Journal of Neuroimmunology 238 (2011) 52–57

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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

Leukotriene synthesis inhibitor decreases vasopressin release in the early phase of sepsis

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ARTICLE INFO

Article history:
Received 17 May 2011
Received in revised form 25 July 2011
Accepted 1 August 2011

Keywords:
Infection
Inflammation
Nitric oxide
Hypotension
Vasopressor
Hormone

ABSTRACT

The aim was to analyze the effect of leukotriene synthesis inhibitor administered intraperitoneally in vasopressin release during sepsis. Male Wistar rats received injections of MK-886 (1.0, 2.0 or 4.0 mg/kg) or vehicle (DMSO 5%) 1 h before cecal ligation and puncture. There was some variation on the survival rate depending on the dose used but the drug did not modify the hematocrit, osmolality, serum sodium and nitrate, plasma protein, and neutrophil recruitment, in any dose. Nevertheless, vasopressin (AVP) release decreased in a dose–response manner in the early phase of sepsis. These results support the suggestion that leukotrienes (LTs) are involved in AVP release during sepsis.

1. Introduction

Clinical and experimental studies report that in the initial phase of sepsis, high AVP levels can be found in plasma of patients or animals. This can be interpreted as a systemic attempt, to restore blood pressure that tends to decrease due to cytokines and NO release. Notwithstanding, in the late phase, when the observed hypotension would be expected to stimulate AVP secretion, its plasma levels were inappropriately low (Landry et al., 1997; Landry and Oliver, 2001; Sharshar et al., 2003). Low plasma AVP levels can be considered a deleterious consequence of an abnormal pituitary response due to baroreflex impairment and high levels of nitric oxide that will also contribute to the hypotension, progression of sepsis, multiple organ failure and death (Landry et al., 1997; Holmes et al., 2001; Sharshar et al., 2003; Maxime et al., 2007). Understanding in detail the mechanisms responsible for the release of this hormone during sepsis should, thus, be of clinical importance.

During sepsis, proinflammatory cytokines are released in an uncontrolled manner, and a large number of these, in particular tumor necrosis factor (TNF)-α, interleukin (IL)-1α and interferon (IFN)-γ, as well as nitric oxide (NO), have been implicated in the pathophysiological process of this disease (Rios-Santos et al., 2003; Benjamim et al., 2005; Sriskandan and Altmann, 2008). At present, a growing body of evidence suggests that also lipid-derived inflammatory mediators, such as leukotrienes (LTs), may play a key role in sepsis-associated disorders (Sprague et al., 1989; Morlion et al., 2000; Benjamim et al., 2005; Baenkler et al., 2006). The capacity for complete biosynthesis of LTs from arachidonic acid (AA) is largely confined to leukocytes, and the biosynthesis pathway can be triggered by a variety of stimuli, including lipopolysaccharide (LPS), IL-1 and TNF-α. These stimuli activate signal transduction cascades that, in turn, activate LTs-forming enzymes (Clark et al., 1995; Hirabayashi and Shimizu, 2000). Briefly, a cytosolic phospholipase A_2 ($cPLA_2$) initiates the synthesis of LTs by cleavage of AA from membrane phospholipids. The subsequent interaction of AA with 5-lipoxygenase (5-LO) and 5-lipoxygenase activating protein (FLAP) forms the intermediate 5-HPETE (5-hydroxyperoxy-6,8,11,14-eicosatetraenoic acid), which is rapidly converted to LTA_4. This unstable intermediate can be hydrolyzed to form LTB_4 or it can be conjugated with glutathione by LTC_4 synthase to produce the cysteinyl leukotrienes (cys-LTs) LTC_4, LTD_4 and LTE_4 (Peters-Golden and Brock, 2003; Peters-Golden and Henderson, 2007). Furthermore, LTA_4 generated in one cell can be taken up by another one that expresses LTA_4 hydrolase or LTC_4 synthase to there continue its biochemical transformation (Murphy and Gijon, 2007). This process named transcellular biosynthesis suggests that the cellular environment exerts an important control over LTs production (Di Gennaro et al., 2004). In functional terms, LTB_4 promotes chemotaxis, activation and adhesion of leukocytes to endothelial cells, thus being the

