste em uma caixa de acrílico (50 x 25 x 25 cm) na qual o piso é formado por barras paralelas de metal (1 mm de diâmetro). Os espaços entre as barras medem 1 cm. Uma plataforma com 7 cm de largura e 2,5 cm de altura é colocada junto à parede esquerda do aparelho (Quevedo et al., 1997; 1999; Roesler et al., 2003; 2004).

Na sessão de treino os animais foram colocados sobre a plataforma e mediu-se o tempo que o animal levava para descer com as quatro patas da plataforma. Esse tempo é denominado latência. Imediatamente após descer da plataforma (com as 4 patas), o animal recebeu um choque de 0,4 µA durante 2 segundos.

Na sessão de teste, o animal foi novamente colocado na plataforma e mediu-se o tempo que ele levava para descer (latência), porém não foi administrado choque.

A latência é um parâmetro clássico de retenção de memória. Os intervalos entre o treino e o teste foram de 1,5 horas para medir memória de curta duração (Izquierdo et al., 1998; Bevilaqua et al., 2003) e 24 horas para memória de longa duração (Izquierdo et al., 1998; Quevedo et al., 1997; 1999; Roesler et al., 2003; 2004).

5.2.4 Esquiva inibitória de treinos contínuos

Nesse teste usa-se também a esquiva inibitória. Na sessão de

treinamento o animal foi colocado na plataforma e imediatamente depois de pisar nas barras de metal recebeu um choque 0.3 µA, durante 2 segundos. Este procedimento continua até que o animal permaneça na plataforma por 50 segundos. O animal é devolvido então a sua caixa moradia (Barichelo et al.; 2005). O teste de memória de longo prazo é realizado 24 horas após.

Figura 04: Esquiva Inibitória

5.2.5 Labirinto em cruz elevado (Elevated Plus-Maze)

É um teste utilizado para avaliar ansiedade. O aparelho consiste em dois braços abertos (50 x 10 cm) e dois braços fechados (50 x 10 x 40 cm) dispostos de forma perpendicular formando uma plataforma central (5 x 5 cm). Os experimentos são conduzidos em sala escura, com luz vermelha posicionada a 50 cm de altura da plataforma central. Os animais foram colocados na plataforma central e tinham 5 minutos para explorar o aparelho. Os parâmetros avaliados são: número de entradas e tempo de permanência no braço aberto e fechado, e número total de entradas em ambos (Pellow et al.,1985).

Figura 05: Labirinto em cruz elevado

5.2.6 Nado forçado (Forced Swimming Test)

Este teste avalia sintomas de comportamento depressivo. Consiste em dois dias de procedimentos, no qual cada rato é posto em um tanque cilíndrico de plástico com 80 cm de altura e 30 de diâmetro, com água a 23º C, sendo que a água é suficiente para o animal não conseguir apoiar as patas no fundo ou escapar. No primeiro dia os ratos são forçados a nadar 15 minutos. No segundo dia (passadas 24 horas), cada animal é novamente forçado a nadar durante 5 minutos. São avaliados os parâmetros de imobilidade, nos quais se incluem imobilidade total ou movimentos para manter a cabeça flutuando da água sem intenção de escapar (Porsolt et al., 1977; Detke et al., 1995; Viana et al., 2005).

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ANEXO A – Artigo "Time-dependent behavioral recovery after sepsis in rats", publicado na "Intensive Care Med" - DOI 10.1007/s00134-008-1129-1.

Intensive Care Med
DOI 10.1007/s00134-008-1129-1

EXPERIMENTAL

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Time-dependent behavioral recovery after sepsis in rats

Received: 28 November 2007
Accepted: 8 April 2008

Supported, in part, by UNESC (Brazil), FAPESC (Brazil) and CNPq (Brazil).

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Abstract *Objective:* To evaluate the cognitive performance in rats that survived sepsis induced by cecal ligation and puncture (CLP) after 10, 30 and 60 days. *Design:* Prospective, controlled experiment. *Setting:* Animal basic science laboratory. *Subjects:* Male Wistar rats, weighing 300–350 g. *Interventions:* The rats were sham-operated or submitted to CLP (sepsis group) with "basic support" (saline, s.c. at 50 mL/kg immediately and 12 h after CLP plus ceftriaxone, s.c. at 30 mg/kg and clindamycin, s.c. at 25 mg/kg 6, 12 and 18 h after CLP). *Measurements and main results:* The animals underwent six behavioral tasks 10, 30 and 60 days after surgery: (a) habituation to the open field; (b) inhibitory avoidance task; (c) continuous multiple trials step-down inhibitory avoidance task; (d) object recognition; (e) elevated plus-maze; and (f) forced swimming test. We demonstrated that survivors 10 days after CLP presented deficits on the habituation to the open field, step-down inhibitory avoidance, continuous multiple-trials step-down inhibitory avoidance, object recognition and forced swimming. After 30 days of sepsis induction, survivors maintained deficits on the step-down inhibitory avoidance, continuous multiple-trials step-down inhibitory avoidance and forced swimming. However, after 60 days all behavior deficits were reversed. *Conclusions:* These results indicate that the impairment of memory and learning, demonstrated 10 days after the induction of sepsis, persist 30 days after the CLP. The cognitive impairments did not persist after 60 days suggesting that this model can help in the understanding of the biological mechanisms associated with sepsis-induced sickness behavior.

Keywords Sepsis · Learning · Memory · Depression · Anxiety · Central nervous system

Introduction

Recently, some studies demonstrated that survivors from intensive care units (ICU) presented cognitive impairment in long-term period, including alterations in memory, attention, concentration and/or the global loss of cognitive function and impairment in the quality of life [1–5].

The mechanisms associated with these findings are not well understood, but could include brain inflammation, oxidative stress and neuron apoptosis [6–8]. In this context, murine models of cecal ligation and perforation (CLP) are clinically relevant since they induce a polymicrobial sepsis that mimics human sepsis [9–13], contributing to the elucidation of the pathogenesis and to the determination of new therapies in sepsis [8, 11]. We

had previously demonstrated that survivors from the CLP model had several deficits on cognitive skills [9, 10], but no previous study has examined a time-dependent pattern of cognitive deficits in CLP survivors. Thus, we present here data regarding a time-dependent recovery on several cognitive deficits, and depression and anxiety like behavior in CLP survivors.

Materials and methods

Animals

Male Wistar rats (3–4 months, 220–310 g) were obtained from our breeding colony (UNESC). The animals were housed five in a cage with food and water available ad libitum and maintained on a 12-h light/dark cycle (lights on at 7:00 a.m.). All experimental procedures involving animals were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care.

Cecal ligation and perforation surgery

The animals were subjected to CLP as previously described [11]. Briefly, rats were anesthetized with a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg) given intraperitoneally. The cecum was tightly ligated with a 3.0-silk suture at its base, below the ileocecal valve, and perforated once with a 14-gauge needle. The cecum was then gently squeezed to extrude a small amount of feces from the perforation site, returned to the peritoneal cavity, and the laparotomy was closed with 4.0-silk sutures. The animals were resuscitated with normal saline (50 mL/kg subcutaneous) immediately and 12 h after CLP. All animals were returned to their cages with free access to food and water. In the sham-operated group, the rats were submitted to all surgical procedures, but the cecum was neither ligated nor perforated. After surgery, the sepsis group received 30 mg/kg ceftriaxone, s.c. and 25 mg/kg clindamycin, s.c., every 6 h for a total of 3 days. The sham-operated group received the volume of saline corresponding to antibiotic administration.

