available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Alterations in the brain electrical activity in a rat model of sepsis-associated encephalopathy

*Ilker M. Kafa**, *Sinan Bakirci*, *Murat Uysal*, *M. Ayberk Kurt*

Uludag University, School of Medicine, Department of Anatomy, Bursa, Turkey

ARTICLE INFO

Article history:
Accepted 15 July 2010
Available online 29 July 2010

Keywords:
Sepsis-associated encephalopathy
Electrocorticography
Somatosensory-evoked potentials
Cecal ligation and puncture
Animal model

ABSTRACT

Sepsis and septic shock are the commonest causes of death in the intensive care units. Although recent research have improved our understanding of the progress and pathophysiology of sepsis and septic shock, underlying mechanisms in sepsis-associated encephalopathy is still poorly understood. The incidence of sepsis-associated encephalopathy has been reported to vary from 8% to 70% of septic patients. We aimed at investigating the brain's electrical activity using somatosensory-evoked potentials and electrocorticographical recordings in cecal ligation and puncture rat model of sepsis. Significant decrease in mean arterial pressure, increase in heart rate, deteriorated neurological reflexes together with positive blood cultures results, thrombocytopenia and increased blood lactate levels suggesting the successful induction of sepsis in the present study. Elongated latencies and increased amplitudes were observed in somatosensory recordings of septic group, while electrocorticograms revealed slight decrease in median and spectral edge frequencies amplitudes and significantly increased delta activities in 50% of the septic rats. These results would suggest that the studies based on the investigation of the sepsis-associated encephalopathy in animal models needs to be combined with the electrophysiological confirmations of the brain dysfunction following the induction of sepsis.

1. Introduction

Sepsis is the most frequent cause of mortality in intensive care units with its complications. 750,000 sepsis cases per year had been reported in the United States with a mortality rate of 30% (Angus et al., 2001; Sands et al., 1997). The term of sepsis compromise wide range of definitions which are related to systemic infection and characterized by the presence of at least two of the following; >38 °C (100.4 °F) or <36 °C (96.8 °F) temperature; >90 beats per minute heart rate; >20/min respiratory rate or <32 mm Hg $PaCO_2$; >12,000/mm^3 or <4000/mm^3 white blood cell count. Severe sepsis with aggravated hypotension non-responsive to fluid resuscitation and deficiency in organ perfusion was defined as septic shock (Bone et al., 1992; Green et al., 2004; Levy et al., 2003).

Sepsis-associated encephalopathy (SAE), septic encephalopathy and critical illness encephalopathy are analogous terms that define diffuse or multifocal brain dysfunction related to sepsis and manifest itself with a range of symptoms ranging from lack of attention to confusion, lethargy and coma (Sharshar et al., 2004; Siami et al., 2008; Streck et al., 2008; Wilson and Young, 2003). Despite of high and variable incidence

* *Corresponding author*. Uludag University, Faculty of Medicine, Anatomy Department, Gorukle, 16059, Bursa, Turkey. Fax: +90 224 441 75 35.
E-mail address: imkafa@uludag.edu.tr (I.M. Kafa).

0006-8993/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2010.07.049

of brain dysfunction that develops in sepsis, underlying mechanisms and diagnostic criteria for SAE are still in debate (Green et al., 2004). Various mechanisms had been proposed for pathophysiology of SAE including the bacteriemia or the effect of the endotoxins (Chelazzi et al., 2008; Jackson et al., 1985; Kadoi et al., 1996; Young et al., 1992), blood–brain barrier disruption (Ari et al., 2006; Hasselgren and Fischer, 1986; Jeppsson et al., 1981; Kafa et al., 2007), microvascular disorders such as microinfarctions and microthrombosis (Maekawa et al., 1981), microabscesses (Gray et al., 2002), alterations in amino acid, cytokine and neurotransmitter levels (Basler et al., 2002; Freund et al., 1979; Sprung et al., 1990; Winder et al., 1988), apoptosis (Gray et al., 2002), excitotoxicity and oxidative stress (Guerra-Romero et al., 1993; Sewerynek et al., 1995), impairment of auto regulatory power (Moller et al., 2002) and calcium deregulation (Zhan et al., 1996). Iatrogenically induced encephalopathy or complications of medical therapy have also been proposed as a cause of SAE (Aminoff, 1995). However, it should be noted that previously proposed mechanisms of SAE and each assumption on the characterization of disorder has its own limitations (Siami et al., 2008).

