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BRIEF REPORT

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Imipramine reverses the depressive symptoms in sepsis survivor rats

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Abstract *Objective:* To evaluate the antidepressant effect of imipramine on depressive symptoms observed in sepsis survivors rats. *Design and setting:* Prospective, controlled experiment in an animal basic science laboratory. *Subjects:* Male Wistar rats weighing 300–350 g. *Interventions:* The rats underwent cecal ligation and perforation (CLP; sepsis group) with "basic support" (saline at 50 ml/kg immediately and 12 h after CLP plus ceftriaxone at 30 mg/kg and clindamycin at 25 mg/kg 6, 12, and 18 h after CLP) or sham-operated (control group). After 10 days of recovery rats received intraperitoneal injections of imipramine 10 mg/kg or saline and were subjected to the forced swimming test. *Measurements and results:* The observed increase in the immobility time in the forced swimming test in animals subjected to CLP, as a parameter of depressive behavior, was reversed by imipramine. *Conclusions:* The depressive symptoms evaluated by forced swimming test had been reversed after imipramine administration. Our data provide evidence that CLP-induced depressive symptoms are sensitive to antidepressants.

Keywords Sepsis · Survivors · Cecal ligation and puncture · Depressive-like symptoms · Rat

Introduction

Despite major improvements in intensive care and antibiotic therapy mortality and morbidity due to severe sepsis and septic shock remain high. Critical illness survivors present long-term cognitive impairment, including alterations in memory, attention, concentration, and/or global loss of cognitive function and beyond some clinical studies that show depressive symptoms in survivors from severe diseases as sepsis and septic shock [1–5]. Cecal ligation and perforation (CLP) models have contributed to elucidate the pathogenesis and to determine new therapies in sepsis [2–4]. Previous studies have shown that sepsis survivors rats after 10 days of operation presented symptoms of depression in the forced swimming task [5]. We evaluated whether depressive symptoms induced by CLP are sensitive to antidepressant drug imipramine.

Materials and methods

Under anesthesia (80 mg/kg ketamine, 10 mg/kg xylazine) 105 male Wistar rats (300–350 g) underwent CLP (sepsis group) and 60 rats underwent sham operation (control group) as previously described [3]. After surgery the sepsis group received "basic support" (saline at 50 ml/kg

immediately and 12 h after CLP plus 30 mg/kg ceftriaxone and 25 mg/kg clindamycin every 6 h over a total of 3 days). The sham-operated group received only saline (50 ml/kg) immediately and 12 h after surgery, and the volume of saline corresponded to antibiotic administration. Survival in the sham group was 100% and in the sepsis group 40% (40 rats). The number of survivals is in accordance with our previous reports [3–5]. Ten days after surgery the animals separately underwent two behavioral tasks: (a) forced swimming test (FST) to evaluate depressive-like symptoms, and (b) the open-field task as a control experiment to evaluate locomotor activity.

The behavioral tests were performed by the same person, who was blinded as to group (SHAM or CLP). All experimental procedures involving animals were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and with the approval by the local ethics committee. The forced swim test was conducted according to previous reports [6, 7]. In brief, the test involves two exposures to a cylindrical tank of water in which rats cannot touch the bottom of the tank or escape. The tank is made of transparent Plexiglas, 80 cm tall, 30 cm in diameter, and was filled with water (22–23 °C) to a depth of 40 cm. Water in the tank was changed after each rat. For the first exposure rats were placed in the water for 15 min (pretest session). The rats had been treated with imipramine in the doses of 10 mg/kg or saline 5 min and 19 and 23 h after the first swimming exposure. This procedure resulted in four experimental groups: SHAM + SAL, SHAM + IMI, CLP + SAL, and CLP + IMI. After 24 h the rats were placed in the water again for a 5 min session (test session). Behavior was videotaped for later analysis, and the periods of immobility and swimming time were recorded.

The behavior in the open field was carried out as a control for locomotor activity in a 40 × 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 into 12 equal rectangles by black lines. Animals were gently placed on the left rear quadrant, and left to explore the arena for 5 min, and their number of crossings was measured. Crossing of the black lines performed in this session were counted [8].

Data are presented as mean ± SEM and were analyzed by analysis of variance followed by Tukey's post-hoc test if necessary.