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doi:10.1016/j.jneuroim.2011.08.001

mediator responsible for recruiting leucocytes from the circulation to the infection locus (Cunningham et al., 1980; Ford-Hutchinson et al., 1980; Gimbrone et al., 1984; Flamand et al., 2007). In contrast, the cys-LTs are better known for their bronchoconstriction effects, characteristic symptoms of patients with asthma (Dahlen et al., 1980), and in blood vessels they increase vascular permeability and cause hypotension (Dahlen et al., 1980; Smedegard et al., 1982; Henderson and Klebanoff, 1983; Goulet et al., 2000; Flamand et al., 2007). Cys-LTs are also involved in a complex network of interactions with a variety of inflammatory mediators, such as NO. Several studies have demonstrated the effect of inhibitors or antagonists of LTs in NO production. Montelukast (antagonist of Cys-LT1 receptor) has been shown to reduce levels of exhaled NO in clinical trials with asthmatic adults (Wilson et al., 2001; Sandrini et al., 2003) and children (Bisgaard et al., 1999; Bratton et al., 1999). This antagonist was also shown to reduce the expression of iNOS in the lung of rats (Knigge et al., 2003). In glial cells, the MK-886 (an LT biosynthesis inhibitor) was responsible for reducing the LPS-induced iNOS expression and NO production, suggesting that 5-LO mediates iNOS gene expression during endotoxemia (Won et al., 2005).

A growing body of evidence suggests that LTs also play an important role in the central nervous system (Lindgren et al., 1985; Shimada et al., 2005) due to the expression of 5-LO and FLAP protein (Lammers et al., 1996), as well as the expression of $CysLT_2$ (cys-LT receptor) in several brain regions, including the hypothalamus (Heise et al., 2000). One of the first reports describing a biological effect of LTs in the brain involved the neuroendocrine system. *In vitro* studies showed that the release of luteinizing hormone (LH) from pituitary cells in response to gonadotrophins releasing hormone (GnRH) was partly mediated by LTs. The response appears to be LTC_4 specific since LTB_4 did not alter the release of LH (Hulting et al., 1985). In the brain, LTC_4 also seems to be related with the vasopressinergic system of the hypothalamic–neurohypophyseal axis. LTC_4 synthase, but not LTA_4 hydrolase, was selectively localized in vasopressinergic neurons of the hypothalamic paraventricular, supraoptic and suprachiasmatic nuclei and in the retrochiasmatic area. In addition it was detected in axons emanating from these neurons to the neurohypophysis (Shimada et al., 2005).

In a recent study from our laboratory we suggested that LTs are involved in arginine vasopressin (AVP) release. The central administration of an inhibitor of leukotriene synthesis in rat abolished the secretion of AVP in the initial phase of the sepsis. Furthermore, its injection improved survival and attenuated the increase of LTC_4 enzyme in the hypothalamus and of serum nitrate levels seen during sepsis (Athayde et al., 2009). As already shown, NOS inhibitor may also affect vasopressin secretion during sepsis, though the results depend on the route of inhibitor administration (Carnio et al., 2006; Corrêa et al., 2007). The objective of this work was to investigate the effects of an intraperitonially administered inhibitor of leukotriene synthesis on AVP release and NO production in the course of experimental sepsis.