To minimize the possibility of the animals not truly developing sepsis, the CLP procedure was always performed by the same investigators. In addition, all animals were observed after CLP to determine signs of infection (pyloerection, lethargy, tachypnea and weight loss), and the number of animals that survived was in accordance with our previous reports [9, 11]. To perform memory experiments, 140 animals underwent sham operation, and the survival in this group was 100%. A total of 360 animals were submitted to CLP; around 40% of these animals survived to perform behavioral tests. The death of the animals was within the first 5–7 days after CLP, and those that survived were free of infection after 10 days with normal motor activity [9].

Behavioral tests

The animals separately underwent six behavioral tasks: habituation to an open field, step-down inhibitory avoidance, continuous multiple-trials step-down inhibitory avoidance task, object recognition, elevated plus-maze and forced swimming test 10, 30 and 60 days after surgery. Thus, using this design we do not assess time-dependent memory, but assess memory over time (with new training at each test session). All behavioral procedures were conducted between 13:00 and 16:00 hours in a sound-isolated room, and a single animal performed only one behavior test in only one time point after surgery. All behavioral tests were recorded by the same person who was blind to the animal group.

Habituation to the open field task

This task evaluates motor performance in the training section and non-associative memory in the retention test session. Habituation to an open field was carried out in a 40 × 60 cm open field surrounded by 50 cm high walls made of brown plywood with a frontal glass wall. The floor of the open field was divided into 12 equal rectangles by black lines. The animals were gently placed on the left rear quadrant and left to explore the arena for 5 min (training session). Immediately following this, the animals were taken back to their home cage and 24 h later submitted again to a similar open-field session (test session). Crossing of the black lines and rearing performed in both sessions were counted. The decrease in the number of crossings and rearings between the two sessions was taken as a measure of the retention of habituation [14].

Step-down inhibitory avoidance task

This task evaluates aversive memory. The apparatus and procedures have been described in previous reports [15]. Briefly, the training apparatus was a 50 × 25 × 25 cm acrylic box (Albarsch, Porto Alegre, Brazil) whose floor consisted of parallel caliber stainless steel bars (1 mm diameter) spaced 1 cm apart. A 7 cm-wide, 2.5 cm-high platform was placed on the floor of the box against the left wall. In the training trial, animals were placed on the platform and their latency to step down on the grid with all four paws was measured with an automatic device. Immediately after stepping down on the grid, the animals received a 0.4 mA, 2.0 s foot shock and returned to their

home cage. A retention test trial was performed 24 h after training (long-term memory). The retention test trial was procedurally identical to training, except that no foot shock was presented. The retention test step-down latency (maximum, 180 s) was used as a measure of inhibitory avoidance retention.

Reactivity to the foot shock was evaluated in the same apparatus used for inhibitory avoidance, except that the platform was removed. Each animal was placed on the grid and allowed a 1-min habituation period prior to the start of a series of shocks (0.5 s), delivered at 10-s intervals. Shock intensities ranged from 0.1 to 0.5 mA in 0.1-mA increments. The adjustments in shock intensity were made in accordance to each animal's response. The intensity was raised by 1 unit when no response occurred and lowered by 1 unit when a response was made. A "flinch" response was defined as withdrawal of one paw from the grid floor, and a "jump" response was defined as rapid withdrawal of three or four paws. Two measurements of the "flinch" threshold were made and then two measurements of the "jump" threshold were made. For each animal the mean of the two scores for the flinch and the jump thresholds was calculated.

Continuous multiple-trials step-down inhibitory avoidance task

This task evaluates aversive memory in the test section and learning when analyzing the number of training trials required for the acquisition criterion (see below). It was performed in the same step-down inhibitory avoidance apparatus; however, in the training session, the animal was placed on the platform and immediately after stepping down on the grid, received a 0.3 mA, 2.0 sec foot shock. This procedure continued until the rat remained on the platform for 50 s. The animal was then returned to the home cage. The number of training trials required to reach the acquisition criterion of 50 s on the platform was recorded. The retention test was performed 24 h later (long-term memory) [9].

Object recognition

This task evaluates non-aversive, non-spatial memory. The apparatus and procedures for the object recognition task have been described elsewhere [16, 17]. Briefly, the task took place in a 40 × 50 cm open field surrounded by 50 cm-high walls made of plywood with a frontal glass wall. The floor of the open field was divided into 12 equal rectangles by black lines. All animals were submitted to a habituation session where they were allowed to freely explore the open field for 5 min. No objects were placed in the box during the habituation trial. Crossings of the black lines and rearings performed in this session were evaluated as locomotors and exploratory activity, respectively. Different times after habituation, training was conducted by placing individual rats for 5 min in the field, in which two identical objects (objects A1 and A2, both being cubes) were positioned in two adjacent corners, 10 cm from the walls. In a short-term recognition memory test given 1.5 h after training, the rats explored the open field for 5 min in the presence of one familiar (A) and one novel (B, a pyramid with a square-shaped base) object. All objects had similar textures (smooth), colors (blue), and sizes (weight 150–200 g), but distinctive shapes. A recognition index calculated for each animal is reported as the ratio TB/(TA + TB) (TA = time spent exploring the familiar object A; TB = time spent exploring the novel object B). In a long-term recognition memory test given 24 h after training, the same rats were allowed to explore the field for 5 min in the presence of the familiar object A and a novel object C (a sphere with a square-shaped base). Recognition memory was evaluated as done for the short-term memory test. Exploration was defined as sniffing (exploring the object 3–5 cm away from it) or touching the object with the nose and/or forepaws.

Elevated plus-maze

The apparatus used in animal models for anxiety has been described in detail elsewhere [18, 19]. Briefly, the apparatus consisted of two open arms (50 × 10 cm) and two closed arms (50 × 10 × 40 cm) arranged in such a way that the two arms of each type were opposite to each other, with a central platform (5 × 5 cm). The maze's height was 50 cm and the tests were conducted under dim red light. Animals were exposed for 5 min to the red light in their own home cages before the testing procedure. Next, they were placed individually on the central platform of the plus-maze facing an open arm. During a 5-min test period, the following measurements were recorded by two observers: the number of entries, the time spent in the open and closed arms, and the total number of arm entries.

Forced swimming test

The test was conducted according to previous reports [20, 21], and was used as a model for depressive behavior. Briefly, the test involves two exposures to a cylindrical water tank in which rats cannot touch the bottom or from which they cannot escape. The tank is made of transparent plexiglass, 80 cm tall, 30 cm in diameter, and filled with water (22–23°C) to a depth of 40 cm. Water in the tank was changed for each rat. For the first exposure, the rats were placed in the water for 15 min (pre-test session). After 24 h, the rats were placed in the water again for

a 5-min session (test session). The periods of immobility were analyzed. The rats were judged to be immobile whenever they stopped swimming and remained floating in the water, with their head just above water level.