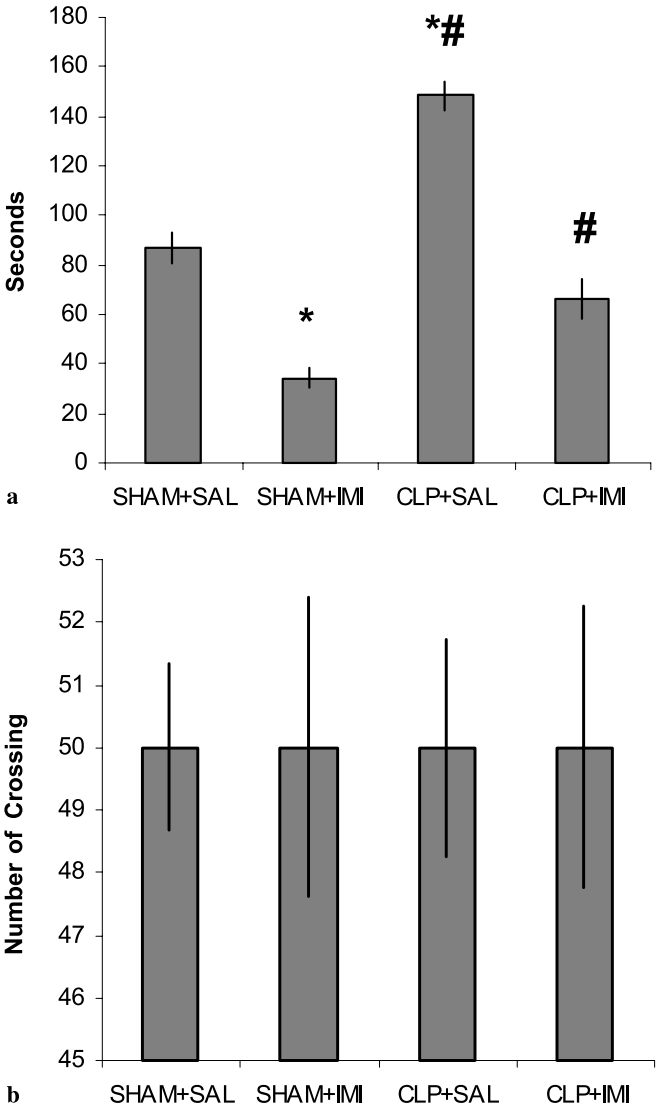

Fig. 1 **a** Forced swimming task. Sepsis group showed a significant increase in the time of immobility, and this behavior was reversed by imipramine. Data are expressed as mean ± SEM, $n = 15$ animals per group; * $p < 0.05$ vs. CLP + SAL and SHAM + IMI with SHAM + SAL, # $p < 0.05$ vs. CLP + IMI and CLP + SAL with SHAM + IMI. **b** Behavior in the open-field. No significant difference was seen in the numbers of crossings between groups in the open-field behavior. Data are expressed as mean ± SEM, $n = 15$ animals per group

Results

In the forced swimming test (Fig. 1A) session depressive-like behavior was observed as a significant increase in the immobility time in the CLP + SAL group ($p < 0.05$) as our group previous described [5]. As we also expected SHAM + IMI group presented a reduction in the immobility time ($p < 0.05$) as a demonstration of validity of our protocol. Our main result was a reduction in the immobility time observed in the CLP + IMI group ($p < 0.05$) was not similar that observed for SHAM + IMI group ($p > 0.05$). These results demonstrate that imipramine reverses the depressivelike symptoms induced by CLP.

In the open-field test (Fig. 1B) no difference in motor activity was demonstrated between groups as observed by the number of crossings ($p > 0.05$), demonstrating

no impairment in motor activity secondary to CLP or imipramine.

Discussion

Previous studies have shown that sepsis survivors presented depressivelike symptoms assessed in the FST [5]. The original view of the FST offered by Porsolt [6] was that of a model of depression with similar features to the learned helplessness model but technically easier to produce. The internal affective state of rodents after exposure to the initial swim in the forced swimming task was labeled as "behavioral despair." The pretest swim induction procedure was similar procedurally to the initial session that induces learned helplessness by exposing rats to inescapable stress. Induction of learned helplessness produces broad-ranging behavioral deficits in affect, cognition, sleep, and motor performance that closely resemble many of the symptoms of depression [8]. Additionally, as described above, the sepsis survivors group did not present locomotor activity impairment, reinforcing that higher immobility time in sepsis group was related to depressivelike symptoms [7].

The FST is undoubtedly the most extensively used rodent model of depression [9]. This model has a high degree of pharmacological validity, as evidenced by its sensitivity to major classes of antidepressants, tricyclic compounds, monoamine oxidase inhibitors, atypical antidepressants, selective serotonin reuptake inhibitors, and electroconvulsive shock [9]. Some researchers believe that the FST should be considered no more than a simple screen for antidepressant drugs [10]. However, because of its sensitivity to antidepressants and to stimuli that provoke depressive behavior, the FST seems to measure a behavioral dimension that is relevant to depression [10]. Some disagreement may arise between the original claim that the FST is a rodent model of human depression and the more careful consideration and use of the FST as an objective marker for a behavioral state associated with depression. Thus our findings should be considered with these limitations in mind.

Several cognitive skills are impaired in animals and humans survivors from sepsis [1–5], and we here demonstrated that the depressive behavior induced by sepsis was reversed by imipramine. In addition, the efficacy of imipramine suggested that the immobility in the FST observed in sepsis survivors was not a nonspecific finding related to the effects of sepsis in the central nervous system but a disorder with typical physiopathological alterations that could be pharmacologically explored. In this way we believe that the CLP model of sepsis will help us to investigate the biological mechanisms involved in the symptoms of depression associated with sepsis and to determine therapeutic approaches to this problem.

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