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Evaluation of energetic metabolism in the rat brain after meningitis induction by *Klebsiella pneumoniae*

Barichello T, Simões LR, Generoso JS, Carradore MM, Moreira AP, Panatto AP, Costa CS, Filho AS, Jeremias IC, Bez GD, Streck E. Evaluation of energetic metabolism in the rat brain after meningitis induction by *Klebsiella pneumoniae*.

Background: Bacterial meningitis is an infection of the central nervous system characterised by strong inflammatory response. The brain is highly dependent on ATP, and the cell energy is obtained through oxidative phosphorylation, a process which requires the action of various respiratory enzyme complexes and creatine kinase (CK) as an effective buffering system of cellular ATP levels in tissues that consume high energy.
Objectives: Evaluate the activities of mitochondrial respiratory chain complexes I, II, III, IV and CK activity in hippocampus and cortex of the Wistar rat submitted to meningitis by *Klebsiella pneumoniae*.
Methods: Adult Wistar rats received either 10 µl of sterile saline as a placebo or an equivalent volume of *K. pneumoniae* suspension. The animals were killed in different times at 6, 12, 24 and 48 h after meningitis induction. Another group was treated with antibiotic, starting at 16 h and continuing daily until their decapitation at 24 and 48 h after induction.
Results: In the hippocampus, the meningitis group without antibiotic treatment, the complex I was increased at 24 and 48 h, complex II was increased at 48 h, complex III was inhibited at 6, 12, 24 and 48 h and in complex IV all groups with or without antibiotic treatment were inhibited after meningitis induction, in the cortex there was no alteration.
Discussion: Although descriptive, our results show that antibiotic prevented in part the changes of the mitochondrial respiratory chain. The meningitis model could be a good research tool to study the biological mechanisms involved in the pathophysiology of the *K. pneumoniae* meningitis.

Tatiana Barichello[1], Lutiana Roque Simões[1], Jaqueline S. Generoso[1], Mirelle M. Carradore[1], Ana Paula Moreira[1], Ana Paula Panatto[1], Caroline S. Costa[1], Álvaro Steckert Filho[1], Isabela C. Jeremias[2], Gisele D. Bez[2], Emilio Streck[2]

[1]Laboratório de Microbiologia Experimental and Instituto Nacional de Ciência e Tecnologia Translacional em Medicina, Programa de Pós-Graduação em Ciências da Saúde, Unidade Acadêmica de Ciências da Saúde, Universidade do Extremo Sul Catarinense, Criciúma, SC, Brazil; and [2]Laboratório de Fisiopatologia and Instituto Nacional de Ciência e Tecnologia Translacional em Medicina, Programa de Pós-Graduação em Ciências da Saúde, Unidade Acadêmica de Ciências da Saúde, Universidade do Extremo Sul Catarinense, Criciúma, SC, Brazil

Keywords: creatine kinase; *Klebsiella pneumoniae*; meningitis; mitochondrial respiratory chain

Professor Tatiana Barichello, PhD,
Laboratório de Microbiologia Experimental,
PPGCS, UNASAU,
Universidade do Extremo Sul Catarinense,
88806–000 Criciúma, SC, Brazil.
Tel: +55 48 34312643;
Fax: +55 48 3443 4817;
E-mail: tba@unesc.net

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Significant outcomes

- The influence of meningitis by *Klebsiella pneumoniae* in the creatine kinase (CK) and mitochondrial respiratory chain activity.
- The meningitis by *K. pneumoniae* is associated with permanent neurological damage; understand the illness pathophysiology is the main factor that contributes to the therapeutic success.
- Our study helps to better understand the pathophysiology of meningitis by *K. pneumoniae*.

Limitations

- Animal model.
- The results in animal models should be interpreted with caution before correlate with the clinic.
- This study evaluates the animal model of adult rats.

