Review Article

Establishment and evaluation of animal models of sepsis-associated encephalopathy

Mubing Qin[1], Yanxia Gao[2], Shigong Guo[3], Xin Lu[1], Qian Zhao[4], Zengzheng Ge[1], Huadong Zhu[1], Yi Li[1]

[1] *Emergency Department, State Key Laboratory of Complex Severe and Rare Diseases, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing 100730, China*
[2] *Emergency Department, the First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China*
[3] *Department of Rehabilitation Medicine, Southmead Hospital, Southmead Road, Bristol BS10 5NB, UK*
[4] *Health Service Department of the Guard Bureau of the Joint Staff Department, Beijing 100017, China*

Corresponding Author: Yi Li, Email: billliyi@126.com

BACKGROUND: Sepsis-associated encephalopathy (SAE) is a critical disease caused by sepsis. In addition to high mortality, SAE can also adversely affect life quality and lead to significant socioeconomic costs. This review aims to explore the development of evaluation animal models of SAE, giving insight into the direction of future research in terms of its pathophysiology and therapy.

METHODS: We performed a literature search from January 1, 2000, to December 31, 2022, in MEDLINE, PubMed, EMBASE, and Web of Science using related keywords. Two independent researchers screened all the accessible articles based on the inclusion and exclusion criteria and collected the relevant data of the studies.

RESULTS: The animal models for sepsis are commonly induced through cecal ligation and puncture (CLP) or lipopolysaccharide (LPS) injection. SAE can be evaluated using nervous reflex scores and sepsis evaluation during the acute phase, or through Morris water maze (MWM), open-field test, fear condition (FC) test, inhibitory avoidance, and other tests during the late phase.

CONCLUSION: CLP and LPS injection are the most common methods for establishing SAE animal models. Nervous reflexs cores, MWM, FC test, and inhibitory avoidance are widely used in SAE model analysis. Future research should focus on establishing a standardized system for SAE development and analysis.

KEYWORDS: Sepsis; Sepsis-associated encephalopathy; Animal model; Systematic review

INTRODUCTION

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection, which leads to the mortality of millions of patients per year.[1,2] Sepsis-associated encephalopathy (SAE) or sepsis-associated delirium (SAD) is a critical disease caused by sepsis. According to a recent study, 30%–70% of sepsis patients develop SAE.[3,4] The acute phase of SAE is associated with high mortality,[5] while the later phase is associated with cognitive dysfunction. In addition to high mortality, SAE can also adversely affect life quality and lead to significant socioeconomic costs.[6-8]

SAE is a disease with complex pathophysiologic changes.[9] Currently, there is no consensus on pathophysiology or targeted pharmacological therapy.[10] Animal models can provide information on SAE etiology and treatment. However, there is a lack of a standard SAE animal model.[11] The aim of this systematic review is to investigate available SAE animal models, which may provide directions for future research.

METHOD

We performed a literature search from January 1, 2000, to December 31, 2022, in MEDLINE, PubMed, EMBASE, and Web of Science using the following

keywords: "sepsis" "sepsis-associated encephalopathy" "sepsis-associated delirium" "cognition OR cognitive dysfunction", combined with the terms "models, animal" "animals" "mouse" and "rat". Studies were excluded if the following exclusion criteria were met: (1) animal studies on the lung, liver, and other organ dysfunctions and (2) clinical studies. Two independent researchers screened all the accessible articles based on the above-mentioned inclusion and exclusion criteria and collected the relevant data of the studies. If any controversies about studies arose between the two researchers in the process of data collection, a third researcher was consulted.

RESULTS
Animal models of sepsis
Cecal ligation and puncture (CLP)

The CLP model is considered the "gold standard" animal model for sepsis in preclinical studies.[12-14] C57BL/6 mice and Long-Evans rats are the most commonly used experimental animals. After general anesthesia, rats or mice undergo laparotomy, and their cecum are isolated. Using a 4-0 silk nonabsorbable surgical thread, the cecum is ligated and punctured by 21G (mice)/18G (rats) needles. The severity of sepsis is determined by the proportion of ligation. For low-grade sepsis, 40% of the cecum is ligated, while for high-grade sepsis, 75% of the cecum is ligated. The rats or mice are resuscitated with 0.9% sterile saline solution after placing the cecum back into the abdominal cavity.[15] This model can induce similar reactions in terms of inflammatory, immune, hemodynamic, and biochemical alterations. Therefore, the CLP model is commonly used in sepsis-induced cognitive impairment studies.

