Inhibition of indoleamine 2,3-dioxygenase prevented cognitive impairment in adult Wistar rats subjected to pneumococcal meningitis

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Streptococcus pneumoniae **is a common cause of forms of bacterial meningitis that have a high mortality rate and cause long-term neurologic sequelae. We evaluated the effects of an indoleamine 2,3-dioxygenase (IDO) inhibitor on proinflammatory mediators and memory in Wistar rats subjected to pneumococcal meningitis. The animals were divided into 4 groups: sham, sham treated with IDO inhibitor, meningitis, and meningitis treated with IDO inhibitor. During the first experiment, the animals were killed 24 hours later, and the hippocampus was isolated for the analysis of tumor necrosis factor (TNF)-α, interleukin (IL)-4, IL-6, IL-10, and cytokine-induced neutrophil chemoattractant 1 (CINC-1) levels. The survival rate was 56.296% in the meningitis group and 29.616% in the meningitis group with IDO inhibitor. In the control group, we found a mean of 14.29 white blood cells/mL cerebrospinal fluid, whereas the mean was 80.00 white blood cells/mL cerebrospinal fluid in the sham IDO inhibitor group, 1167.00 white blood cells/mL cerebrospinal fluid in the meningitis group, and 286.70 white blood cells/mL cerebrospinal fluid in the meningitis IDO inhibitor group. In the meningitis group with IDO inhibitor, the levels of TNF-α and CINC-1 were reduced. In the second experiment, animals were subjected to a behavioral task and cytokine analysis 10 days after meningitis induction. In the meningitis group, there was an impairment of aversive memory. However, in the meningitis group that received adjuvant treatment with the IDO inhibitor, animals demonstrated preservation of aversive memory. These findings showed dual effects of the IDO inhibitor on a pneumococcal meningitis animal model because the inhibitor impaired survival but also produced beneficial effects, including anti-inflammatory activity and neuroprotection against the latter behavioral deficits. (Translational Research 2013;162:390–397)**

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Abbreviations: CINC-1 = cytokine-induced neutrophil chemoattractant 1; CSF = cerebral spinal fluid; IDO = indoleamine 2,3-dioxygenase; IL = interleukin; TNF = tumor necrosis factor; WBC = white blood cell

AT A GLANCE COMMENTARY

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Background

Streptococcus pneumoniae is a common cause of forms of bacterial meningitis that have a high mortality rate and cause long-term neurologic sequelae. Pneumococcal compounds are proinflammatory mediators that induce an innate immune response that triggers the production of cytokines. Cytokines activate the metabolic enzyme indoleamine 2,3-dioxygenase (IDO), which catabolizes the amino acid L-tryptophan to kynurenine, which is metabolized in a succession of novel compounds that have neurotoxic properties. In the current study, we evaluated the effects of an IDO inhibitor on proinflammatory mediators and memory in rats subjected to pneumococcal meningitis.

Translational Significance

The rat model permits a refined evaluation of clinical and neurologic symptoms. The training and learning abilities of rats can also be used to study post-meningitis: learning and memory disabilities; depressive-like and anxiety-like behaviors. In addition, this model allows study new adjunctive therapy approaches.

Streptococcus pneumoniae is a common cause of forms of bacterial meningitis that have a high mortality rate and cause long-term neurologic sequelae in adults,[1] which affect up to 50% of survivors.[2] Pneumococcal compounds are proinflammatory mediators that induce an innate immune response that activates nuclear factor kappa B, and subsequently trigger the production of proinflammatory cytokines and chemokines, and the expression of costimulatory molecules.[3] These proinflammatory cytokines activate the metabolic enzyme indoleamine 2,3-dioxygenase (IDO),[4] which is widely expressed in all tissues, including epithelial cells, macrophages, and dendritic cells.[5] IDO in microglia catabolizes the amino acid L-tryptophan to kynurenine.[6,7] Kynurenine is then metabolized in a succession of novel compounds that have neurotoxic properties. One of its metabolites, quinolinic acid, causes oxidative stress in rat brain synaptosomes,[8] and long-term exposure of human neurons to this acid equivalent in the cerebrospinal fluid of patients with complex acquired immune deficiency syndrome dementia leads to modification of the dendritic ultrastructure.[9] In addition, within the central nervous system, macrophages and microglia in the brain may convert L-tryptophan into the neurotoxic quinolinic acid under conditions of inflammatory disease.[10] In previous studies, we verified that pneumococcal meningitis increases cytokine-induced neutrophil chemoattractant 1 (CINC-1) levels in arterial blood more than in venous blood.[11] In addition, tumor necrosis factor (TNF)-α, interleukin (IL) 1β, IL-6, and CINC-1 are produced primarily in the first 6–24 hours after the induction of pneumococcal meningitis.[12] Furthermore, it has been well established that survivors of pneumococcal meningitis exhibit long-term cognitive impairment. We have already shown that rats that survive pneumococcal meningitis exhibit learning and memory impairment 10 days after the induction of meningitis.[13] In the current study, we evaluated the effects of an IDO inhibitor on proinflammatory mediators and memory in Wistar rats subjected to pneumococcal meningitis.

EXPERIMENTAL PROCEDURE

Infecting organism. *Streptococcus pneumoniae* (serotype 3) was cultured overnight in 10 mL Todd Hewitt broth. Cultures were then diluted in fresh medium and grown to the logarithmic phase. These cultures were centrifuged for 10 minutes at 5000*g* and resuspended in sterile saline at a concentration of 5×10^9 cfu/mL. The size of the inoculum was confirmed using quantitative cultures.[14,15]

Animal model of meningitis. Adult male Wistar rats (body weight, 250–350 g) from our breeding colony were used for the experiments. All procedures were approved by the Animal Care and Experimentation Committee of UNESC/88/2012, Brazil, and were in accordance with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*.[16] All surgical procedures and bacterial inoculations were performed with the animal under anesthesia, which consisted of the intraperitoneal administration of 6.6 mg/kg ketamine, 0.3 mg/kg xylazine, and 0.16 mg/kg acepromazine. Rats underwent a cisterna magna tap with a 23-gauge needle. The animals received either 10 μL sterile saline or an equivalent volume of *S. pneumoniae* suspension. At the time of inoculation, animals received fluid replacement and were then returned to their cages.[17,18] After 18 hours, the induction