Introduction

Bacterial meningitis remains a major cause of death and long-term neurologic sequelae worldwide. However, mortality and morbidity vary by causative organism, age and geographical location of the patient (1,2). *Klebsiella pneumoniae* is a capsulated gram-negative pathogen that is known to cause infection both in community and mainly as a hospital-acquired infection presenting as pneumonia, septicemia and meningitis in patients with some predisposing factors (3,4). *Klebsiella pneumoniae* has recently become an increasingly common cause of the meningitis acute (5) affecting 14–61% of nosocomial meningitis (6), being particularly devastating among immunocompromised patients (7) with mortality rates ranging between 30 and 40% (8), however in Singapore and parts of northern Taiwan the mortality rate is higher. Furthermore, this pathogen was the most frequent causative of the meningitis, bacteremia and septic shock in patients with liver cirrhosis (9). Furthermore, bacterial meningitis in young adults in south Taiwan, and (10) in some Asian areas, there has been an increased incidence in adults (11). Bacterial invasion in the cerebral spinal fluid (CSF) promotes the release of bacterial components like polysaccharide capsule, peptidoglycan, bacterial DNA and lipopolysaccharide (12,13), leading to the activation of the brain innate immune defence, releasing a cascade of inflammatory mediators and leukocytes recruitment (14,15). An excessive release of pro-inflammatory mediators and reactive oxygen species could contribute to interrupt the bioenergetic activity or the metabolic activity in injured neurons (16,17). The CK is vital for normal energy homeostasis by exerting some integrated functions, such as temporary energy buffering, metabolic capacity, energy transfer and metabolic control. The brain, like other tissues with high and variable rates of ATP metabolism, presents high phosphocreatine concentration and CK activity (18–20). Furthermore, another generating source of ATP is the oxidative phosphorylation, that is the predominant mitochondria physiological function but additional functions include the production and detoxification of reactive oxygen species, which is involved in various forms of apoptosis, cytoplasmic regulation and mitochondrial matrix calcium, synthesis and metabolites catabolism, so, abnormality any of these processes can lead to mitochondrial dysfunction (21). Therefore, the main factor that contributes to the therapy success is to understand the pathogenesis and pathophysiology of the bacteria in the central nervous system (CNS) (16). Thus, to clarify a little more the pathophysiology of this illness, the aim of our study was to investigate the energetic metabolism in the rat brain after meningitis induced by *K. pneumoniae.*

Materials and methods

Infecting organism

Klebsiella pneumoniae was cultured overnight in 10 ml of Todd Hewitt broth, diluted in fresh medium and grown to logarithmic phase. The culture was centrifuged for 10 min at (5000 × g) and resuspended in sterile saline to the concentration of 1×10^6 cfu/ml. The size of the inoculum was confirmed by quantitative cultures (22,23).

Animal model of meningitis

Adult male Wistar rats (250–300 g body weight), from our breeding colony were used for the experiments. All procedures were approved by the Animal Care and Experimentation Committee of UNESC, Brazil, and followed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23) revised in 1996. All surgical procedures and bacterial inoculations were performed under anaesthesia, consisting of an intraperitoneal administration of ketamine (6.6 mg/kg), xylazine (0.3 mg/kg) and acepromazine (0.16 mg/kg) (24,25). Rats underwent a cisterna magna tap with a 23-gauge needle. The animals received either 10 µl of sterile saline as a placebo or an equivalent volume of *K. pneumoniae* suspension. At the time of inoculation, animals received fluid replacement (2 ml of saline subcutaneously) and were subsequently returned to their cages. The animals were killed in different times at 6, 12, 24 and 48 h after meningitis induction by *K. pneumoniae* ($n = 6$ in each group). Another group was treated with antibiotic (ceftriaxone 100 mg/Kg twice a day, i.p.) starting at 16 h and continuing daily until their decapitation at 24 and 48 h after meningitis induction, $n = 6$ in each group (22,23). The control group did not receive antibiotic treatment (26).

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Tissue assessment and homogenate preparation

Hippocampus and cortex were homogenised (1:10, w/v) in SETH buffer, pH 7.4 (250 mM sucrose, 2 mM EDTA, 10 mM Trizma base, 50 IU/ml heparin). The homogenates were centrifuged at 800 × g for 10 min and the supernatants kept at −70 °C until used for enzymes activity determination. The maximal period between homogenate preparation and enzyme analysis was always less than 5 days. Protein content was determined by the method described by Lowry et al. (27) using bovine serum albumin as standard.