Statistical analysis

Data from the open-field task were analyzed with ANOVA followed by Tukey post hoc and expressed as mean ±SEM. Data from the inhibitory avoidance task, object recognition task and the number of training trials from continuous multiple trials step-down inhibitory avoidance are reported as median and interquartile ranges and comparisons among groups were performed using Mann–Whitney U tests. The within individual groups were analyzed by Wilcoxon tests. The data for the elevated plus-maze and forced swimming tests are reported as means ±SEM and were analyzed by the Student's t test. Unpaired t tests were performed on the flinch and jump scores, comparing the sham and septic groups. In all comparisons, $P < 0.05$ indicated statistical significance.

Results

The experiments conducted here replicate part of our previous published results [9, 10, 22]. In behavior tests that did not present impairments on 10 or 30 days after sepsis, we did not perform the 60-day analyses (to avoid unnecessary use of experimental animals).

Habituation to the open-field task

In the open-field task, there were no differences in the number of crossings and rearings between groups in the habituation to the open-field training session ($P > 0.05$), demonstrating no difference in motor and exploratory activity between groups. In the test session, there was a significant reduction in both crossings ($df = 28.9$, $P < 0.001$) and rearings ($df = 26.1$, $P < 0.001$) of the sham group compared to the sepsis group at 10 days (suggesting memory impairment), but not 30 days after sepsis (Fig. 1).

The step-down inhibitory avoidance

The step-down latency in the inhibitory avoidance in the test section, 10 ($Z = -3.07$, $P = 0.002$) and 30 days ($Z = -2.93$, $P = 0.003$) after CLP, but not 60 days, was significantly decreased in the sepsis group when compared with the sham group (Fig. 2), suggesting impaired aversive memory.

Since sepsis could affect sensory processing during training, such as the rats' reactivity to the foot shock, rather than memory, we evaluated the effects of sepsis on foot shock sensitivity in all analyzed time points, and there were no significant differences between groups in the flinch or the jump nociceptive thresholds, showing that sepsis did not affect the animal's reactivity to the foot shock (data not shown).

Continuous multiple-trials step-down inhibitory avoidance task

In the continuous multiple trials step-down inhibitory avoidance, 10 and 30 days, but not 60 days, after CLP we demonstrated a significant increase in the number of training trials required to reach the acquisition criterion (50 s on the platform) in the sepsis group compared to the sham group (Fig. 3a). The results of this task suggest that the sepsis group required approximately two times more

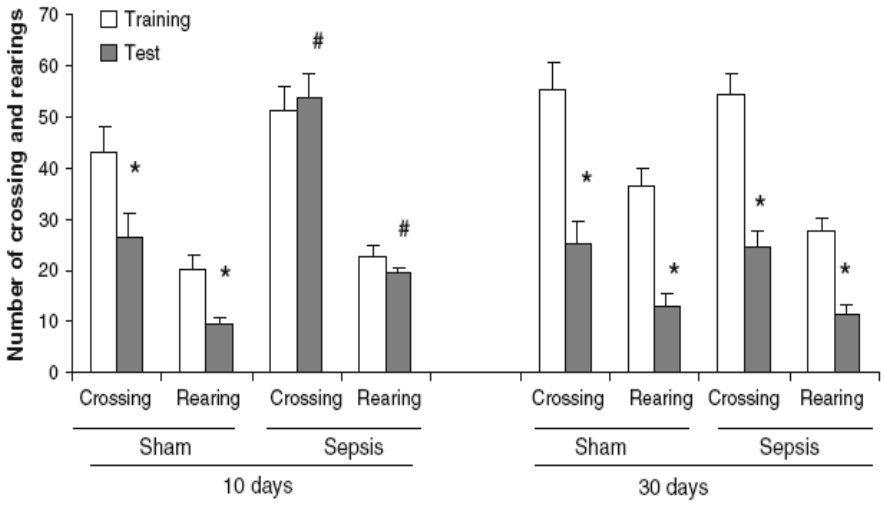

Fig. 1 Habituation on the open-field. Animals were submitted to CLP or sham-operated. They underwent the training test on the open-field task 10 or 30 days after surgery and were tested 24 h later. Data are presented as mean ± SEM, $n = 10$ rats per group. # $P < 0.05$ versus sham and * $P < 0.05$ versus training

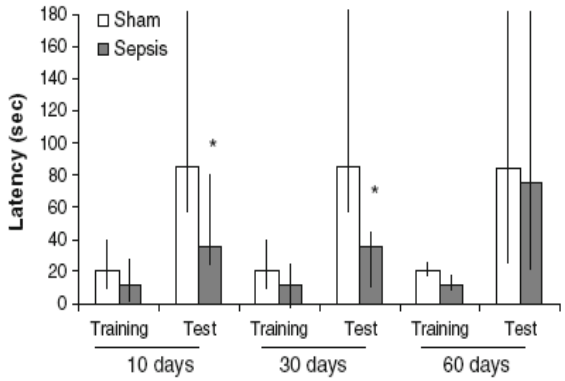

Fig. 2 The step-down inhibitory avoidance. Animals were submitted to CLP or sham-operated. They underwent the training test on the step-down inhibitory avoidance task 10, 30 or 60 days after surgery and were tested 24 h later. Data are presented as median and interquartile ranges, $n = 10$ rats per group. * $P < 0.05$ versus sham

stimulus to reach the acquisition criterion compared with the sham group 10 and 30 days after CLP, indicating learning impairment. In the retention test, there was no difference between groups for all the times tested (Fig. 3b).

Object recognition

At 10, but not 30 days, after CLP, the animals presented impairment of novel object recognition memory, i.e., they did not spend a significantly higher percentage of time exploring the novel object during short ($Z = -3.07$, $P = 0.002$) and long-term ($Z = -2.77$, $P = 0.006$) retention test sessions in comparison to the training trial (Fig. 4). In addition, sepsis group (10 days) presented a significant reduction in the recognition index in short and long-term recognition retention tests compared to the sham group (Fig. 4).

Elevated plus-maze task

There were no statistically significant differences in the number of entries (Fig. 5a, $df = 21.6$, $P = 0.63$) or in the time spent (Fig. 5b, $df = 21.9$, $P = 0.32$) in the arms between groups 10 days after surgery, suggesting that CLP survivors did not present anxiety-like symptoms.