Lipopolysaccharide (LPS) injection

LPS-induced sepsis is another common sepsis model. LPS is one of the main mediators of the clinical manifestations of infection and systemic inflammation. LPS is a highly toxic molecule derived from gram-negative bacterial membranes. *Escherichia coli* is the most common gram-negative bacterium in sepsis studies. The mechanism of LPS-induced sepsis is the release of cytokines, leading to a systemic cytokine storm. LPS is usually injected into the abdominal cavity; however, in an animal model by Lee et al,[16] the researchers directly injected LPS into the lateral ventricle of a rat brain that resulted in memory dysfunction, indicating cognitive impairment.

SAE evaluation during the acute phase

In supplementary Table 1, we created a scoreboard to analyze SAE during the acute phase, including nervous reflex scores and sepsis evaluation.

Nervous reflex scores

After CLP surgery or LPS injection, the acute phase of SAE can be detected by abnormal neurobehaviors. Five categories need to be considered: corneal reflex, righting reflex, auricular reflex, tail-flick reflex, and avoidance reflex.[17] The corneal reflex is a reflex action of the eye resulting in automatic closing of the eyelid when the cornea is stimulated. The righting reflex is induced by placing a mouse/rat in a supine position and observed whether the animal can turn to the prone position. The auricular reflex is induced by touching the auditory meatus to observe if the animal turns its head. The tail-flick reflex is induced by stimulating the tails of a mouse/rat to observe whether the animal attempts to withdraw. The avoidance reflex is used to observe whether a mouse/rat attempts to escape from a tail-flick reflex. If a mouse/rat has normal reflexes, it receives two points. If a mouse/rat has delayed reflexes within 10 s, it receives 1 point. Finally, if a mouse/rat has no response, it receives 0 point (supplementary Table 2).

Sepsis evaluation

Previous studies using animal models evaluate the responses from sepsis or septic shock using scoring systems such as the Mouse Clinical Assessment Score for Sepsis (M-CASS) and the Murine Sepsis Score (MSS).[18-20] The septic manifestations of any animal model are based on the behaviors of the animals, including consciousness, respiratory rate, and response to stimulation. Weight loss has also been observed in most studies on sepsis. In addition to behavior observation, biomarkers of sepsis may also be used. Cytokines, such as interleukin (IL) and monocyte chemotactic protein-1 (MCP-1), which are common biomarkers of infection, can be detected in plasma, abdominal ascitic fluid, or brain tissue (i.e., hippocampus) through enzyme-linked immunosorbent assay (ELISA).[21]

SAE evaluation during the late phase
Morris water maze (MWM) and other mazes

The MWM test[22] is one of the most commonly used behavioral tests to measure spatial memory and long-term memory by observing and recording escape latency, thigmotaxis duration, distance moved, and velocity during the time spent in a circular water tank. MWM includes a training phase and a probe test.

In addition, other mazes have also been used in many

studies, such as the Y maze,[23] T maze,[24] elevated plus maze,[25] and radial maze.[26] These mazes are composed of 3 to 8 plexiglas arms and divided into open arms and closed arms. Except for the elevated plus maze, all the mazes are located on the ground. The number of entries, time spent, speed, and percentage of the total distance traveled in each arm were quantified.

Open-field test

In an open-field test,[21] an animal is placed in the middle of the bottom surface, and its movements are record for minutes to hours as it moves around and explores its environment. After the experiment is completed, the computer tracking program will analyze the animal's movement over time. This test can measure horizontal activity, time spent in various areas of the open field, and total distance traveled. This test can also be used to measure the anxiety and short-term memory of an animal.

Fear condition (FC) test

An FC test is conducted with the help of a fear-regulating device. The device is divided into two phases: a training phase and a testing phase.[27] During the training phase, animals are exposed indoors for 3 min and then stimulated with tone (30 s, 65 dB, 3 kHz) and foot electrical stimulation (3 s, 0.75 mA). During the testing phase, animals are given tone without foot electrical stimulation, and the freezing time (no visible movement other than respiration) during the 3-minute tone stimulation period is recorded. The fear memory of animals is evaluated based on the recorded freezing time.

Inhibitory avoidance

Inhibitory avoidance is a neurobehavioral test that focuses on fear memory and is the best studied form of memory.[28] There are two types of tests: the step-down test[29] and the step-through test.[30] Both tests use electric stimulants and a retention test after 24 h. The training equipment is an acrylic box with a floor composed of parallel arranged stainless steel bars. When an animal jumps onto the platform, it will undergo an electric shock of 0.4 mA for 5 s, which will be repeated 1 h later. The incubation period is recorded, with over 60 s indicating that fear avoidance has been established. After the electric shock is removed at 24 h, the retention test was conducted, and the latency time is recorded. The step-through test has the same principle – the electric shock is combined with a dark box due to the animals' preference for darkness. Animals are initially placed into a light box. When they enter a dark box, they undergo a 5-second electric shock, and the latency time is recorded as mentioned above.

