Journal of Neuroimmunology 278 (2015) 44–52

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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

Sepsis progression to multiple organ dysfunction in carotid chemo/baro-denervated rats treated with lipopolysaccharide

Gino Nardocci [a], Aldo Martin [a], Sebastián Abarzúa [a], Jorge Rodríguez [a], Felipe Simon [b,c], Edison P. Reyes [d,e], Claudio Acuña-Castillo [f], Cristina Navarro [a], Paula P. Cortes [a], Ricardo Fernández [a,*]

[a] Laboratorio de Fisiología, Departamento de Ciencias Biológicas, Facultad de Ciencias Biológicas y Facultad de Medicina, Universidad Andrés Bello, Santiago, Chile
[b] Laboratorio de Fisiopatología Integrativa, Departamento de Ciencias Biológicas, Facultad de Ciencias Biológicas y Facultad de Medicina, Universidad Andrés Bello, Santiago, Chile
[c] Millenium Institute on Immunology and Immunotherapy, Santiago, Chile
[d] Centro de Fisiología Celular e Integrativa, Facultad de Medicina, Clínica Alemana — Universidad del Desarrollo, Santiago, Chile
[e] Dirección de Investigación, Universidad Autónoma de Chile, Santiago, Chile
[f] Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile

ARTICLE INFO

Article history:
Received 11 September 2014
Received in revised form 29 November 2014
Accepted 1 December 2014
Available online xxxx

Keywords:
Sepsis
Carotid body
TNF-α
Glucocorticoids
Epinephrine
Tissue damage
Bilateral carotid/sinus neurotomy
Multiple organ dysfunction

ABSTRACT

Sepsis progresses to multiple organ dysfunction (MOD) due to the uncontrolled release of inflammatory mediators. Carotid chemo/baro-receptors could play a protective role during sepsis. In anesthetized male rats, we measured cardiorespiratory variables and plasma TNF-α, glucocorticoids, epinephrine, and MOD marker levels 90 min after lipopolysaccharide (LPS) administration in control (SHAM surgery) and bilateral carotid chemo/baro-denervated (BCN) rats. BCN prior to LPS blunted the tachypneic response and enhanced tachycardia and hypotension. BCN-LPS rats also showed blunted plasma glucocorticoid responses, boosted epinephrine and TNF-α responses, and earlier MOD onset with a lower survival time compared with SHAM-LPS rats. Consequently, the complete absence of carotid chemo/baro-sensory function modified the neural, endocrine and inflammatory responses to sepsis. Thus, carotid chemo/baro-receptors play a protective role in sepsis.

1. Introduction

Sepsis syndrome is closely associated with many pathological processes such as systemic inflammation, coagulopathies, hemodynamic abnormalities, and multiple organ dysfunction syndrome (MODS) (Riedemann et al., 2003). Sepsis syndrome includes systemic inflammatory response syndrome (SIRS) and its consequences (severe sepsis and septic shock). The progression of MODS associated with systemic inflammation is mainly caused by an uncontrolled release of pro-inflammatory mediators, which damage parenchymatous organs. Additionally, sepsis activates and/or depresses numerous other systems within the body, including neural, hormonal, and metabolic pathways (Carre and Singer, 2008; Deutschman and Tracey, 2014; Singer et al., 2004). Thus, systemic inflammation initiates the disruption of communication between different organ systems, and, subsequently, MODS reflects a progressive uncoupling that might become irremediable.

Despite many efforts and significant advances in maintaining therapies, sepsis syndrome and MODS are the main causes of death in critical care patients (Martin et al., 2003). This is mainly due to the absence of a truly effective therapy (Riedemann et al., 2003), along with the increasing projected incidence in the United States of 1.5% per annum and average costs per case of US$22,100 (Angus et al., 2001). Thus, the knowledge of the immunometabolic and neurophysiological mechanisms and the pathophysiology of sepsis progression to organ dysfunction and death would help us to improve current therapies and to identify new pharmacological therapeutic targets.