Forced swimming test

In the test session (5 min), we observed a significant increase in the immobility time in the sepsis group compared to the sham group 10 ($df = 11.5$, $P = 0.01$) and 30 ($df = 13.7$, $P < 0.001$) days after surgery (Fig. 6),

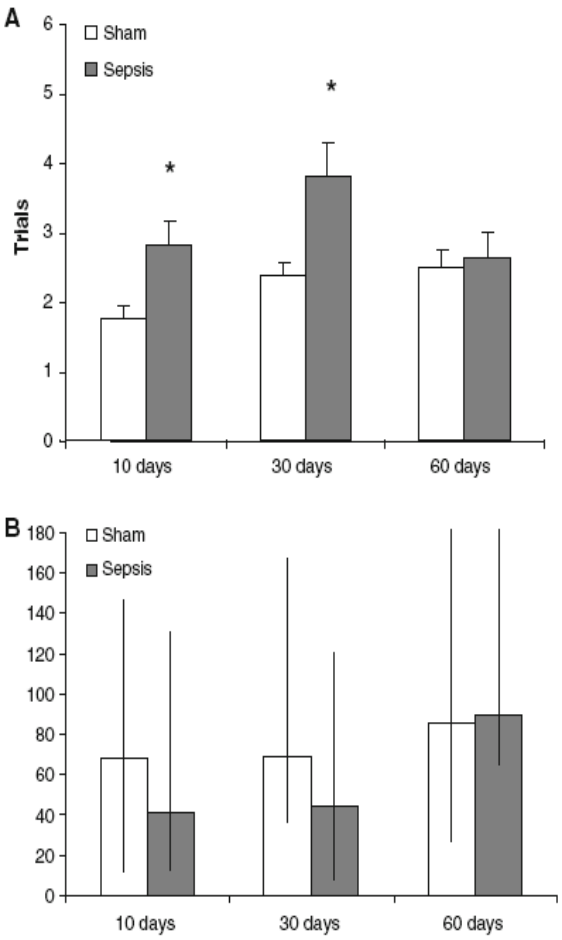

Fig. 3 Continuous multiple-trials step-down inhibitory avoidance task. Animals were submitted to CLP or sham-operated. They underwent the training test on the continuous multiple-trials step-down inhibitory avoidance task 10, 30 or 60 days after surgery and were tested 24 h later. **a** Training trials required to reach the acquisition criterion (50 s on the platform). Data are presented as mean ± SEM, $n = 10$ rats per group. * $P < 0.05$ versus sham. **b** Retention in the test section. Data are presented as median and interquartile ranges, $n = 10$ rats per group

suggesting depressive-like behavior. In contrast, after 60 days there were no statistically significant differences between groups (Fig. 6).

Discussion

Our results demonstrated that septic animals 10 days after the surgery presented memory and learning deficits and depressive-like symptoms. These impairments were time dependent, since after 30 days of sepsis rats presented a normal performance on the object recognition test and after 60 days animals presented a normal performance in all behavior tests performed. These results resemble some

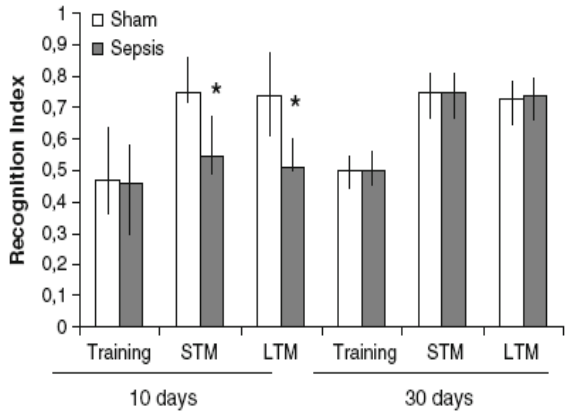

Fig. 4 Object recognition. Animals were submitted to CLP or sham-operated. They underwent the training test on the object recognition task 10 or 30 days after surgery and were tested 1.5 h (short-term memory, STM) or 24 h (long-term memory, LTM) later. Data are presented as median and interquartile ranges, $n = 10$ rats per group, * $P < 0.05$ versus sham

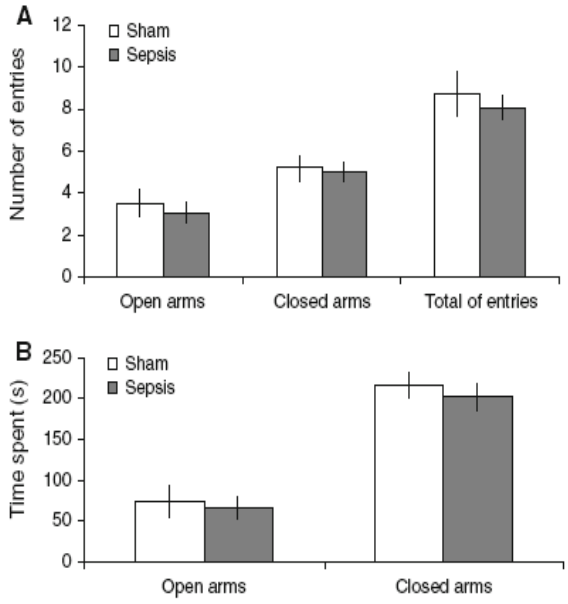

Fig. 5 Elevated plus-maze task. Animals were submitted to CLP or sham-operated. They underwent the elevated plus-maze task 10 days after surgery. **a** Number of entries on the closed and open arms. **b** Time spent on the closed and open arms. Data are presented as mean ± SEM, $n = 10$ rats per group

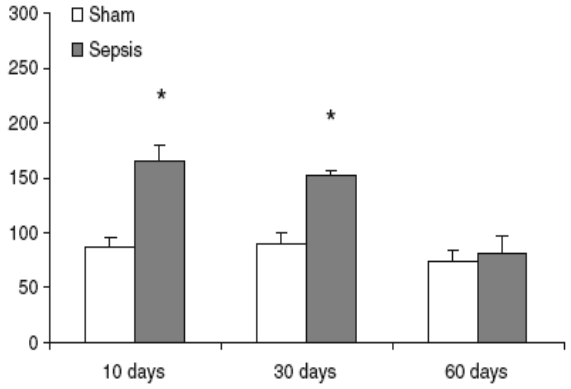

Fig. 6 The forced swimming test. Animals were submitted to CLP or sham-operated. They underwent the forced swimming test 10, 30 or 60 days after surgery and the immobility time was recorded. Data are presented as mean ± SEM, $n = 10$ rats per group. * $P < 0.05$ versus sham

of the findings observed in prospective studies on survivors from ICU that demonstrated a gradual, but not complete, recovery from behavior deficits during follow-up [2].

Several critically ill patients presented significant chronic neurocognitive impairments at 2, 6 and 9 months, and 1, 2 and up to 6 years after leaving the hospital. These neurocognitive impairments improved during the first 6–12 months, but were severe enough to disturb the patients' daily function and quality of life [3, 23]. For example, 55% of surviving ARDS/sepsis patients recover their neurocognitive impairments after 1 year [24]. Patients who did not recover in the first year from ICU discharge maintain the neurocognitive deficits after 2 years [3]. In general, these impairments include mental processing speed, memory, language and visuospatial abilities, of which only memory was assessed in our animal model.

Depressive symptoms were also relevant after ICU discharge [25], with an incidence in 10–58% of the patients [24, 26–28]. In addition, it was estimated that 47% of these patients had clinically significant anxiety symptoms [25], but when compared to the general ICU survivors, sepsis patients reported significantly fewer problems in the anxiety/depression dimension [1]. We demonstrated here that depressive and anxiety-like symptoms also occurred in our animal model, and, at least for anxiety symptoms, these alterations were reversible early after CLP.