Activities of mitochondrial respiratory chain enzymes

NADH dehydrogenase (complex I) was evaluated according to the method described by Cassina and Radi (28) by the rate of NADH-dependent ferricyanide reduction at 420 nm. The activities of succinate: DCIP oxidoreductase (complex II) and succinate: cytochrome *c* oxidoreductase (complex III) was determined according to the method of Fischer et al. (29). Complex II activity was measured by following the decrease in absorbance due to the reduction of 2,6-DCIP at 600 nm. Complex III activity was measured by cytochrome *c* reduction from succinate. The activity of cytochrome *c* oxidase (complex IV) was assayed according to the method described by Rustin et al. (30), measured by following the decrease in absorbance due to the oxidation of previously reduced cytochrome *c* at 550 nm. The activities of the mitochondrial respiratory chain complexes were expressed as nmol/min × mg protein.

CK activity assay

CK was measured in brain homogenates pre-treated with 0.625 mM lauryl maltoside. The reaction mixture consisted of 60 mM Tris–HCl, pH 7.5, containing 7 mM phosphocreatine, 9 mM MgSO4 and approximately 0.4–1.2 µg protein in a final volume of 100 µl. After 15 min of pre-incubation at 37 °C, the reaction was started by the addition of 0.3 µmol of ADP plus 0.08 µmol of reduced glutathione. The reaction was stopped after 10 min by the addition of 1 µmol of *p*-hydroxymercuribenzoic acid. The creatine formed was estimated according to the colorimetric method of Hughes (31). The colour was developed by the addition of 100 µl 2% μ-naphtol and 100 µl 0.05% diacetyl in a final volume of 1 ml and read spectrophotometrically after 20 min at 540 nm. Results were expressed as units/min × mg protein.

Statistics. Data about CK and mitochondrial respiratory chain complexes were analysed by Student's *t*-test and are expressed as mean ± SME of five to six animals in each group. All analyses were performed using the Statistical Package for the Social Science (SPSS) software version 16.0.

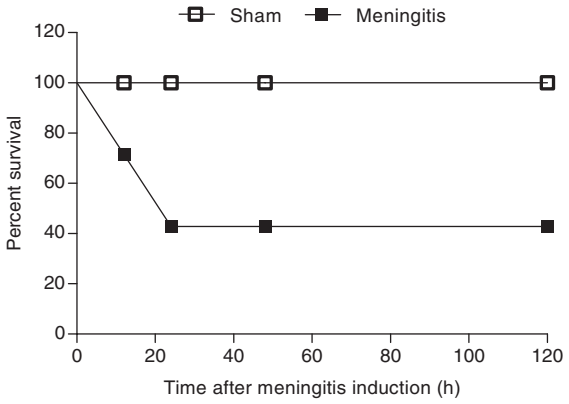

Fig. 1. Kaplan–Meier survival curves of adults Wistar rats infected by intracisternal inoculation with *Klebsiella pneumoniae*. Results are expressed as percentage of survival over time with spontaneous death. Rats had a mortality rate of 57.143%.

Results

In this study, we evaluated the CK activities and mitochondrial respiratory chain complexes I, II, III and IV in hippocampus and cortex of rats submitted to meningitis by *K. pneumoniae*.

Survival was analysed by Kaplan–Meier curves (Fig. 1), including all infected animals from the time of infection up to 120 h ($n = 38$). The animals started dying at 12 h (28.571%) and 24 h (57.143%), after that time surviving animals were killed until 120 h.

We verified that complex I was increased at 24 and 48 h in hippocampus in meningitis group without antibiotic treatment (Fig. 2a; $p < 0.05$); complex II was increased in hippocampus at 48 h in meningitis group without antibiotic treatment (Fig. 2b; $p < 0.05$); complex III was inhibited at 6, 12, 24 and 48 h in hippocampus in meningitis group without antibiotic treatment (Fig. 2c; $p < 0.05$) and in complex IV all groups were inhibited in hippocampus after meningitis induced by *K. pneumoniae* (Fig. 2d; $p < 0.05$). We also verified that there was no change in CK activity in both structures (Fig. 3).