The nervous system, acting through the autonomic nervous system, coordinates the fine-tuning of cardiorespiratory interplay to maintain cellular bioenergetics and appropriate oxygen delivery to the tissues (Abboud and Thames, 1983; Eyzaguirre et al., 1983). Autonomic

Abbreviations: MODS, multiple organ dysfunction syndrome; CB, carotid body; LPS, lipopolysaccharide; IP, intraperitoneally; BCN, bilateral carotid neurotomy; P_S, systolic blood pressure; f_H, instantaneous heart frequency; V_T, tidal volume; f_R, instantaneous respiratory frequency; V_E, minute ventilatory volume; ECG, electrocardiogram; TNF-α, tumor necrosis factor-alpha; IL, interleukin; ELISA, enzyme-linked immunosorbent assay; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TBIL, total bilirubin; GGT, gamma-glutamyl transferase; CRE, creatinine; BUN, blood urea nitrogen; CK, creatine kinase; LDH, lactic dehydrogenase; ALP, alkaline phosphatase; AMY, amylase; GLU, glucose; LAC, lactic acid; NTS, *nucleus tractus solitarii*

* Corresponding author at: Laboratorio de Fisiología, Departamento de Ciencias Biológicas, Facultad de Ciencias Biológicas y Facultad de Medicina, Universidad Andrés Bello. Av. República 252, 8370134 Santiago, Chile.
E-mail address: rfernandez@unab.cl (R. Fernández).

http://dx.doi.org/10.1016/j.jneuroim.2014.12.002
0165-5728/

(sympathetic–parasympathetic) balance is maintained by several reflex arcs such as arterial baroreflexes (Kirchheim, 1976), central chemoreflexes, peripheral arterial chemoreflexes, and pulmonary stretch reflexes (Liljestrand, 1958). Therefore, the interactions among these reflexes are of special clinical interest because the overactivity of a single reflex, which pathophysiologically occurs in several disorders, can lead to the suppression of opposite reflex responses (Schmidt et al., 2001).

Increasing evidence obtained by us and other researchers has shown that a particular neural reflection, carotid body (CB) reflexes, not only serves as a chemoreceptor for respiratory reflex responses, as traditionally accepted, but also as a sensor for the immune status (Fan et al., 2009; Fernandez et al., 2011; Reyes et al., 2012; Wang et al., 2002, 2006; Zapata et al., 2011; Zhang et al., 2007) and as a modulator of autonomic balance, tending to coordinate the cardiorespiratory interplay (Del Rio et al., 2011, 2012) devoted to maintaining oxygen homeostasis in different pathologies. On the other hand, we found that the CB develops acute inflammation induced by local and systemic lipopolysaccharide (LPS) administration. Acute CB inflammation manifests itself as diminished chemosensory activity, ventilatory chemoreflexes and the ventilatory chemosensory drive (Fernandez et al., 2008). Shi et al. (2007) also found that the survival time in sinoaortic-denervated rats is significantly reduced when compared with sham-operated animals in a model of sepsis evoked by cecal ligation and puncture (Shi et al., 2007). The objective of this study was to assess the role played by carotid chemo/baro-receptors in the progression from sepsis to multiple organ dysfunction in LPS-induced septic rats. Consequently, we propose that carotid chemo/baro-receptors play a protective role during sepsis syndrome and MODS.

2. Materials and methods

2.1. Animals and surgical procedures

Young (5-week-old) male Sprague–Dawley rats, weighing from 90 to 130 g, were used. Experimental protocols were approved by the Commission of Bioethics and Biosafety of the Universidad Andres Bello. The animals were anesthetized with 60 mg/kg sodium pentobarbitone administered intraperitoneally (IP) (kindly provided by Dr. Patricio Zapata), with supplementary doses (12 mg kg^{-1} h^{-1}) given IP to maintain a light level of surgical anesthesia (stage 3, plane 2), and were placed in a supine position. The animals breathed spontaneously throughout the experiment. The body temperature, assessed with a rectal thermistor probe, was maintained at approximately 37.0 ± 0.1 °C, by placing a regulated heating pad under the rat.

The animals (total n = 50) were separated into four groups: control groups with a sham surgical intervention that omitted the neurotomy, i.e., with intact carotid/sinus nerves (SHAM), treated IP with either saline (0.9% NaCl) (SHAM-saline, n = 8) or 15 mg/kg lipopolysaccharide (LPS, from *Escherichia coli* serotype 0127:B8; L3129, Sigma-Aldrich Corp., USA) (given in 100 μL saline/100 g body weight) (SHAM-LPS, n = 12); and experimental groups submitted to bilateral carotid neurotomy (BCN), also treated IP with either saline (BCN-saline, n = 9) or 15 mg/kg LPS (BCN-LPS, n = 21). To gain access to the carotid regions on both sides, a ventral midline incision of the neck was performed. Carotid/sinus nerves were sectioned at their entrance to the carotid bodies (in BCN animals). BCN was confirmed by testing the ventilatory chemosensory drive, i.e., the suppression of the decrease in ventilation provoked by breathing 100% O_2 (Fernandez et al., 2003).