In this context, our presented model mimics several aspects of the clinical setting and could be an important tool in the understanding the mechanisms of behavioral deficits and recovery in sepsis survivors. Semmler et al. [29] demonstrated that behavioral alterations were matched by sepsis-induced loss of neurons in the hippocampus and the prefrontal cortex and reduced cholinergic innervation in the parietal cortex in LPS-induced sepsis in rats. In addition, we demonstrated that oxidative damage occurred early in the course of sepsis, mainly in the hippocampus [7], and this could be associated with long-term behavioral deficits [8]. In addition, we previously demonstrated that depressive-like symptoms in sepsis survivors were reversed by imipramine treatment, suggesting that the mechanisms of depressive behavior are not specific to sepsis, but share features with general depressive symptoms [30].

Clearly, the behavior alterations observed in ICU survivors seemed to persist, which is different from that demonstrated by us here. There is probably not a single uniform cause of neurocognitive impairments in the ICU setting, but possible mechanisms may include hypoxemia, the use of sedatives or analgesics, hypotension, delirium and hyperglycemia that interact dynamically with premorbid variables [31]. In addition, sepsis survivors reported significantly fewer problems in the anxiety/depression dimension, and there was a trend towards fewer problems being reported by sepsis survivors although in other dimensions of the EuroQol five-dimension questionnaire [1]. Thus, in our model, we did not study all these potential mechanisms responsible for human long-term alterations, but only the isolated effect of sepsis on neurocognitive impairment and this may explain why our animals had a full recovery, differing from that observed in humans.

In addition, the concept of sickness behavior could help to elucidate some of the mechanisms associated with long-term cognitive (dys)function after CLP. The peripherally produced cytokines in response to an infection could act on the brain leading to behavioral symptoms related to sickness [32]. In this context, it is possible that cytokines produced during septic response could contribute to the development of long-term cognitive impairment, and the use of G-CSF could prevent long-term impairment as well as sickness behavior [33].

The presented model could be a useful tool to study cognitive impairment, depression and anxiety after sepsis, and the mechanisms associated with the recovery of these functions.

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ANEXO B – Artigo "Memory-Enhancing Treatments Reverse the Impairment of Inhibitory Avoidance Retention in Sepsis-Surviving Rats", submetido à "Intensive Care Med".

Memory-Enhancing Treatments Reverse the Impairment of Inhibitory Avoidance Retention in Sepsis-Surviving Rats

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Supported by UNESC (Brasil) and CNPq (Brasil).

None of the authors have any financial interests to disclose.

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ABSTRACT

Objective: To evaluate the effects of memory enhancers in sepsis survivors rats.

Design*:* Prospective, controlled experiment.

Setting*:* Animal basic science laboratory.

Subjects: Male Wistar rats, weighing 300–350 g.

Interventions: The rats underwent cecal ligation and perforation (CLP) (sepsis group) with "basic support" (saline at 50 mL/kg immediately and 12 hrs after CLP plus ceftriaxone at 30 mg/kg and clindamycin at 25 mg/kg 6, 12, and 18 hrs after CLP) or sham-operated (control group). After 10 or 30 days rats was submitted to an inhibitory avoidance task and after training received injections of saline (SAL), epinephrine (EPI), naloxone (NAL), dexamethasone (DEX) or glucose (GLU) and after 24 hours were submitted to the inhibitory avoidance test.

Measurements and Main Results: We demonstrated that memory-enhancers reversed impairment in the sepsis group 10 and 30 days after sepsis induction. This effect was of lower magnitude when compared to sham animals 10, but not 30, days after sepsis.

Conclusion: Using different pharmacologic approaches we conclude that the adrenergic memory formation pathways are responsive in sepsis survivors animals.

Key Words: rat sepsis survivors, cognitive impairment, memory enhancers, inhibitory avoidance task

INTRODUCTION

Central nervous system (CNS) dysfunction secondary to sepsis can occur in 8-70% of septic patients (1). In addition, it has been demonstrated that survivors from sepsis presented long-term cognitive impairment, including alterations in memory, attention, concentration and global loss of the cognitive function (2). However, the mechanisms associated to these alterations are still unclear. We had previously demonstrated that sepsis survivors after ten and thirty days of cecal ligation and perforation (CLP) presented memory impairment and behavior alterations, and we proposed this model as an useful tool to determine the mechanisms associated to long-term cognitive impairment in sepsis survivors (3-7).

The aversively motivated learning is influenced by neuromodulators and hormones related to emotional aspects of the training experience. Emotionally arousing events cause a release of epinephrine (EPI) and an increase in corticosterone, and both EPI and corticosteroids are known to modulate memory (8). Other systems could modulate the formation of emotionally motivated memory. For example, opioid receptors are involved in memory modulation, and post-training injections of the opioid antagonist naloxone (NAL) enhance retention of inhibitory avoidance in rats (9).

In this context, we investigated if some of the molecular mechanisms associated to memory formation are preserved in sepsis survivors using the post-training administration of EPI, NAL, dexamethasone (DEX) and glucose (GLU) using a step-down inhibitory avoidance task in rats.

MATHERIALS AND METHODS

Animals: Two hundred and forty (240) adult male Wistar rats (220–300 g), were obtained from our breeding colony. They were housed five to a cage with food and water available ad libitum and were maintained on a 12-h light/dark cycle (lights on at 7:00 AM). Behavioral procedures were conducted between 8:00 and 12:00. All experimental procedures involving animals were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care, and approval for the study was given by the ethics committee from our University.

Cecal ligation and perforation surgery: Animals were subjected to CLP as described (10) with adaptations (11-13). Briefly, rats were anesthetized with a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg), given intraperitoneally. Under aseptic conditions, a 3-cm midline laparotomy was performed to allow exposure of the cecum with the adjoining intestine. The cecum was tightly ligated with a 3.0-silk suture at its base, below the ileocecal valve, and was perforated once with a 14-gauge needle. The cecum was then gently squeezed to extrude a small amount of feces from the perforation site returned to the peritoneal cavity, and the laparotomy was closed with 4.0-silk sutures. Animals were resuscitated with normal saline (50 mL/kg subcutaneous) immediately and 12 h after CLP. All animals were returned to their cages with free access to food and water. In the sham-operated group the rats were submitted to all surgical procedures but the cecum was neither ligated nor perforated. After surgery, the sepsis group received “basic support” (30 mg/kg ceftriaxone and 25 mg/kg clindamycin, subcutaneous, every 6 h for a total of 3 days). The sham-operated group received the volume of saline corresponding to antibiotic administration. Survival in the sham group was 100%, and in the sepsis group was

Inhibitory avoidance: The inhibitory avoidance procedure was described in previous reports (14). The apparatus was a 50 x 25 x 25-cm acrylic box whose floor consisted of parallel caliber stainless-steel bars (1 mm diameter) spaced 1 cm apart, 7-cm-wide, 2.5-cm-high platform was placed on the floor of the box against the left wall. Animals were placed on the platform and their latency to step down on the grid with all four paws was measured with an automatic device. Training sessions were performed 10 or 30 days after surgery, and immediately after stepping down on the grid, animals received a 0.3-mA, 2-s foot shock. In test sessions, carried out 24 h after training, no foot shock was given and the step-down latency (maximum 180 s) was used as a measure of retention. The behavioral tests were performed by the same person who was blinded to the experimental group.