Discussion

There has been an increased incidence of meningitis by *Klebsiella* sp in adults (11), especially in Asian countries. Among the gram-negative pathogens implicated in bacterial meningitis, in Taiwan, *K. pneumoniae* most common in adults (32), in great part this increase in the number of cases is related to the frequency of neurosurgical procedures,

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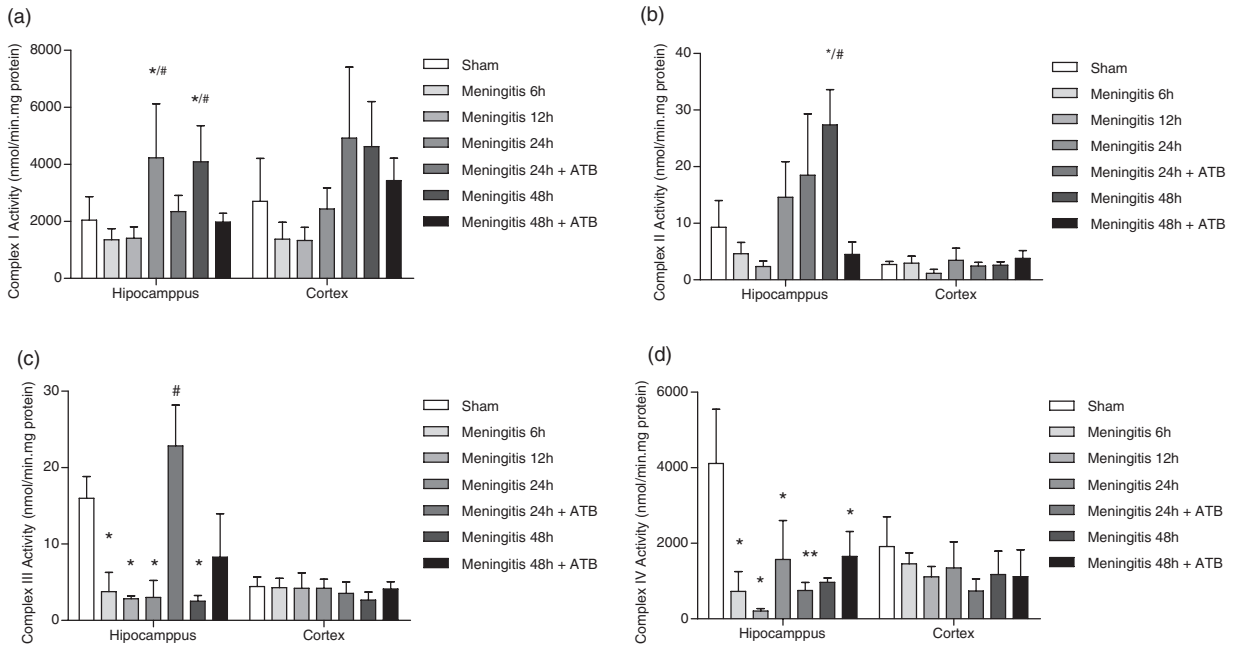

Fig. 2. Activity of the mitochondrial respiratory chain complexes I (a), II (b), III (c) and IV (d) in hippocampus and cortex of rats after meningitis by *Klebsiella pneumoniae*. Results are expressed as mean ± SD ($n = 6$) (nmol/min × mg protein). *Statistically significant when compared with sham group, $p < 0.05$. #Statistically significant when compared between meningitis groups with and without antibiotic in the same period.

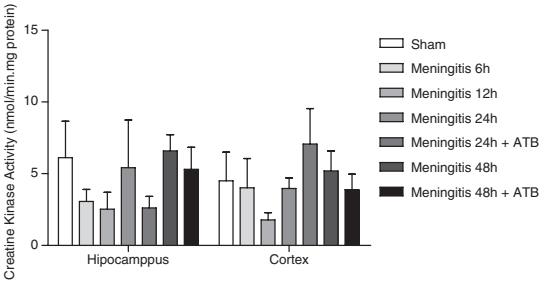

Fig. 3. CK activity in hippocampus and cortex of rats after meningitis by *Klebsiella pneumoniae*. Results are expressed as mean ± SD ($n = 6$) (nmol/min × mg protein). *Statistically significant when compared with sham group, $p < 0.05$. #Statistically significant when compared between meningitis groups with and without antibiotic in the same period, $p < 0.05$.