2.2. Cardiorespiratory recordings

To confirm the effectiveness of the LPS treatment (i.e., the induction of severe sepsis), the systolic blood pressure (P_S), instantaneous heart frequency (f_H), tidal volume (V_T), and instantaneous respiratory frequency (f_R) were recorded before surgery (either SHAM or BCN) and for up to 90 min after saline or LPS treatment (usually administered 15 min after simulated or effective surgery) with a physiological recording acquisition system.

The heart frequency was derived through a tachograph fed by the ECG signal obtained at Einthoven's lead II. P_S was measured using a pressure tail cuff for a non-invasive blood pressure recording system for rats (ML125/R) coupled to a MLT125/R pulse transducer (AD Instruments, Castle Hill, Australia). Transient introductions (1 min) of the rat head into a plastic mask connected to a respiratory flow head (MLT1L, AD Instruments) were conducted to measure the ventilatory flow ($\delta V/\delta t$), which was converted into V_T through a volumetric differential pressure transducer. Ventilatory signals were derived through a tachograph to determine f_R. All transducers were connected to a PowerLab® 8/30 (AD Instruments), and physiological variables were instantaneously displayed through the Chart® software (AD Instruments). In addition, raw signals were stored and subsequently analyzed.

The reported physiological values were obtained by averaging raw data recorded 2 min before surgery (Basal), 2 min before saline or LPS injections (post-surgery, either SHAM or BCN) or 2 min after the mentioned time point. At the end of the experiments, animals that did not die as a result of the treatment were euthanized by an overdose of pentobarbitone.

2.3. Blood samples and plasma measurements

At the end of the experiments (90 min after saline or LPS administration), cardiac puncture was performed for blood extraction into lithium heparin-containing tubes. The blood was immediately centrifuged at 5000 rpm for 10 min at 4 °C to separate the plasma, which was then used to measure tumor necrosis factor-alpha (TNF-α), corticosterone, cortisol, epinephrine, and different markers of multiple organ dysfunction (MOD).

Plasma TNF-α was measured by an enzyme-linked immunosorbent assay (ELISA), according to the protocol described elsewhere (Christodoulides et al., 2000), using a monoclonal anti-rat TNF-α capture antibody (R&D Systems Inc., MN, USA), matched biotinylated anti-rat TNF-α detecting antibody (R&D Systems, Inc.) and recombinant rat TNF-α standard (R&D Systems Inc.). The reaction was revealed with streptavidin-conjugated alkaline phosphatase and *p*-nitrophenyl phosphate, and the absorbance was determined at 405 nm. Plasma corticosterone and cortisol were measured with competitive EIA kits (Cayman Chemical Company, MI, USA) according to the manufacturers' instructions. Plasma epinephrine was also measured using a commercially available competitive ELISA kit (ALPCO Immunoassay, NH, USA).

2.4. Blood biochemical analyses

For evaluating organ functions, we measured plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TBIL), gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) for the liver; creatinine (CRE) and blood urea nitrogen (BUN) for the kidney; creatine kinase (CK) and lactic dehydrogenase (LDH) for the heart and other organs (such as muscle); amylase (AMY) for the exocrine pancreas; and glucose (GLU) and lactic acid (LAC) for metabolic function. MOD markers were measured by the Piccolo Xpress Chemistry Analyzer (General Chemistry, 13 panel) (Abaxis, CA, USA), the iSTAT System (CG4+ cartridge) (Abbott Laboratories, IL, USA) or by commercial kits (Valtek Diagnostics, Santiago, Chile), according to the manufacturers' instructions.

2.5. Statistical analysis

Data are expressed as the mean ± standard error of the mean (SEM) or as the mean ± 95% confidence interval (CI) for the relative risk. For the cardiorespiratory recordings, significant differences were assessed by a one-way ANOVA followed by Dunnett's post-test to compare