Intervention: The animals were divided in 15 per group and received an i.p. injection of saline (control), epinephrine (25μg/kg), naloxone (0.4 mg/kg), dexamethasone (0.3 mg/kg), or glucose (320 mg/kg) immediately after training. Those doses were selected on the basis of previous reports (14).

Statistical Analyses: Data for inhibitory avoidance is presented as median (interquartile range) of retention test latencies. Differences between training and test session latencies within each group were determined using a Wilcoxon test. The Kruskal-Wallis test was performed in comparisons between groups. To the

comparison between various treatments the Mann-Whitney with the Bonferroni adjust modified by Finner was used.

RESULTS

As expected, classical memory enhancers, epinephrine (Z=-3.05, p=0.002 for both 10 and 30 days after surgery, comparing training and test), naloxone (Z=-3.06, p=0.002 for both 10 and 30 days after surgery, comparing training and test), dexamethasone (Z=-3.05, p=0.002 for both 10 and 30 days after surgery, comparing training and test) or glucose (Z=-3.06, p=0.002 for both 10 and 30 days after surgery, comparing training and test) improved memory in the inhibitory avoidance 10 and 30 days after sham surgery (Figure 1 and 2). Ten days after CLP epinephrine (Z=-3.18, p=0.001, comparing training and test), naloxone (Z=-3.06, p=0.002, comparing trainings and test), dexamethasone (Z=-3.06, p=0.002, comparing trainings and test) or glucose (Z=-3.06, p=0.002, comparing trainings and test) reversed memory impairment, but this effect was of lower magnitude when compared to sham animals (Figure 1). In addition, memory enhancers reversed memory impairment 30 days after sepsis induction, in the same magnitude when compared to sham animals (Z=-3.18, p=0.001 to epinephrine, comparing training and test; Z=-3.06, p=0.002 to naloxone, comparing training and test; Z=-2.93, p=0.003 to dexamethasone, comparing training and test; Z=-3.06, p=0.002 to glucose, comparing training and test) (Figure 2).

DISCUSSION

The present study demonstrated that the administration of memory enhancers (epinephrine, naloxone, dexamethasone, or glucose) in sepsis survivors

reverses long-term cognitive impairment. These suggest that, instead of the demonstrated neuronal loss after sepsis (15), the molecular mechanisms associated to memory formation are preserved in sepsis survivors. The effect of cognitive enhancers seemed to be of different magnitude 10 or 30 days after sepsis suggesting that the mechanisms responsible to memory formation were more compromised early after sepsis recovery. This observation is consistent with our previous results that demonstrated a time-dependent recuperation of memory deficits in sepsis survivors rats (3-7).

All the used memory enhancers seemed to exert its effect by modulate adrenergic system, and there is extensive evidence that catecholamines have profound effects on cognitive function (16). Immediate post-training systemic injections of epinephrine or norepinephrine enhance the consolidation and/or storage of novel information in rats (16). The enhancing effects of glucocorticoids on memory consolidation depend on the integrity of the amygdala noradrenergic system (17) as do the enhancing effects of naloxone (18). The effects of noradrenergic system on memory formation seemed to be dependent on glucose, since a noradrenergic agonist enhances memory formation by facilitation glucose uptake at the time of memory consolidation (19). These effects are not restricted to animal models. Recent evidence indicates that epinephrine enhances memory consolidation in humans (20). In addition, it is now well established that glucocorticoid hormones enhance memory consolidation (21) and that glucose modulates memory formation in humans (22). Opioid peptides mediates alterations in human memory during heightened emotional states, and help to explain why memories may be selectively deficient under conditions of stress (23). Thus, since survivors from of ICU presented long-term cognitive impairment, including alterations in memory, and this was associated to a

decrease on quality of life (24) our results opens the perspective to improve long-term outcome in sepsis survivors.

Some limitations of our study must be pointed. First, septic animals in comparison to sham controls received antibiotics, which could have neuroprotective properties (25). We had demonstrated previously that the antibiotics used in our model did not modify memory performance in our model (4), thus we believe that this limitation is of minor importance. Second, it would be interesting to examine the effects of other drugs more promising as clinically useful cognitive enhancers (i.e. rolipram) (26), but since this is the first demonstration of enhancing memory after CLP we decided to use more "classical" memory enhancers. Third, only single doses of the memory enhancers were evaluated, thus, instead of a normal response observed using these doses, we could not rule out the possibility that in sepsis survivors the dose response curve to these enhancers may be altered. Forth, we demonstrated that sepsis altered memory of an emotional event (i.e. foot shock). One may suggest that the response to a new stimulus depends on the intensity of a previous emotional challenge and that we are not observing a true sepsis effect, but a procedure-related effect. We tried to avoid this limitation randomly dividing animals between groups, and animals were subjected to the same surgical procedure, being sepsis the solely difference between groups. In addition, these animals presented deficits not only related to emotional memory, but also related to object and environmental recognition (3,4,5). There are also some clues that suggest that animals are similar regarding the stress-response. First, in the open-field task, there were no differences in the number of crossings and rearings between groups in the training session, demonstrating no difference in motor and exploratory activity between groups (3,4), and stressed animals presented alterations in the exploratory

activity (27). Second, when analyzing at 10 and 30 days after CLP sham and septic animals presented no differences on foot shock sensitivity as assessed by the “flinch and jump” response test (28).

CONCLUSION

We demonstrated, for the first time, using different pharmacologic approaches, that the adrenergic system is responsive in sepsis survivors animals, in different intensities 10 and 30 days after sepsis. Since this system is relevant to memory formation in humans and animals our results opens the perspective that the modulation of adrenergic system could be a suitable tool to the treatment of memory deficits observed in sepsis survivors.

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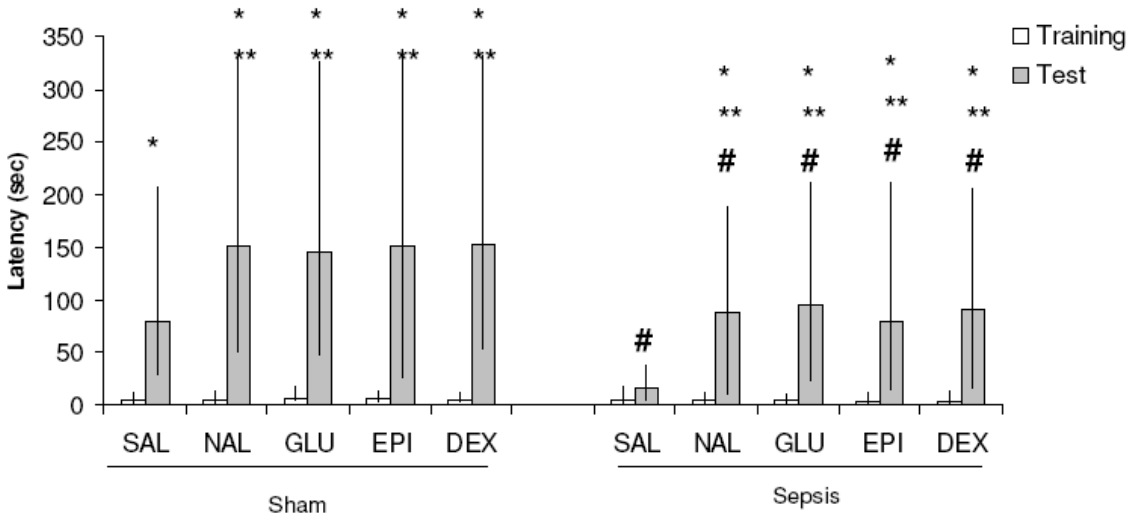
LEGEND TO FIGURES