and the large number of patients with head injury from motorcycle accidents (33); however, chronic diseases like diabetes and liver cirrhosis predispose to meningitis (5). Bacterial invasion of the meninges induces a complex immune response (34), being that glial cells are an important early source of pro-inflammatory cytokines during the CNS infection by *K. pneumoniae* (35). The complex host inflammatory response from the white blood cells leads to mitochondrial damage initiating the release of the cytochrome *c* into the cytosol. There are many evidences that mitochondria participates in the caspase-dependent pathway resulting caspase activation and neuronal damage development in the bacterial meningitis (16,36). Furthermore, the brain is highly dependent on ATP; most cell energy is obtained through oxidative phosphorylation, a process that requires the action of various respiratory enzyme complexes situated in a special structure of the inner mitochondrial membrane, the mitochondrial respiratory chain (37). Another way to get energy is through the creatine/phosphocreatine/CK system that is essential for normal energy homeostasis by exerting some integrated functions, such as, temporary energy buffering, metabolic capacity, energy transfer and metabolic control (18,38). Meningitis caused by *K. pneumoniae* increased complex I at 24 and 48 h and complex II at 48 h in hippocampus. In a previous study, we also showed increased complex II at 24 and 48 h in hippocampus among surviving rats by pneumococcal meningitis (6). The increase of the complexes I and II could be compensation mechanisms, because of the decreased activity of complex III and IV. There was an activity decrease in complex III at 6, 12, 24 and 48 h; however at 24 and 48 h with antibiotic treatment the levels did not change in the hippocampus. In complex IV, there was also an activity decrease in all the times in the hippocampus. Furthermore, complex III deficiencies are among the least common respiratory chain abnormalities identified to date in humans (39), mutations in the cytochrome *b* gene constitute a major cause of complex III deficiency, and underlie a variety of

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disorders, such as encephalopathy, optic neurophathy (39), encephalomyopathy (40) although, there are not clinical findings which are specific for complex III deficiency (41), likewise, meningitis caused by *K. pneumoniae* inhibited the activity of complex III and IV in the hippocampus. The mitochondrial dysfunction can be responsible of oxidative stress due to the lack of reactive oxygen species detoxification and neurological clinical symptoms (21). In autopsy studies on patients who died from bacterial meningitis, injure in the CNS was characterised by tissue necrosis in the cortical hemispheres and by apoptotic cell death in the dentate gyrus (42). Hippocampal apoptosis is associated with learning and memory deficits observed in survivors of bacterial meningitis (43).

The meningitis by *K. pneumoniae* also is associated with permanent neurological damage (44), moreover, understand the illness pathophysiology is the main factor that contributes to the therapeutic success (16). Although descriptive and with high rate mortality our results show that antibiotic prevented in part the changes of the mitochondrial respiratory chain. White blood cells and oxidative stress are responsible to apoptosis activation; furthermore, treatment with antibiotics decreases immunogenic components in the CSF. The complete sterilisation of *Neisseria meningitidis* from CSF occurs within 2 h of given a parenteral third-generation cephalosporin and the beginning of sterilisation of *Streptococcus pneumoniae* from CSF by 4 h into treatment (1). In previous studies, we verified that early antibiotic administration prevented cognitive impairment induced by meningitis in rats (45) and prevented in part oxidative stress (22).

We believe that the damage by *K. pneumoniae* meningitis is related to mitochondrial respiratory chain dysfunction. The statistic shows differences between groups; however, the work do not have statistical power to generalise the findings. Although descriptive, our findings suggest that the meningitis model could be a good research tool to study the biological mechanisms involved in the pathophysiology of this illness and the secondary alterations of the *K. pneumoniae* meningitis.