Novel object recognition (NOR) test

The NOR test is designed to assess short- or long-term recognition memory in mice and rats and relies on the innate preference of rodents for novelty.[31] The animals are ideally placed in a dimly lit arena to reduce anxiety and encouraged to explore objects. The arena contains two identical objects (similar size and texture), which are placed in opposite corners of the arena, with enough space for animals to move past these objects without interacting with them. Animals can freely explore objects and the arena during training. After free exploration, there is a delay between 3 min and 4 h (evaluate short-term memory) or between 24 h and 72 h (evaluate long-term memory). The familiar objects are put back into the arena together with novel objects. The time spent sniffing each object and rearing on each object is recorded by experimenters who are blinded to the group of the tested animals.

Neuroinflammation and brain–blood barrier (BBB) integrity

SAE is associated with excessive microglial activation, impaired endothelial barrier function, and BBB dysfunction.[9,32] In the brain, microglia are the main mononuclear phagocytes and astrocytes, which are critical in neuronal microenvironment homeostasis and maintenance of BBB function. Many experimental methods can be used for detecting cytokines to evaluate microglial and astrocyte activation and neuroinflammation.[33,34] In addition, the assessment of BBB integrity has been considered in many studies.[21,25] A total of 200 μL Evans blue dye (4% in saline or 1% in phosphate-buffered saline [PBS]) is injected via the tail vein 2 h before euthanasia. Under general anesthesia, animals are perfused with physiological saline or PBS to flush residual dyes and blood in the veins. After perfusion, brain samples are collected, weighed, and homogenized in PBS. To detect Evans blue dye in the tissue, 650 nm excitation and 700 nm emission wavelength filters are used. Data are expressed as micrograms per milligram of brain weight. Following this, the animals cannot be further analyzed, so the order of steps in the experiment is important, especially if further exploration of the BBB integrity is desired.

Dynamic positron emission tomography/ computed tomography (PET/CT) imaging and magnetic resonance imaging (MRI)

The PET/CT scan can be used to detect the activation of glial cells with [11C] PBR28 due to its function of binding

the mitochondrial translocator protein (TSPO).[31,35] The pharmacokinetic parameters of 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F] F-FDG) from dynamic PET are sensitive in detecting SAE in rats.[36] In addition, 2-[6-chloro-2-(4-[^{125}I] iodophenyl)-imidazo[1,2-a]pyridin-3-yl]-N-ethyl-N-methyl-acetamide ([^{125}I] CLINME) and [^{99m}Tc][2,2-dimethyl-3-[(3E)-3-oxidoiminobutan-2-yl]azanidylpropyl]-[(3E)-3-hydroxyiminobutan-2-yl]azanide ([^{99m}Tc]HMPAO) can also be used to detect microglial activation and brain hypoperfusion, respectively, in the early phase of systemic inflammation.[37] Multiparametric MRI can be used to detect BBB function.[36] When quantifying the T1 value of every specific brain region, the researchers find that the SAE group has a significantly higher intensity in 12 regions than the control group, which indicates that BBB permeability increased.

DISCUSSION

The SAE animal models are based on the sepsis model with the investigation of neurological abnormalities. In this study, we summarize the common SAE models in terms of methodology and evaluation. The CLP- and LPS-induced models are the most well-recognized animal models of sepsis. The nervous reflex test is a part of neurobehavioral scores and is conducted to evaluate reactions in the acute phase of sepsis. In addition, there are various scoring systems for sepsis evaluation, such as the M-CASS and MSS. The behavior test is mainly used in the late phase of SAE after surgery or injection,[21,38-41] which is widely applied in neurological disorders to analyze the anxiety state, memory and other cognitive dysfunction. BBB integrity assessment by Evans blue and imaging methods can be applied in SAE detection. Considering the variability of research designs, an individual experimental protocol should be chosen carefully. Future research can develop a better scoring system for SAE animal models based on existing research.[42]

CONCLUSION

CLP and LPS injection are the most common methods for establishing SAE animal models. Nervous reflex, inhibitory avoidance, MWM, and FC examinations are widely used in SAE model analysis. Future research should focus on establishing a standardized system for SAE development and analysis.

REFERENCES

1 Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). JAMA. 2016;315(8):801-10.