2. Material and methods

2.1. Animals

Male Wistar rats (250 ± 30 g) from the Animal Care Facility of the Universidade de São Paulo (USP), Campus Ribeirão Preto were used in the present study. The rats were housed in controlled temperature (25 ± 1 °C) and photoperiod (12-h light/dark cycle) conditions, with food (Nuvilab CR-1, Nuvital Nutrientes, Paraná, Brazil) and water available *ad libitum*. All experimental protocols were performed according to the international guidelines on the ethical use of animals and approved by the Animal Ethics Committee of USP (CEUA)-Campus Ribeirão Preto, defining the number of animals used and minimizing their suffering. Humane endpoints in shock research (Nemzek et al., 2004) were used as criteria to euthanize CLP animals in high suffering immediately before or soon after the time-points defined in this study.

2.2. Experimental protocol

The animals received an intraperitoneal (i.p.) injection of 1 ml containing dimethyl sulfoxide (DMSO) 5% as vehicle or an inhibitor of leukotriene synthesis, MK-886 (3-[1-(p-chlorobenzyl)-5-(isopropyl)-3-tert-butylthioindol-2-yl]-2,2-dimethylpropanoic acid, Merck Frosst Canada Ltd., Quebec, Canada) 1 h before CLP or sham operation. The biological half life of MK-886 is reported to be 2 h (Friedman et al., 1993). In the first group of animals ($n = 80$), the survival of the animals was monitored for 3 days. In the second group ($n = 200$) the animals were decapitated at 0, 4 (early phase of sepsis) or 24 h (late phase of sepsis) after surgery and blood was collected for determination of hematocrit, serum nitrate and sodium, plasma osmolality and proteins, and plasma AVP measurements. The peritoneal exudate was carefully collected for evaluating neutrophil migration into the peritoneal cavity.

2.3. Cecal ligation and puncture (CLP)

Severe sepsis was induced by CLP, as previously described (Corrêa et al., 2007; Pancoto et al., 2008; Athayde et al., 2009; Oliveira-Pelegrin et al., 2009). Briefly, rats were anesthetized with 2, 2, 2-tribromoethanol (Acros Organics, Geel, Belgium, 250 mg/kg i.p.). Under sterile surgical conditions, a 2 cm midline incision was made on the ventral surface of the abdomen, and the cecum was exposed and ligated below the ileocecal junction without causing bowel obstruction. The cecum was punctured 10 times with a 16-gage needle, and fecal contents were allowed to spill into the peritoneum. The cecum was repositioned in the abdomen, and the peritoneal wall and skin incisions were closed. Sham animals underwent an identical laparotomy, but did not undergo ligation and puncture and served as controls. All animals received a subcutaneous injection of saline (20 ml/kg body weight) immediately after the surgery. The animals were allowed to recover in their cages with free access to food and water.

2.4. Neutrophil migration into the peritoneal cavity

Neutrophil migration was assessed 4 h after CLP. The animals were killed and the cells present in the peritoneal cavity were harvested by introducing 15 ml of phosphate-buffered saline (PBS). Total counts were performed with Turk solution in a Neubauer chamber. Differential cell counts were obtained using a cytocentrifuge (Cytospin 3, Shandon Southern Products Ltd., Cheshire, UK) and staining with Panotico (Laborclin LTDA, Pinhais Parana, Brazil). The results were expressed as the number of neutrophils per ml of peritoneal exudate.

2.5. Determination of hematocrit, serum nitrate and sodium, plasma osmolality and protein

Hematocrit was measured by centrifugation, serum sodium by flame photometry (Micronal, São Paulo, Brazil), and plasma osmolality by freezing-point depression (Precision System, Inc., Natick, MA, USA). Plasma protein was determined by a Bradford colorimetric assay in a Microplate reader (Bio-Rad Laboratories, Hercules, CA, USA), and nitrate was quantified by chemoluminescence system (Sievers 280 NOA, Sievers, Boulder, CO, USA).

2.6. Radioimmunoassay (RIA) for AVP

A RIA for AVP was performed as previously described (Corrêa et al., 2007). Briefly, plasma samples (1.0 ml) were extracted using the acetone/petroleum ether method, lyophilized and stored at −70 °C until analysis. Standard reagents and incubation protocols were used