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References

1. Kim KS. Acute bacterial meningitis in infants and children. Lancet Infect Dis 2010;**10**:32–42.
2. Klinger G, Chin CN, Beyene J, Perlman M. Predicting the outcome of neonatal bacterial meningitis. Pediatrics 2000;**106**:477–482.
3. Hussen AS, Shafran SD. Acute bacterial meningitis in adults. A 12-year review. Medicine (Baltimore) 2000;**79**:360–368.
4. Ko WC, Paterson DL, Sagnimeni AJ et al. Community-acquired *Klebsiella pneumoniae* bacteremia: global differences in clinical patterns. Emerg Infect Dis 2002;**8**:160–166.
5. Lu CH, Chang WN, Chang HW. Klebsiella meningitis in adults: clinical features, prognostic factors and therapeutic outcomes. J Clin Neurosci 2002;**9**:533–588.
6. Barichello T, Savi GD, Simões LR et al. Evaluation of mitochondrial respiratory chain in the brain of rats after pneumococcal meningitis. Brain Res Bull 2010;**82**:302–307.
7. Sahly H, Podschun R. Clinical, bacteriological, and serological aspects of Klebsiella infections and their spondylarthropathic sequelae. Clin Diagn Lab Immunol 1997;**4**:393–399.
8. Liu YC, Cheng DL, Lin CL. *Klebsiella pneumoniae* liver abscess associated with septic endophthalmitis. Arch Intern Med 1986;**146**:1913–1916.
9. Su CM, Chang WN, Tsai NW, Huang CR, Wang HC, Lu CH. Clinical features and outcome of community-acquired bacterial meningitis in adult patients with liver cirrhosis. Am J Med Sci 2010;**340**:452–456.
10. Tsai MH, Lu CH, Huang CR et al. Bacterial meningitis in young adults in Southern Taiwan: clinical characteristics and therapeutic outcomes. Infection 2006;**34**:2–8.
11. Tang LM, Chen ST, Hsu WC, Chen CM. Klebsiella meningitis in Taiwan: an overview. Epidemiol Infect 1997;**119**:135–142.
12. Hirst RA, Kadioglu A, O'Callaghan C, Andrew PW. The role of pneumolysin in pneumococcal pneumonia and meningitis. Clin Exp Immunol 2004;**138**:195–201.
13. Leib SL, Tauber MG. Pathogenisis of bacterial meningitis. Infect Dis Clin North Am 1999;**13**:527–548.
14. Grandgirard D, Leib SL. Meningitis en Neonatos: bench to bedside. Clin Perinatol 2010;**37**:655–676.
15. Klein M, Koedel U, Pfister HW. Oxidative stress in pneumococcal meningitis: a future target for adjunctive therapy? Prog Neurobiol 2006;**80**:269–280.
16. Sellner J, Täuber MG, Leib SL. Pathogenesis and pathophysiology of bacterial CNS infections. Handb Clin Neurol 2010;**96**:1–16.
17. Tauber MG, Moser B. Cytokines and chemokines in meningeal inflammation: biology and clinical implications. Clin Infect Dis 1999;**28**:1–12.
18. Bessman SP, Carpenter CL. The creatine-creatine phosphate energy shuttle. Annu Rev Biochem 1985;**54**: 831–865.
19. Schnyder T, Winkler H, Gross H, Eppenberger HM, Wallimann T. Crystallization of mitochondrial creatine kinase. Growing of large protein crystals and electron microscopic investigation of microcrystals consisting of octamers. J Biol Chem 1991;**266**:5318–5322.
20. Wallimann T, Wyss M, Brdiczka D, Nicolay K, Eppenberger HM. Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the 'phosphocreatine circuit' for cellular energy homeostasis. Biochem J 1992;**281**:21–40.
21. Brand MD, Nicholls DG. Assessing mitochondrial dysfunction in cells. Biochem J 2011;**435**:297–312.