2 Sahu P, Raj Stanly EA, Simon Lewis LE, Prabhu K, Rao M, Kunhikatta V. Prediction modelling in the early detection of neonatal sepsis. World J Pediatr. 2022;18(3):160-75.

3 Tauber SC, Djukic M, Gossner J, Eiffert H, Brück W, Nau R. Sepsis-associated encephalopathy and septic encephalitis: an update. Expert Rev Anti Infect Ther. 2021;19(2):215-31.

4 Gofton TE, Young GB. Sepsis-associated encephalopathy. Nat Rev Neurol. 2012;8(10):557-66.

5 Chung HY, Wickel J, Brunkhorst FM, Geis C. Sepsis-associated encephalopathy: from delirium to dementia? J Clin Med. 2020;9(3):703.

6 Pezzullo L, Streatfeild J, Hickson J, Teodorczuk A, Agar MR, Caplan GA. Economic impact of delirium in Australia: a cost of illness study. BMJ Open. 2019;9(9):e027514.

7 Pandharipande PP, Girard TD, Jackson JC, Morandi A, Thompson JL, Pun BT, et al. Long-term cognitive impairment after critical illness. N Engl J Med. 2013;369(14):1306-16.

8 Saczynski JS, Marcantonio ER, Quach L, Fong TG, Gross A, Inouye SK, et al. Cognitive trajectories after postoperative delirium. N Engl J Med. 2012;367(1):30-9.

9 Peng XY, Luo ZX, He S, Zhang LH, Li Y. Blood-brain barrier disruption by lipopolysaccharide and sepsis-associated encephalopathy. Front Cell Infect Microbiol. 2021;11:768108.

10 Robba C, Crippa IA, Taccone FS. Septic encephalopathy. Curr Neurol Neurosci Rep. 2018;18(12):82.

11 Savi FF, de Oliveira A, de Medeiros GF, Bozza FA, Michels M, Sharshar T, et al. What animal models can tell us about long-term cognitive dysfunction following sepsis: a systematic review. Neurosci Biobehav Rev. 2021;124:386-404.

12 Hubbard WJ, Choudhry M, Schwacha MG, Kerby JD, Rue LW, Bland KI, et al. Cecal ligation and puncture. Shock. 2005;24(Supplement 1):52-7.

13 Wichterman KA, Baue AE, Chaudry IH. Sepsis and septic shock—a review of laboratory models and a proposal. J Surg Res. 1980;29(2):189-201.

14 Dejager L, Pinheiro I, Dejonckheere E, Libert C. Cecal ligation and puncture: the gold standard model for polymicrobial sepsis? Trends Microbiol. 2011;19(4):198-208.

15 Rittirsch D, Huber-Lang MS, Flierl MA, Ward PA. Immunodesign of experimental sepsis by cecal ligation and

puncture. Nat Protoc. 2009;4(1):31-6.

16 Lee B, Sur B, Park J, Kim SH, Kwon S, Yeom M, et al. Ginsenoside Rg3 alleviates lipopolysaccharide-induced learning and memory impairments by anti-inflammatory activity in rats. Biomol Ther (Seoul). 2013;21(5):381-90.

17 Yang Y, Yun D, Dong B, Geng Y, Wan Y. VIP alleviates sepsis-induced cognitive dysfunction as the TLR-4/NF-κB signaling pathway is inhibited in the hippocampus of rats. J Mol Histol. 2022;53(2):369-77.

18 Osuchowski MF, Ayala A, Bahrami S, Bauer M, Boros M, Cavaillon JM, et al. Minimum Quality Threshold in Pre-Clinical Sepsis Studies (MQTiPSS): an international expert consensus initiative for improvement of animal modeling in sepsis. Infection. 2018;46(5):687-91.

19 Huet O, Ramsey D, Miljavec S, Jenney A, Aubron C, Aprico A, et al. Ensuring animal welfare while meeting scientific aims using a murine pneumonia model of septic shock. Shock. 2013;39(6):488-94.

20 Shrum B, Anantha RV, Xu SX, Donnelly M, Haeryfar SM, McCormick JK, et al. A robust scoring system to evaluate sepsis severity in an animal model. BMC Res Notes. 2014;7:233.

21 Xu XE, Liu L, Wang YC, Wang CT, Zheng Q, Liu QX, et al. Caspase-1 inhibitor exerts brain-protective effects against sepsis-associated encephalopathy and cognitive impairments in a mouse model of sepsis. Brain Behav Immun. 2019;80:859-70.

22 Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods. 1984;11(1):47-60.

