Erythropoietin prevents cognitive impairment and oxidative parameters in Wistar rats subjected to pneumococcal meningitis

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Pneumococcal meningitis is characterized by a severe inflammatory reaction in the subarachnoid and ventricular space of the brain, disruption of the blood-brain barrier, hearing loss, and neurologic sequelae in as many as 27% of surviving patients. Several experimental studies have shown that erythropoietin (EPO) and its receptor are expressed in the central nervous system and have neuroprotective properties through the inhibition of apoptosis, as well as anti-inflammatory, antioxidant, angiogenic, and neurotrophic effects. In the current study, we demonstrated the effect of erythropoietin (EPO) on lipid peroxidation, protein carbonylation, superoxide dismutase (SOD), catalase (CAT), myeloperoxidase (MPO), and behavioral parameters in rats with pneumococcal meningitis. EPO decreased lipid peroxidation and protein carbonylation, and it prevented protein degradation in the hippocampus and frontal cortex. MPO activity was decreased, and both SOD and CAT activity were increased in the first 6 hours after pneumococcal meningitis induction. Novel object recognition memory was impaired in the meningitis group; however, adjuvant treatment with EPO prevented memory impairment during both the short- and long-term retention tests. The meningitis group showed no difference in motor and exploratory activity between training and test sessions in the open-field task, which indicates that habituation memory was impaired; however, adjuvant treatment with EPO prevented habituation memory impairment. Although there are some limitations with respect to the animal model of pneumococcal meningitis, this study suggests that adjuvant treatment with EPO contributed to decreased oxidative stress and prevented cognitive impairment. (Translational Research 2014;163:503–513)

Abbreviations: BBB = blood-brain barrier; CAT = catalase; CNS = central nervous system; CSF = cerebrospinal fluid; DTNB = 5,5-dithiobis (2-nitrobenzoic acid); EPO = erythropoietin; MPO = myeloperoxidase; NF-κB = nuclear transcription factor kappa B; ROS = reactive oxygen species; SOD = superoxide dismutase; TBARS = thiobarbituric acid reactive species; TNB = 5-thio-2-nitrobenzoic acid; TLR = toll-like receptor

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AT A GLANCE COMMENTARY

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Background

Pneumococcal compounds are proinflammatory mediators that induce an innate immune response that triggers the production of cytokines and reactive oxygen species (ROS). Several experimental studies have shown that erythropoietin (EPO) and its receptors are expressed in the central nervous system and have neuroprotective properties, such as anti-inflammatory and antioxidant.

Translational Significance

Although there are some limitations to the study of therapeutic treatments in an animal model of pneumococcal meningitis, our results suggest that adjuvant treatment with EPO can decrease ROS, increase enzymatic defense, and prevent cognitive impairment resulting from pneumococcal meningitis infection.

Pneumococcal meningitis is characterized by a severe inflammatory reaction in the subarachnoid and ventricular space of the brain, disruption of the blood-brain barrier (BBB), and hearing loss and neurologic sequelae in as many as 27% of survivors.[1-3] Pneumococcal compounds are highly immunogenic, which induce a cascade of inflammatory reactions through different sensors, including Toll-like receptors (TLRs).[4] TLRs are activated by pneumococcal cell wall compounds such as lipoteichoic acid, the lipoprotein pneumolysin, and pneumococcal DNA.[4,5] These receptors transduce their signals through a common intracellular adapter protein known as myeloid differentiation factor 88, or MyD88,[4,6] which results in the activation and nuclear translocation of nuclear transcription factor kappa B (NF-κB).[7,8] NF-κB plays a key role in the expression of genes that are involved in the development of accessory cell and leukocyte populations, inducing the expression of many proteins that are implicated in inflammation and the immune response.[9] In addition, polymorphonuclear leukocytes produce nitric oxide ($NO^{\bullet}$), superoxide anion radicals ($O_2^{-\bullet}$), and hydrogen peroxide (H_2O_2). $O_2^{-\bullet}$ and $NO^{\bullet}$ can lead to the formation of peroxynitrite (ONOO), which is a strong oxidant.[10] This oxidant exerts cytotoxic effects on endothelial cells,[11] increases the permeability of the BBB and lipid peroxidation, and induces many other complex interactions that may be involved in the pathophysiology of pneumococcal meningitis.[10,12] In previous studies, we observed BBB breakdown between 12 hours and 24 hours in the hippocampus, and at 12 hours and 18 hours in the cortex after pneumococcal meningitis induction in adult Wistar rats.[13] In neonatal Wistar rats, we observed the BBB breakdown in the hippocampus at 18 hours and in the cortex at 12 hours after pneumococcal meningitis induction.[14] Erythropoietin (EPO) was identified originally as a hematopoietic cytokine that is involved in the process of erythropoiesis.[15] Several experimental studies have shown that EPO and its receptor are expressed in neurons, astrocytes, oligodendrocytes, microglia, and endothelial cells.[16] Additional evidence suggests that EPO has neuroprotective properties within the nervous system through the inhibition of apoptosis,[17] as well as anti-inflammatory, antioxidant, angiogenic, and neurotrophic effects.[16,18] In the current study, we evaluated the effects of EPO on inflammatory mediators, and learning and memory in Wistar rats infected with pneumococcal meningitis.

MATERIALS AND METHODS

Infecting organism. *Streptococcus pneumoniae* (serotype 3) was cultured overnight in 10 mL Todd Hewitt broth, then diluted in fresh medium and grown to the logarithmic phase. This culture was 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 by quantitative cultures.[19]

Animal model of meningitis. Male Wistar rats (body weight, 250–300 g) from our breeding colony were used for the experiments. All procedures were approved by the Animal Care and Experimentation Committee of Universidade do Extremo Sul Catarinense, Brazil, comitê de ética no Uso de Animais:86/2012, and were followed in accordance with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals*.[20] All surgical procedures and bacterial inoculations were performed with the animal under anesthesia, and consisted of an intraperitoneal administration of ketamine (6.6 mg/kg), xylazine (0.3 mg/kg), and acepromazine (0.16 mg/kg).[13] Rats underwent a cisterna magna tap with a 23-gauge needle. The animals received either 10 μL artificial cerebrospinal fluid (CSF) as a placebo or an equivalent volume of *S. pneumoniae* in suspension. At the time of inoculation, the animals received fluid replacement and were returned to their cages.[13,21] Infection with meningitis was documented by a quantitative culture of 5 μL CSF, which was obtained by a puncture of the cisterna magna.

Organization of the experimental groups. The experiment was conducted at 2 different time points. At the first time point, the animals were divided into 4 groups with a total of 8 animals per group: control, control