Neuroscience Letters 465 (2009) 71–73

Contents lists available at ScienceDirect

Neuroscience Letters

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

Early antibiotic administration prevents cognitive impairment induced by meningitis in rats

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ARTICLE INFO

Article history:
Received 25 May 2009
Received in revised form 8 August 2009
Accepted 29 August 2009

Keywords:
Meningitis
S. pneumoniae
Memory
Central nervous system

ABSTRACT

Neurological deficit and alterations in the hippocampus still frequently occur following bacterial meningitis in children, despite the antibiotic treatment. We investigated the long-term outcomes using early versus late antibiotic therapy in experimental pneumococcal meningitis. To this aim, male Wistar rats underwent a basilar cistern tap receiving either sterile saline as a placebo or an equivalent volume of a *Streptococcus pneumoniae* suspension. Antibiotics were started 8 or 16 h after infection and the animals were followed for 10 days to the determination of long-term cognitive outcomes. The animals were submitted to the habituation of an open-field as an index of long-term cognitive function. Early antibiotic administration (8 h after inoculation) when compared to late antibiotic administration (16 h after inoculation) prevented cognitive impairment induced by *pneumococcal* meningitis in Wistar rats. The findings from this study suggest that early antibiotic administration is an effective strategy to prevent long-term cognitive impairment in a meningitis animal model.

The estimated annual incidence of bacterial meningitis is 4–6 per 100,000 adults, and *Streptococcus pneumoniae* and *Neisseria meningitides* are the causative bacteria in 80% of the cases [6]. Meningitis is associated with a significant mortality rate of up to 30% and persisting neurologic sequelae including sensory–motor deficits, and seizures. Moreover, impairment of learning and memory occur in up to 50% of the survivors [6,19]. In addition, patients after pneumococcal meningitis have a higher rate of cognitive dysfunction [6].

The host reaction contributes to the unfavorable outcome from meningitis leading to neuronal injury that includes cortical necrosis and hippocampal apoptosis [4]. In addition, secondary brain injury continues through the initial 2–4 days after antibiotic therapy is initiated, and is due to an exacerbation of the inflammatory process subsequent to the liberation of bacterial products [13]. Thus, it is plausible to suppose that the timing of antibiotic administration could interfere in the occurrence of long-term cognitive impairment. However, to the best of our knowledge, there are no published data that demonstrated this. In order to investigate the putative role of the time of antibiotic treatment and the occurrence of cognitive dysfunction we evaluated the long-term performance to the open-field task in an animal model of *S. pneumoniae* meningitis. All procedures were approved by the Animal Care and Experimentation Committee of the UNESC, Brazil, and followed the National Institute of Health's guidelines. Seventy males Wistar rats (250–300 g of body weight) were divided in two groups (Meningitis group, 42 animals; Sham group, 30 animals). All surgical procedures and bacteria administrations were performed under anesthesia consisting of an intraperitoneal (i.p.) administration of ketamine (6.6 mg/kg), xylazine (0.3 mg/kg), and acepromazine (0.16 mg/kg) [6,4]. *S. pneumoniae* (ATCC 6303) was cultured overnight in Todd Hewitt broth and grown to logarithmic phase. In the morning of the experiment, the bacteria were washed and resuspended in sterile 0.9% NaCl, and grown to logarithmic phase, 5×10^9 cfu/mL [6,13]. On day 1, the rats underwent a basilar cistern tap with a 23-gauge needle. The position of the needle was verified by the free flow of clear cerebrospinal fluid. Cerebrospinal fluid was withdrawn and the animals received either 10 μL of sterile saline as a control (sham) or an equivalent volume of the *S. pneumoniae*. At the time of inoculation, the animals received fluid replacement (10 mL of saline subcutaneously) and analgesics (0.5 mL of Buprenorphine subcutaneously), and were returned to their cages. Following their recovery from anesthesia, the animals were supplied with food and water *ad libitum*.

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doi:10.1016/j.neulet.2009.08.072

Meningitis was documented by a quantitative culture of 5 μL of CSF obtained by puncture of the cisterna magna and cultured quantitatively on sheep blood agar plates. The number of bacteria in the CSF was determined by plating serial dilutions of CSF on blood agar plates at 8 (early antibiotic administration) and 16 (late antibiotics administration) hours after inoculation, and followed by the initiation of the antibiotic treatment (ceftriaxone 100 mg/kg twice a day, i.p.), dividing the animals in four groups: Sham 8 h; Sham 16 h; Meningitis 8 h; and Meningitis 16 h with 15 animals per group (40% mortality in the Meningitis group).

Ten days after inoculation the animals seemed to be free from infection. We performed blood cultures that were all negative in this period, the animals recovered their weight and grooming habits, blood counts returned to control levels, and reactive protein C values were negative. At this time, animals underwent the open-field task. Habituation to an open-field was carried out in a 40 cm × 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 by black lines into 12 equal rectangles. 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 cages, 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 [1,18]. All data are presented as mean ± S.D. Data were analyzed by Student's *t*-test, considered $p < 0.05$ to be significant.