22. Barichello T, Savi GD, Silva GZ et al. Antibiotic therapy prevents, in part, the oxidative stress in the rat brain after meningitis induced by *Streptococcus pneumoniae*. Neurosci Lett 2010;**478**:93–96.
23. Irazuzta JE, Pretzlaff RK, Zingarelli B, Xue V, Zemlan F. Modulation of nuclear factor-kB activation and decreased markers of neurological injury associated with hypothermic therapy in experimental bacterial meningitis. Crit Care Med 2002;**30**:2553–2559.
24. Grandgirard D, Schürch C, Cottagnoud P, Leib SL. Prevention of brain injury by the nonbacteriolytic antibiotic daptomycin in experimental pneumococcal meningitis. Antimicrob Agents Chemother 2007;**51**:2173–2178.
25. Hoogman M, van de Beek M, Weisfelt M, de Gans J, Schmand B. Cognitive outcome in adults after bacterial meningitis. J Neurol Neurosurg Psychiatry 2007;**78**:1092–1096.
26. Barichello T, Silva GZ, Savi GD et al. Brain creatine kinase activity after meningitis induced by *Streptococcus pneumoniae*. Brain Res Bull 2009;**80**:85–88.
27. Lowry OH, Rosebough NG, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;**193**:265–275.
28. Cassina A, Radi R. Differential inhibitory action of nitric oxide and peroxynitrite on mitochondrial electron transport. Arch Biochem Biophys 1996;**328**:309–316.
29. Fischer C, Ruitenbeek W, Berden JA. Differential investigation of the capacity of succinate oxidation in human skeletal muscle. Clin Chim Acta 1985;**153**:23–36.
30. Rustin P, Chretien D, Bourgeron T et al. Biochemical and molecular investigations in respiratory chain deficiencies. Clin Chim Acta 1994;**228**:35–51.
31. Hughes BP. A method for estimation of serum creatine kinase and its use in comparing creatine kinase and aldolase activity in normal and pathologic sera. Clin Chim Acta 1962;**7**:597–604.
32. Chang WN, Lu CH, Huang CR et al. Clinical characteristics of post-neurosurgical *Klebsiella pneumoniae* meningitis in adults and a clinical comparison to the spontaneous form in a Taiwanese population. J Clin Neurosci 2010;**17**:334–338.
33. Lu CH, Chang WN, Lin YC et al. Bacterial brain abscess: microbiological features, epidemiological trends and therapeutic outcomes. Q J Med 2002;**95**:501–509.
34. Coimbra RS, Voisin V, Saizieu Ab DE et al. Gene expression in cortex and hippocampus during acute pneumococcal meningitis. BMC Biol 2006;**4**:15.
35. Wen LL, Chiu CT, Huang YN, Chang CF, Wang JY. Rapid glia expression and release of proinflammatory cytokines in experimental *Klebsiella pneumoniae* meningoencephalitis. Exp Neurol 2007;**205**:270–278.
36. Mitchell L, Smith SH, Braun JS, Herzog KH, Weber JR, Tuomanen EI. Dual phases of apoptosis in pneumococcal meningitis. J Infect Dis 2004;**190**:2039–2046.
37. Grimwood K, Anderson P, Anderson V, Tan L, Nolan T. Twelve year outcomes following bacterial meningitis: further evidence for persisting effects. Arch Dis Child 2000;**83**:111–116.
38. Andres RH, Ducray AD, Schlattner U, Wallimann T, Widmer HR. Functions and effects of creatine in the central nervous system. Brain Res Bull 2008;**76**:329–343.
39. Bénit P, Lebon S, Rustin P. Respiratory-chain diseases related to complex III deficiency. Biochim Biophys Acta 2009;**1793**:181–185.
40. Keightley JA, Anitori R, Burton MD, Quan F, Buist NR, Kennaway NG. Mitochondrial encephalomyopathy and complex III deficiency associated with a stop-codon mutation in the cytochrome b gene. Am J Hum Genet 2000;**67**:1400–1410.
41. Mourmans J, Wendel U, Bentlage HA et al. Clinical heterogeneity in respiratory chain complex III deficiency in childhood. J Neurol Sci 1997;**49**:111–117.
42. Nau R, Soto A, Bruck W. Apoptosis of neurons in the dentate gyrus in humans suffering from bacterial meningitis. J Neuropathol Exp Neurol 1999;**58**:265–274.
43. Leib SL, Heimgartner C, Bifrare YD, Loeffler JM, Täauber MG. Dexamethasone aggravates hippocampal apoptosis and learning deficiency in pneumococcal meningitis in infant rats. Pediatr Res 2003;**4**:4.
44. Wu UI, Mai FD, Sheu JN et al. Melatonin inhibits microglial activation, reduces pro-inflammatory cytokine levels, and rescues hippocampal neurons of adult rats with acute *Klebsiella pneumoniae* meningitis. J Pineal Res 2011;**50**:59–70.
45. Barichello T, Silva GZ, Batista AL et al. Early antibiotic administration prevents cognitive impairment induced by meningitis in rats. Neurosci Lett 2009;**465**:71–73.

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