1. Inhibitory Avoidance Task ten days cecal ligation and perforation (CLP). Animals were submitted to CLP or sham-operated. Ten days after surgery animals underwent the training test on the inhibitory avoidance task. Immediately after training animals received a single injection of saline (SAL), epineohrine (EPI), naloxone (NAL), dexamethasone (DEX) or glucose (GLU), and animals were tested 24 hours after. Data are presented as median (interquartile range) of retention test latencies. * significantly different between training and test, $p<0.05$, Wilcoxon test; ** significantly different between NAL, GLU, EPI or DEX and SAL in the test section, $p<0.05$, Mann-Whitney test (Kruskal-Wallis chi-square 13.4, $p=0.009$); # significantly different between sham and CLP in the test section, $p<0.05$ Mann-Whitney test (Kruskal-Wallis chi-square 27.48, $p<0.001$).

2. Inhibitory Avoidance Task thirty days cecal ligation and perforation (CLP). Animals were submitted to CLP or sham-operated. Thirty days after surgery animals underwent the training test on the inhibitory avoidance task. Immediately after training animals received a single injection of saline (SAL), epineohrine (EPI), naloxone (NAL), dexamethasone (DEX) or glucose (GLU), and animals were tested 24 hours after. Data are presented as median (interquartile range) of retention test latencies. * significantly different between training and test, $p<0.05$, Wilcoxon test; ** significantly different between NAL, GLU, EPI or DEX and SAL in the test section, $p<0.05$, Mann-Whitney test (Kruskal-Wallis chi-square 27.7, $p<0.001$); # significantly different between sham and CLP in the test section, $p<0.05$ Mann-Whitney test (Kruskal-Wallis chi-square 30.8, $p=0.001$).

FIGURES

1.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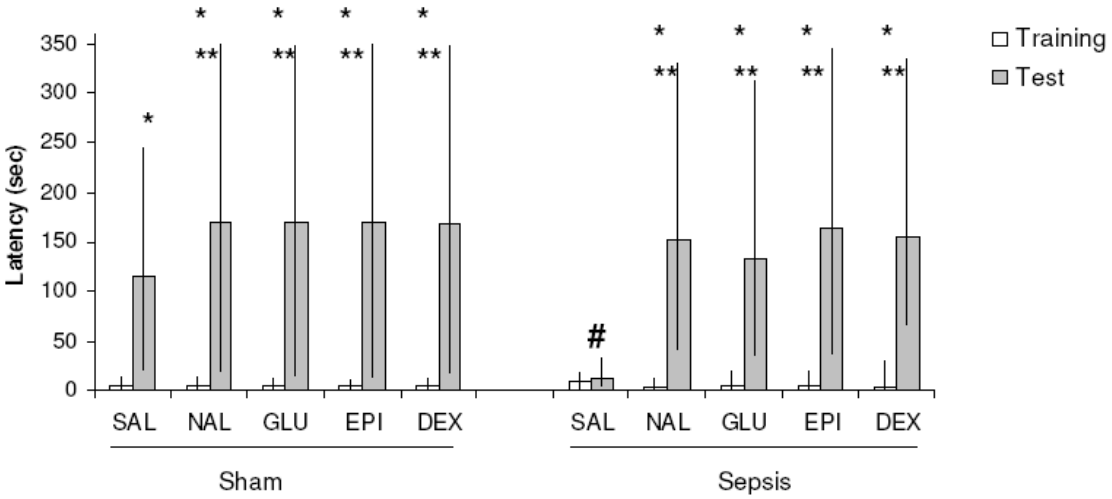
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ANEXO C– Artigo “Imipramine reverses the depressive symptomsin sepsis survivor rats”, publicado na “Intensive Care Med” - DOI 10.1007/s00134-007-0804-y.

Intensive Care Med
DOI 10.1007/s00134-007-0804-y

BRIEF REPORT

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Imipramine reverses the depressive symptoms in sepsis survivor rats

Received: 2 April 2007
Accepted: 6 July 2007

This research was supported in part by UNESC (Brazil), FAPESC (Brazil), and CNPq (Brazil).

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Abstract *Objective:* To evaluate the antidepressant effect of imipramine on depressive symptoms observed in sepsis survivors rats. *Design and setting:* Prospective, controlled experiment in an animal basic science laboratory. *Subjects:* Male Wistar rats weighing 300–350 g. *Interventions:* The rats underwent cecal ligation and perforation (CLP; sepsis group) with “basic support” (saline at 50 ml/kg immediately and 12 h after CLP plus ceftriaxone at 30 mg/kg and clindamycin at 25 mg/kg 6, 12, and 18 h after CLP) or sham-operated (control group). After 10 days of recovery rats received intraperitoneal injections of imipramine 10 mg/kg or saline and were subjected to the forced swimming test. *Measurements and results:* The observed increase in the immobility time in the forced swimming test in animals subjected to CLP, as a parameter of depressive behavior, was reversed by imipramine. *Conclusions:* The depressive symptoms evaluated by forced swimming test had been reversed after imipramine administration. Our data provide evidence that CLP-induced depressive symptoms are sensitive to antidepressants.

Keywords Sepsis · Survivors · Cecal ligation and puncture · Depressive-like symptoms · Rat

Introduction

Despite major improvements in intensive care and antibiotic therapy mortality and morbidity due to severe sepsis and septic shock remain high. Critical illness survivors present long-term cognitive impairment, including alterations in memory, attention, concentration, and/or global loss of cognitive function and beyond some clinical studies that show depressive symptoms in survivors from severe diseases as sepsis and septic shock [1–5]. Cecal ligation and perforation (CLP) models have contributed to elucidate the pathogenesis and to determine new therapies in sepsis [2–4]. Previous studies have shown that sepsis survivors rats after 10 days of operation presented symptoms of depression in the forced swimming task [5]. We evaluated whether depressive symptoms induced by CLP are sensitive to antidepressant drug imipramine.

Materials and methods

Under anesthesia (80 mg/kg ketamine, 10 mg/kg xylazine) 105 male Wistar rats (300–350 g) underwent CLP (sepsis group) and 60 rats underwent sham operation (control group) as previously described [3]. After surgery the sepsis group received “basic support” (saline at 50 ml/kg

immediately and 12 h after CLP plus 30 mg/kg ceftriaxone and 25 mg/kg clindamycin every 6 h over a total of 3 days). The sham-operated group received only saline (50 ml/kg) immediately and 12 h after surgery, and the volume of saline corresponded to antibiotic administration. Survival in the sham group was 100% and in the sepsis group 40% (40 rats). The number of survivals is in accordance with our previous reports [3–5]. Ten days after surgery the animals separately underwent two behavioral tasks: (a) forced swimming test (FST) to evaluate depressive-like symptoms, and (b) the open-field task as a control experiment to evaluate locomotor activity.

