Neurochemistry International 108 (2017) 436–447

Contents lists available at ScienceDirect

Neurochemistry International

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

Alpha-lipoic acid attenuates acute neuroinflammation and long-term cognitive impairment after polymicrobial sepsis

Amanda Della Giustina [a], Mariana Pereira Goldim [a], Lucinéia Gainski Danielski [a], Drielly Florentino [a], Khiany Mathias [a], Leandro Garbossa [a], Aloir Neri Oliveira Junior [a], Maria Eduarda Fileti [a], Graciela Freitas Zarbato [a], Naiana da Rosa [a], Ana Olívia Martins Laurentino [a], Jucélia Jeremias Fortunato [a], Francielle Mina [e], Tatiani Bellettini-Santos [e], Josiane Budni [e], Tatiana Barichello [b, c, d, e], Felipe Dal-Pizzol [f], Fabricia Petronilho [a, *]

[a] Laboratory of Neurobiology of Inflammatory and Metabolic Processes, Graduate Program in Health Sciences, University of South Santa Catarina, Tubarão, SC, Brazil
[b] Translational Psychiatry Program, Department of Psychiatry and Behavioral Sciences, The University of Texas Health Science Center at Houston, McGovern Medical School, Houston, TX, USA
[c] Center of Excellence on Mood Disorders, Department of Psychiatry and Behavioral Sciences, The University of Texas Health Science Center at Houston, McGovern Medical School, Houston, TX, USA
[d] Neuroscience Graduate Program, The University of Texas Graduate School of Biomedical Sciences at Houston, Houston, TX, USA
[e] Laboratory of Neurosciences, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina, Criciúma, SC, Brazil
[f] Laboratory of Experimental Pathophysiology, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina, Criciúma, SC, Brazil

ARTICLE INFO

Article history:
Received 23 February 2017
Received in revised form
31 May 2017
Accepted 7 June 2017
Available online 10 June 2017

Chemical compounds studied in this article:
Alpha-lipoic acid (PubChem CID:6112)
Ceftriaxone (PubChem CID:5479530)
Copper sulfate (PubChem CID:24462)
Dinitrophenylhydrazone (PubChem CID:5367495)
Dipyrone (PubChem CID:522325)
Evans blue dye (PubChem CID:24832074)
Hexadecyltrimethylammonium bromide (PubChem CID:121494050)
Ketamine (PubChem CID:15851)

ABSTRACT

Sepsis is a complication of an infection which imbalance the normal regulation of several organ systems, including the central nervous system (CNS). Evidence points towards inflammation and oxidative stress as major steps associated with brain dysfunction in sepsis. Thus, we investigated the α-lipoic acid (ALA) effect as an important antioxidant compound on brain dysfunction in rats. *Wistar* rats were subjected to sepsis by cecal ligation and perforation (CLP) or sham (control) and treated orally with ALA (200 mg/kg after CLP) or vehicle. Animals were divided into sham + saline, sham + ALA, CLP + saline and CLP + ALA groups. Twelve, 24 h and 10 days after surgery, the hippocampus, prefrontal cortex and cortex were obtained and assayed for levels of TNF-α and IL-1β, blood brain barrier (BBB) permeability, nitrite/nitrate concentration, myeloperoxidase (MPO) activity, thiobarbituric acid reactive species (TBARS) formation, protein carbonyls, superoxide dismutase (SOD) and catalase (CAT) activity and neurotrophins levels. Behavioral tasks were performed 10 days after surgery. ALA reduced BBB permeability and TNF-α levels in hippocampus in 24 h and IL-1β levels and MPO activity in hippocampus and prefrontal cortex in 24 h. ALA reduced nitrite/nitrate concentration and lipid peroxidation in 24 h in all structures and protein carbonylation in 12 and 24 h in hippocampus and cortex. CAT activity increased in the hippocampus and cortex in all times. ALA enhanced NGF levels in hippocampus and cortex and prevented cognitive

Abbreviations: ALA, alpha-lipoic acid; BBB, blood brain barrier; BDNF, brain-derived neurotrophic factor; CAT, catalase; CLP, cecal ligation and perforation; CREB, cAMP-response element-binding protein; CVO, circumventricular organ; IL, interleukin; LPS, lipopolysaccharide; MAPK, mitogen activated protein kinase; MPO, myeloperoxidase; NO, nitric oxide; NF-κB, nuclear factor-κB; NGF, nerve growth factor; SAE, sepsis-associated encephalopathy; SOD, superoxide dismutase; RNS, reactive nitrogen species; ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive species; TNF-α, tumor necrosis factor-α; Trk, tropomyosin receptor kinase.

* Corresponding author. Laboratório de Neurobiologia de Processos Inflamatórios e Metabólicos, Programa de Pós-graduação em Ciências da Saúde, Universidade do Sul de Santa Catarina, Tubarão, SC, Brazil.
E-mail address: fabricia.petronilho@unisul.br (F. Petronilho).

http://dx.doi.org/10.1016/j.neuint.2017.06.003
0197-0186/

Thiopental (PubChem CID:23665410)
Trichloroacetic acid (PubChem CID:6421)
Xylazine (PubChem CID:5707)

Keywords:
Sepsis
Antioxidant
Behavior
Blood brain barrier
Oxidative stress
Neuroinflammation

impairment. Our data demonstrates that ALA reduces the consequences of polymicrobial sepsis in rats by decreasing inflammatory and oxidative stress parameters in the brain.