The diagnosis of SAE based on the evaluation of the state of consciousness, alterations in cognitive function and brain's electrical activity recordable by electroencephalogram (EEG) (Eidelman et al., 1996). Abnormal findings in EEG recordings may represent a mental dysfunction in sepsis and it may show diffuse slowing even if the neurological signs are normal (Wilson and Young, 2003). Slowing in theta waves, diffuse delta waves, generalized triphasic waves and suppression has been reported to develop gradually, as the encephalopathy progress and these changes in EEG recordings have been suggested to be in correlation with the mortality of sepsis (Wilson and Young, 2003). More recently, somatosensory-evoked potential (SEP) recordings have also been used as a reliable technique and a sensitive marker for early changes in cerebral function in SAE (Chelazzi et al., 2008). The intention of this study is not to recount clinical works on electrophysiological changes in sepsis but to shed light on this subject in an animal model, therefore, we aimed at investigating the electrical activity of rat brains through electrocorticography (ECoG) and SEP recordings in cecal ligation and puncture (CLP) rat model, in an attempt to provide further information on the electrophysiological characteristics of septic encephalopathy and its relations with the clinical findings of human sepsis.

2. Results

2.1. *Clinical, vital and hematologic findings*

Symptoms indicating a septic status such as piloerection, shivering, lethargy, ocular and nasal exudation were observed in all animals in CLP group. Post mortem laparotomy revealed a clear peritoneal cavity with a non-septic appearance in sham-operated and unoperated control groups, while diffuse purulent matter, necrosis, adhesions and inflammation were observed in the peritoneal cavity of the animals in CLP group. Within 24 h, none of the rats were dead in sham-operated and unoperated controls, while mortality rate was 33% in CLP group.

Differences in all parameters for three groups (CLP, sham-operated and unoperated controls) were first analyzed using the Kruskal–Wallis Test, in order to discern any significant alterations between the groups and followed by the Mann–Whitney test for multiple comparisons. Repeated Measure (RM) ANOVA (one-way or Friedman) was also used to analyze trends over time for parameters for each group. Sham-operated and unoperated control groups reflected a mean arterial pressure (MAP) outline with a relatively flat pattern which was normalized at the end of the monitoring process (80.5% and 101.6% of preliminary values, respectively) (Fig. 1A). Kruskal–Wallis test showed a significant difference between groups in MAP at 24 h ($p<0.01$) and the drop in MAP was significant compared to sham-operated and unoperated controls at this time point ($p<0.05$ and $p<0.01$ respectively, Mann–Whitney *U* Test). In CLP group, following a slight increase at 6 h, MAP significantly dropped (to 66.7%, $p<0.05$, RM ANOVA) at 12 h compared to baseline values at 0 h and this drop became more evident at 24 h (to 43.4%, $p<0.05$, RM ANOVA). A significant difference between groups was also found for heart rate at 6, 12 and 24 h ($p<0.01$, for all comparisons, Kruskal–Wallis Test). Mann–Whitney *U* Test revealed a significant difference between CLP and unoperated group at 12 and 24 h ($p<0.01$, for both comparisons) and sham-operated group at 6-24 h ($p<0.01$) (Fig. 1B). Heart rate significantly increased in CLP group after 6 h ($p<0.05$ at 6 h and $p<0.01$ at 12 h, RM ANOVA) and increased to 144% of initial values at 24 h ($p<0.05$, RM ANOVA) (Fig. 1B). There was no significant difference between the two control groups for MAP and heart rate ($p>0.05$). Although a significant decline was observed in rectal temperature at 2 h in both CLP and sham-operated groups ($p<0.05$, RM ANOVA), the difference was not significant in the remainder of the monitoring period compared to initial values in both groups ($p>0.05$, for both group) (Fig. 1C).

Neurological scorings were significantly different between the groups with Kruskal–Wallis test at 2, 6, 12 and 24 h ($p<0.01$, for all time points) (Fig. 1D). Neurologic assessment showed remarkable loss in neurological reflexes at 2 h in all anaesthetized animals compared to unoperated control group. RM ANOVA shoved that the neurological responses were then gradually recovered between 2 and 12 h ($p<0.05$) and a full recovery was observed in 24 h ($p>0.05$) in sham-operated group. Although similar scores were also observed in the first 12 h in CLP rats, unlike the sham-operated group, the poor neurological scores persisted at 24 h and the scores were found to be significantly lower compared to both sham-operated and unoperated controls ($p<0.05$, for both comparisons, Mann–Whitney *U* Test).

Blood lactate levels were significantly higher in CLP group (7.32 ± 0.6 mmol/L) compared to controls ($p<0.05$, for both comparisons, Mann–Whitney *U* Test) while there was no significant difference between the two controls ($p>0.05$) (Table 1). Either leucopenia or leukocytosis was observed in rats within the CLP group, however means of leukocyte levels did not reveal a significant difference between CLP group and control groups ($p>0.05$, for both comparisons). Monocyte and erythrocyte levels were significantly higher and thrombocyte level was significantly lower in CLP group compared to unoperated control group ($p<0.05$, for all comparisons).