The behavioral tests were performed by the same person, who was blinded as to group (SHAM or CLP). All experimental procedures involving animals were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and with the approval by the local ethics committee. The forced swim test was conducted according to previous reports [6, 7]. In brief, the test involves two exposures to a cylindrical tank of water in which rats cannot touch the bottom of the tank or escape. The tank is made of transparent Plexiglas, 80 cm tall, 30 cm in diameter, and was filled with water (22–23 °C) to a depth of 40 cm. Water in the tank was changed after each rat. For the first exposure rats were placed in the water for 15 min (pretest session). The rats had been treated with imipramine in the doses of 10 mg/kg or saline 5 min and 19 and 23 h after the first swimming exposure. This procedure resulted in four experimental groups: SHAM + SAL, SHAM + IMI, CLP + SAL, and CLP + IMI. After 24 h the rats were placed in the water again for a 5 min session (test session). Behavior was videotaped for later analysis, and the periods of immobility and swimming time were recorded.

The behavior in the open field was carried out as a control for locomotor activity in a 40 × 60 cm open field surrounded by 50-cm-high walls made of brown plywood with a frontal glass wall. The floor of the open field was divided into 12 equal rectangles by black lines. Animals were gently placed on the left rear quadrant, and left to explore the arena for 5 min, and their number of crossings was measured. Crossing of the black lines performed in this session were counted [8].

Data are presented as mean ± SEM and were analyzed by analysis of variance followed by Tukey's post-hoc test if necessary.

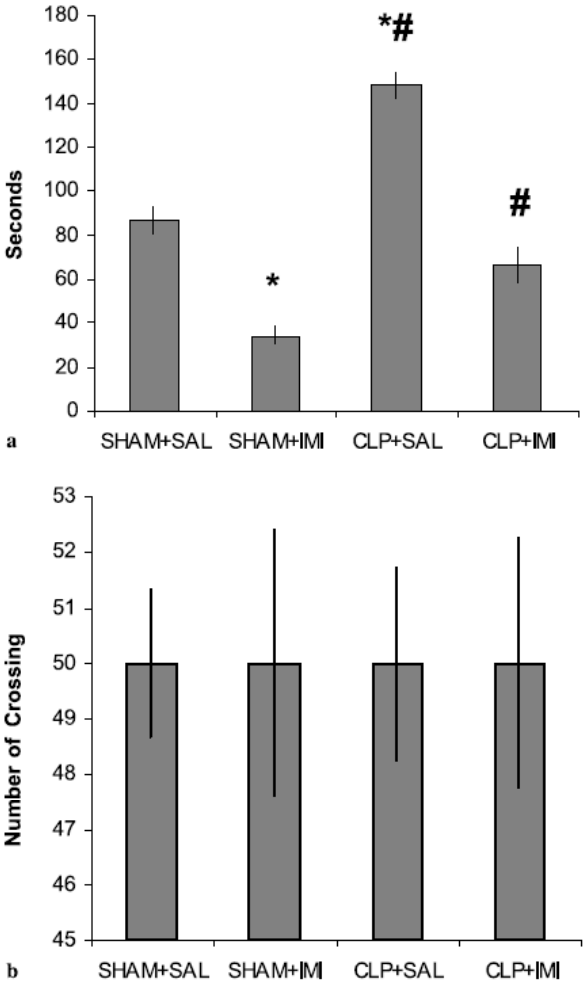

Fig. 1 **a** Forced swimming task. Sepsis group showed a significant increase in the time of immobility, and this behavior was reversed by imipramine. Data are expressed as mean ± SEM, $n = 15$ animals per group; * $p < 0.05$ vs. CLP + SAL and SHAM + IMI with SHAM + SAL, # $p < 0.05$ vs. CLP + IMI and CLP + SAL with SHAM + IMI. **b** Behavior in the open-field. No significant difference was seen in the numbers of crossings between groups in the open-field behavior. Data are expressed as mean ± SEM, $n = 15$ animals per group

Results

In the forced swimming test (Fig. 1A) session depressive-like behavior was observed as a significant increase in the immobility time in the CLP + SAL group ($p < 0.05$) as our group previous described [5]. As we also expected SHAM + IMI group presented a reduction in the immobility time ($p < 0.05$) as a demonstration of validity of our protocol. Our main result was a reduction in the immobility time observed in the CLP + IMI group ($p < 0.05$) was not similar that observed for SHAM + IMI group ($p > 0.05$). These results demonstrate that imipramine reverses the depressivelike symptoms induced by CLP.

In the open-field test (Fig. 1B) no difference in motor activity was demonstrated between groups as observed by the number of crossings ($p > 0.05$), demonstrating

no impairment in motor activity secondary to CLP or imipramine.

Discussion

Previous studies have shown that sepsis survivors presented depressivelike symptoms assessed in the FST [5]. The original view of the FST offered by Porsolt [6] was that of a model of depression with similar features to the learned helplessness model but technically easier to produce. The internal affective state of rodents after exposure to the initial swim in the forced swimming task was labeled as "behavioral despair." The pretest swim induction procedure was similar procedurally to the initial session that induces learned helplessness by exposing rats to inescapable stress. Induction of learned helplessness produces broad-ranging behavioral deficits in affect, cognition, sleep, and motor performance that closely resemble many of the symptoms of depression [8]. Additionally, as described above, the sepsis survivors group did not present locomotor activity impairment, reinforcing that higher immobility time in sepsis group was related to depressivelike symptoms [7].

The FST is undoubtedly the most extensively used rodent model of depression [9]. This model has a high degree of pharmacological validity, as evidenced by its sensitivity to major classes of antidepressants, tricyclic compounds, monoamine oxidase inhibitors, atypical antidepressants, selective serotonin reuptake inhibitors, and electroconvulsive shock [9]. Some researchers believe that the FST should be considered no more than a simple screen for antidepressant drugs [10]. However, because of its sensitivity to antidepressants and to stimuli that provoke depressive behavior, the FST seems to measure a behavioral dimension that is relevant to depression [10]. Some disagreement may arise between the original claim that the FST is a rodent model of human depression and the more careful consideration and use of the FST as an objective marker for a behavioral state associated with depression. Thus our findings should be considered with these limitations in mind.

Several cognitive skills are impaired in animals and humans survivors from sepsis [1–5], and we here demonstrated that the depressive behavior induced by sepsis was reversed by imipramine. In addition, the efficacy of imipramine suggested that the immobility in the FST observed in sepsis survivors was not a nonspecific finding related to the effects of sepsis in the central nervous system but a disorder with typical physiopathological alterations that could be pharmacologically explored. In this way we believe that the CLP model of sepsis will help us to investigate the biological mechanisms involved in the symptoms of depression associated with sepsis and to determine therapeutic approaches to this problem.

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