There were no differences in the number of crossings ($t = -0.175$; df = 12; $p = 0.864$) and rearings ($t = 0.116$; df = 22; $p = 0.0909$) in the training session in both groups in 8 (Fig. 1) and 16 h (Fig. 2), suggesting no impairment on motor function. In the test session, the animals that received antibiotic 8 h after inoculation presented a decreased in the number of crossings ($t = -1.486$; df = 10; $p = 0.168$) and rearings ($t = -0.085$; df = 10; $p = 0.934$) (similar to the sham animals), demonstrating the formation of habituation memory (Fig. 1). In contrast, when antibiotics were given 16 h after infection there were no differences between the training and test sessions in the number of crossings ($t = 5.545$; df = 23; $p = 0.0001$) and rearings ($t = 8.607$; df = 23; $p = 0.0001$) when compared with the sham group, suggesting memory impairment (Fig. 2).

These deficits appear to be directly related to impaired hippocampus-dependent memory processes [17,10]. Additionally, some authors demonstrated that reduced density of neuronal cells

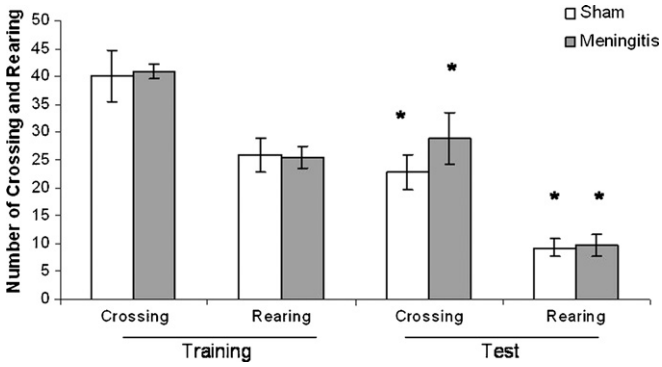

Fig. 1. Habituation to the Open Field Test with antibiotic therapy started 8 h after induction. In the test session, animals presented a decreased in the number of crossings and rearings (similar to sham animals). Bars represent means ± S.D. $n = 15$ rats per group. $^*p < 0.05$ versus training according to Student's *t*-test.

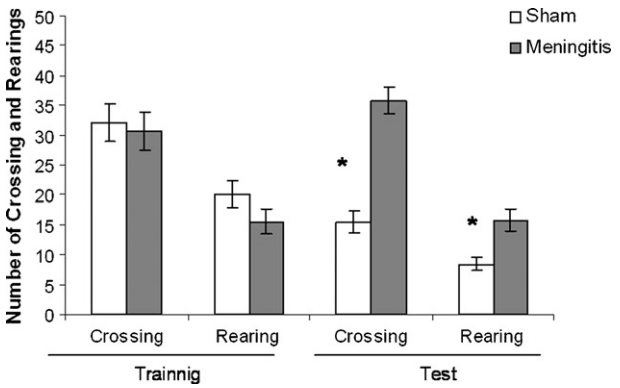

Fig. 2. Habituation to the Open Field Test with antibiotic therapy started 16 h after induction. There was no differences between training and test sections in the number of crossings and rearings when comparison with sham group. Bars represent means ± S.D. $n = 15$ rats per group. $^*p < 0.05$ versus training according to Student's *t*-test.

in the prefrontal cortex, where lesions are known to lead to orientation disturbances and mnestic problems in complex tasks [5,14]. The parietal association cortex is also involved in spatial learning [16]. In experimental meningitis, one of the consequences of the massive inflammatory reaction in the meninges and subarachnoid space is the occurrence of neuronal apoptosis in the hippocampus [2]. The death of these cells appears to be responsible for the learning and memory deficits in patients who survive the disease [12,15].

To date, few experimental strategies that aimed to decrease late cognitive impairment after meningitis were published. The early application of hypothermia or dexamethasone modulates the inflammatory reaction improving short-term neurologic outcome [9,8]. Caspase activation secondary to inflammatory reaction is probably central to the development of late cognitive impairment, since the pharmacologic caspase inhibition by bocaspartyl abolished cognitive impairment after meningitis in animal models [7]. In this context, the use of bactericidal antibiotic regimens can cause a burst of meningeal inflammation during experimental meningitis [3], and could be associated to worsen the long-term outcome. Recently, the effectiveness and safety of a short course of antibiotic therapy for bacterial meningitis was evaluated in a meta-analysis of randomized controlled trials (RCT). The authors evaluated RCT on patients of all ages with acute community-acquired bacterial meningitis that compared treatment with the same antibiotics, in the same daily dosage, administered for a short course (up to 7 days) versus a longer course (2 days or more than corresponding short course). They concluded that there were no differences between short and long-course antibiotic treatment for bacterial meningitis in children [11].We here, using a simple and feasible strategy, demonstrated that early antibiotic administration (8 h after inoculation) when compared to late antibiotic administration (16 h after inoculation) prevents cognitive impairment induced by *Pneumococcal meningitis* in Wistar rats. The exact mechanism to explain these effects was not explored in this study, but these data reinforce the need of early antibiotics administration to improve outcome after bacterial meningitis.

Acknowledgements

This research was supported by grants from CNPq (TB, JQ and FD-P), FAPESC (JQ and FD-P), Instituto Cérebro e Mente (TB, JQ and FD-P) and UNESC (TB, JQ and FD-P). JQ and FD-P are CNPq Research Fellows. CMC is holder of a CNPq Studentship.

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