1. Introduction

Sepsis-associated encephalopathy (SAE) is defined as a diffuse cerebral dysfunction caused by a systemic inflammatory response to infection and is associated with an increased rate of morbidity and mortality in Intensive Care Units (Hotchkiss and Karl, 2003; Warren, 1997). Moreover, SAE is clinically characterized by slowing of mental processes, impaired attention, disorientation, confusion, agitation, lethargy, delirium or coma (Gofton and Young, 2012). Studies show that 71% of septic patients develop irreversible acute brain dysfunction and about 70% of the sepsis survivors present, still in the hospital, neurocognitive impairment, and 40% of this cognitive impairment persists up to one year after hospitalization, evidencing the wide clinical importance of SAE (Gofton and Young, 2012; Sprung et al., 1990; Winters et al., 2010).

The exact mechanisms for brain dysfunction in septic patients are far from clear. Currently, it is known that pathophysiology of SAE involves blood-brain barrier (BBB) disruption (Blom et al., 2015), mitochondrial and endothelial dysfunction and disturbances in neurotransmission (Blom et al., 2015; Bozza et al., 2013), as well as impairment of neuronal metabolism and direct cellular damage to the brain (Yao et al., 2014). However, the involvement of oxidative stress is indeed the most tested and proven hypothesis, which may culminate in these pathophysiological events (Berg et al., 2011; Bozza et al., 2013). With regards to experimental data, brain dysfunction due to sepsis usually promotes behavior alterations in animals and affects the expression of growth factors involved in neuroplasticity, such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), which reflects learning and memory impairment (Lipsky and Marini, 2007). Animal models of sepsis remain essential for early translational research, and to date the murine model that best resembles the human septic response is the cecal ligation and perforation (CLP) (Hubbard et al., 2005; Wichterman et al., 1980). In a model of polymicrobial sepsis induced by CLP, oxidative damage occurs in the hippocampus of animals in 0–96 h after the induction of sepsis due to increased production of free radicals (Barichello et al., 2006) and it may last up for 10 days (Comim et al., 2011a,b).

Previous reports have suggested that administration of antioxidants can protect mitochondria and decrease oxidative stress in brain regions in animal model of sepsis induced by CLP (Barichello et al., 2007a,b; Cassol-Jr et al., 2010a,b; Petronilho et al., 2012; Silvestre et al., 2014). The alpha-lipoic acid (ALA) or 1,2-dithiolane-3-pentanoic acid has been described in the literature as an “ideal” antioxidant. It is a naturally occurring dithiol compound synthesized enzymatically in the mitochondria from octanoic acid and exhibits unique antioxidant aspects. ALA chelates several metal ions, such as Cu^{2+}, Zn^{2+}, Pb^{2+}, Hg^{2+} and Fe^{3} (Ou et al., 1995) and scavenges reactive oxygen species (ROS) and nitrogen species (RNS) *in vitro* and *in vivo* (Rochette et al., 2013). Added to this, it can regenerate antioxidant molecules such as glutathione, vitamin C and vitamin E (Shay et al., 2009). Further, lipoic acid is a potent inhibitor of NF-kappaB (Packer et al., 1995) and decreased the expression of matrix metalloproteinase-9 (Kim et al., 2007) and TNF-α dependent on the transcriptional activity of this nuclear factor (Zhang and Frei, 2001). ALA shows solubility in water and lipids and together with its small chemical structure this facilitates its permeability through biological membranes, including the BBB (Holmquist et al., 2007; Maczurek et al., 2008).

Lipoic acid has been tested in different therapeutic situations, such as weight management (Kim et al., 2016; Li et al., 2017), multiple sclerosis (Khalili et al., 2014), male fertility (Haghighian et al., 2015) and type 2 diabetes (Ametov et al., 2003; Garcia-Alcala et al., 2015) and promoted positive results. Considering that ALA has been shown to have an antioxidant and a neuroprotective role, the potential value in improving long-term cognitive impairment has not been evaluated. Therefore, in this study, we examine the effects of ALA on brain oxidative stress, BBB permeability, inflammatory parameters, neurotrophins expression and cognitive function in rats after polymicrobial sepsis.

2. Materials and methods

2.1. Animals

Adult male Wistar rats, approximately 60 days old, weighing 250–350 g, from breeding colonies maintained at the Universidade do Sul de Santa Catarina were used. The animals were housed four per cage during the entire experiment, under controlled conditions of temperature (22 ± 1 °C), relative humidity (45–55%) and day/light cycle (12:12 h, light on at 06:00 h). Rat chow (standard diet for laboratory animals – NUVILAB CR-1®, Brazil) and tap water were available *ad libitum*. This study was approved by the Animal Research Ethic Committee of the Universidade do Sul de Santa Catarina (protocol # 15.043.4.01.IV). All experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978), making sure that all efforts were made to minimize the number of animals used and their suffering.

2.2. Sepsis induction–CLP model

Rats were subjected to polymicrobial sepsis induction by CLP as previously described (Hubbard et al., 2005). Briefly, they were anesthetized with a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg), given intraperitoneally. Under aseptic conditions, a 3-cm midline laparotomy was performed to expose the cecum and adjoining intestine. The cecum was tightly ligated with a 3.0 silk suture at its base, below the ileocecal valve, and was perforated once with 14-gauge needle. The cecum was then squeezed gently to extrude a small amount of feces through the perforation site. The cecum was then returned to the peritoneal cavity, and the laparotomy was closed with 4.0 silk sutures. Animals were resuscitated with regular saline (50 mL/kg) subcutaneously (s.c.) immediately after CLP. All animals received ceftriaxone (30 mg/kg) and dipyrone (80 mg/kg) subcutaneously (s.c.) immediately after and 12 h after