23 Lin SP, Wei JX, Hu JS, Bu JY, Zhu LD, Li Q, et al. Artemisinin improves neurocognitive deficits associated with sepsis by activating the AMPK axis in microglia. Acta Pharmacol Sin. 2021;42(7):1069-79.

24 Deacon RM, Rawlins JN. T-maze alternation in the rodent. Nat Protoc. 2006;1(1):7-12.

25 Andonegui G, Zelinski EL, Schubert CL, Knight D, Craig LA, Winston BW, et al. Targeting inflammatory monocytes in sepsis-associated encephalopathy and long-term cognitive impairment. JCI Insight. 2018;3(9):99364.

26 Delcourt J, Miller NY, Couzin ID, Garnier S. Methods for the effective study of collective behavior in a radial arm maze. Behav Res Methods. 2018;50(4):1673-85.

27 Ji MH, Qiu LL, Tang H, Ju LS, Sun XR, Zhang H, et al. Sepsis-induced selective parvalbumin interneuron phenotype loss and cognitive impairments may be mediated by NADPH oxidase 2 activation in mice. J Neuroinflammation. 2015;12:182.

28 Izquierdo I, Furini CR, Myskiw JC. Fear memory. Physiol Rev. 2016;96(2):695-750.

29 Tuon L, Comim CM, Petronilho F, Barichello T, Izquierdo I, Quevedo J, et al. Time-dependent behavioral recovery after sepsis in rats. Intensive Care Med. 2008;34(9):1724-31.

30 Blake MG, Boccia MM, Baratti CM. Behavioral differences on memory retrieval between two variants of step-through inhibitory avoidance task in mice. Neurosci Lett. 2008;444(1):102-5.

31 Giridharan VV, Generoso JS, Lence L, Candiotto G, Streck E, Petronilho F, et al. A crosstalk between gut and brain in sepsis-induced cognitive decline. J Neuroinflammation. 2022;19(1):114.

32 Gao QZ, Hernandes MS. Sepsis-associated encephalopathy and blood-brain barrier dysfunction. Inflammation. 2021;44(6):2143-50.

33 Michels M, Sonai B, Dal-Pizzol F. Polarization of microglia and its role in bacterial sepsis. J Neuroimmunol. 2017;303:90-8.

34 Hoogland ICM, Houbolt C, van Westerloo DJ, van Gool WA, van de Beek D. Systemic inflammation and microglial activation: systematic review of animal experiments. J Neuroinflammation. 2015;12:114.

35 Brownell AL, Kuruppu D, Kil KE, Jokivarsi K, Poutiainen P, Zhu AJ, et al. PET imaging studies show enhanced expression of mGluR5 and inflammatory response during progressive degeneration in ALS mouse model expressing SOD1-G93A gene. J Neuroinflammation. 2015;12(1):1-8.

36 Zhu TX, Jiang JY, Xiao YT, Xu D, Liang ZB, Bi L, et al. Early diagnosis of murine sepsis-associated encephalopathy using dynamic PET/CT imaging and multiparametric MRI. Mol Imaging Biol. 2022;24(6):928-39.

37 Szöllösi D, Hegedűs N, Veres DS, Futó I, Horváth I, Kovács N et al. Evaluation of brain nuclear medicine imaging tracers in a murine model of sepsis-associated encephalopathy. Mol Imaging Biol. 2018;20(6):952-62.

38 Barichello T, Martins MR, Reinke A, Constantino LS, Machado RA, Valvassori SS, et al. Behavioral deficits in sepsis-surviving rats induced by cecal ligation and perforation. Braz J Med Biol Res. 2007;40(6):831-7.

39 Ozcan PE, Senturk E, Orhun G, Gumru S, Arican N, Orhan N, et al. Effects of intravenous immunoglobulin therapy on behavior deficits and functions in sepsis model. Ann Intensive Care. 2015;5(1):62.

40 Yan C, Ma Z, Ma H, Li Q, Zhai Q, Jiang T, et al. Mitochondrial transplantation attenuates brain dysfunction in sepsis by driving microglial M2 polarization. Mol Neurobiol. 2020;57(9):3875-90.

41 Zhang N, Zhao W, Hu ZJ, Ge SM, Huo Y, Liu LX et al. Protective effects and mechanisms of high-dose vitamin C on sepsis-associated cognitive impairment in rats. Sci Rep. 2021;11(1):14511.

42 Saito M, Fujinami Y, Ono Y, Ohyama S, Fujioka K, Yamashita K, et al. Infiltrated regulatory T cells and Th2 cells in the brain contribute to attenuation of sepsis-associated encephalopathy and alleviation of mental impairments in mice with polymicrobial sepsis. Brain Behav Immun. 2021;92:25-38.